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Some Improvements in Electrophoresis

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The introduction of the electrophoresis apparatus of Tiselius [1, 2, 3] made available for the first time means for investigating in a convenient manner and with a reasonable accuracy the electrophoretic properties of proteins, enzymes and similar high molecular substances carrying an electric charge. Using this method it is possible not only to follow the migration of the substances in question without interrupting the experiment, but also to obtain rather accurate information about the composition of a mixture of high molecular electrolytes in terms of the electrophoretic properties of its components.

After its introduction the apparatus has found widespread use and some improvements have been described. Originally Toepler's schlieren method or Lamm's scale method were used for observing the migrating boundaries, *cf.* Tiselius and Kabat.[4] Later two methods for directly recording the refraction gradient as a function of the position in the electrophoresis cell were published, *i. e.* the schlieren scanning method described by Longsworth,[5] *cf.* Longsworth, Shedlovsky and MacInnes,[6] and the modified Philpot method described by Svensson.[7, 8] Recently Astrup and Helm [9] have introduced a mirror method in the electrophoresis technic, thereby avoiding the very costly corrected schlieren lens. It is the purpose of this paper to describe some of our experiences with this method and further improvements of the apparatus.

THE OPTICAL ARRANGEMENT

The optical arrangement is shown in fig. 1. The camera arrangement (not shown in the figure) is the commonly used method according to Svensson *(l. c.)* including an objective (Hugo Meyer & Co., Görlitz, Aristoplanat 1 : 77,

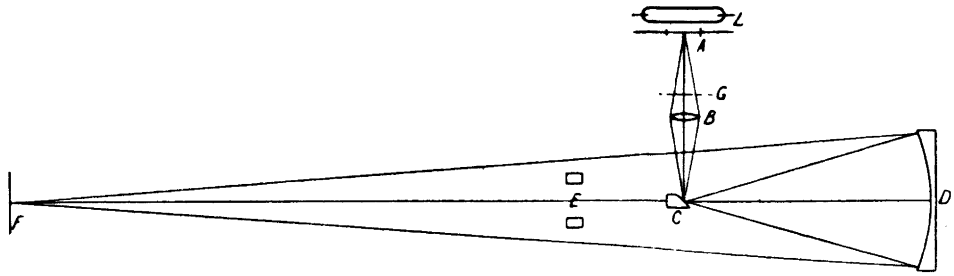

Fig. 1. Optical arrangement of the mirror method for observing the migrating boundaries. A *horizontal slit (0.3 × 20 mm);* B *Voigtländer Anastigmat 1 : 2.5, f = 5 cm;* C *90° reflecting mirror (aluminized);* D *aluminized elliptical mirror, diameter 168 mm, excentricity 3.6;* E *electrophoresis cell;* F *inclined slit;* L *light source.*

f = 45 cm) and a cylindrical lens (a cylindrical spectacle-lens of dioptry + 5 and 0).

As light source is used a Philips »Philora» Hg-lamp (H P 500). The high pressure tube is removed from the bulb and placed horizontally immediately behind the horizontal slit (distance from slit to axis of tube is ~10 mm). In use the tube is red hot and after burning for about 10 minutes the line of maximum light intensity lies a little over the center of the tube; this is to be kept in mind, when the tube is mounted. The horizontal part of the light arc is about 20 mm, with a diameter sufficient to give maximal light intensity during the slit within an angle of 12° — just what is needed. Under these conditions no condenser lens is needed for filling the elliptical mirror with light.

The yellow Hg lines may be used (Zeiss Monochromat Filter A), but the green line gives shorter times of exposure. This line is isolated by combining two filters from Schott & Gen., Jena, namely GG 14 and BG 20. Agfa »Isochrom» plates may be used, but Perutz »Silber-eosin» are better. A hard developer is used.

The objective B forms a diminished image in front of the reflecting mirror C. The size of this mirror allows it to be placed in the optical axis throwing no shadow on the two electrophoresis cells. The front side of the objective is turned against the slit.

The mirror D has ellipsoidal curvature with distances 622 mm and 2.250 mm to the foci, and forms an enlarged image of the horizontal slit on the inclined slit F.

A simple construction of the inclined slit is shown in fig. 2. It may be turned at different angles (α) with the vertical and tan. α is marked on the

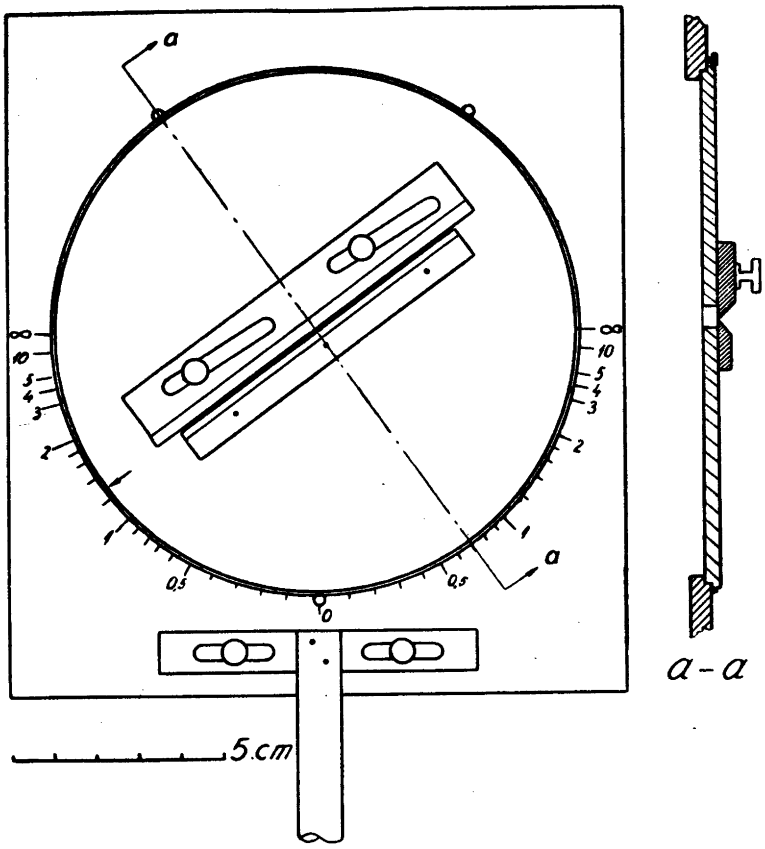

Fig. 2. Construction of the inclined slit.

slit. The areas of the maxima obtained on the photographic plate are proportional to tan. α, and it is thus easy to calculate the exposure times, when the angle is changed. The inclined slit may also be moved until 30 mm in horizontal direction, thus allowing several exposures on the same plate to be made.

The most important advantage of the mirror method over the usual method is that the elliptical mirror is far cheaper than a corrected lens of similar dimensions. A disadvantage is, that the arrangement is more susceptible to shaking. It may be mentioned that a mirror method also has been described for use in the ultracentrifuge.[10]

The waterbath containing the electrophoresis apparatus introduces some errors. The image is formed at a distance of, in our case, about 10 cm further

away from the cells, at which point the inclined slit must be placed. At the same time it becomes unsharp as pencils of light originating from the periphery of the elliptical mirror do not meet at the same points as pencils coming from the center of the mirror. This error may according to our experience be corrected by placing the inclined slit at some distance from the focus in the direction of the water bath, and the vertical slit at a larger distance from the objective B.

The water in the water bath must be kept clear, which is obtained by circulating the water through granulated charcoal (by means of a mammuth pump) at a speed of about 100 liters per hour. The charcoal is changed once a month.

THE ELECTROPHORESIS APPARATUS

In the original apparatus of Tiselius the electrode vessels have a comparative large surface area. Very small movements in vertical direction, as for instance produced by walking on the floor close to the apparatus, will suffice completely to destroy the experiment by liquid passing from one electrode vessel to the other through the cells. This is due to the small cross section of the cells in comparison with the surface area of the liquid in the electrode vessels. Tiselius proposed to decrease this surface area by placing an ebonite rod or stopper in the mouth of the vessels. In our experience we have had much trouble on this point, and we therefore prefer electrode vessels of the closed type described by Longsworth and MacInnes [11] as later used also by Svensson.[12] These vessels also permit the emptying and filling of the apparatus to be carried out without removing it from the water bath.

As the correct dimensions are very important the construction of the apparatus is shown in fig. 3. The electrode vessels are made of »Duran» or »Pyrex» glass and the electrode consists of 50 g of 1 mm silver wire.

We always perform the filling of the apparatus, when it is placed in the water bath. When this filling is carried out at room temperature and the apparatus then placed in the water bath at about 0° the contraction of the solution in the lower closed compartment of the cells may allow liquid to pass in from the surrounding water bath, thus disturbing the experiment. After being placed in the water bath the lower compartment of the cells are filled with the solution under investigation (cooled in the water bath) and closed, whereupon the rest of the apparatus is filled with buffer. Buffer solution is sucked up to above the stopcock in the electrode tube, and 50 ml of saturated NaCl solution is filled in the funnels. The ground glass stoppers over the cells are placed in position and the salt solution in the funnels cautiously poured

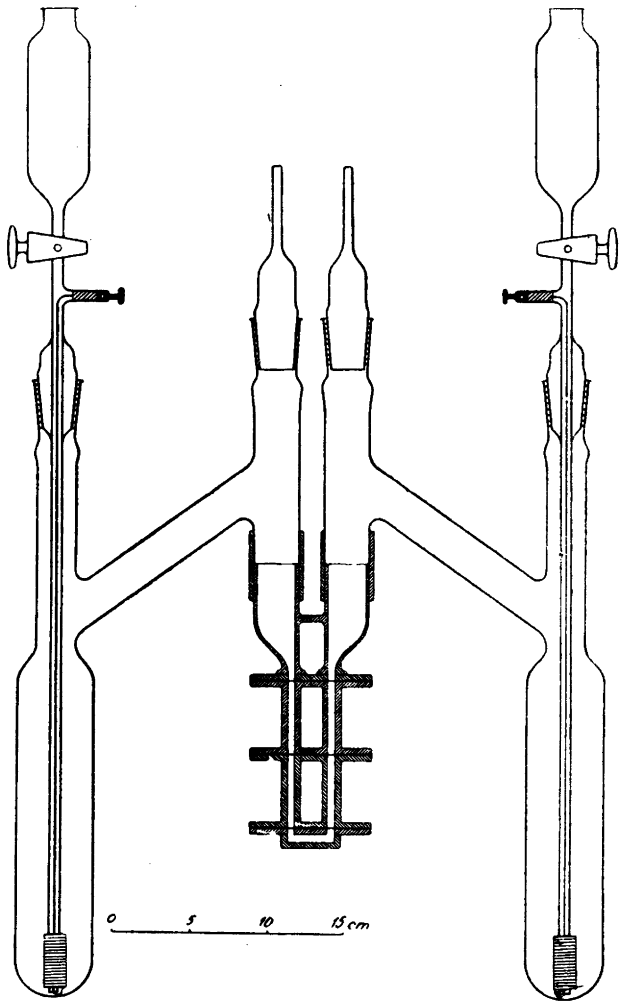

Fig. 3. Design of the electrode vessels.

down over the electrode, whereby the glass stoppers are filled and closed, *e. g.* by means of glass stopcocks (not shown).

In this manner it is possible to prepare for a new experiment in 8—10 minutes, and to run about 15 experiments without removing the apparatus for regreasing of the cells. This relates to qualitative experiments, for the quantitative determination of the migration velocity greasing must be carried out more often in order to secure complete insolation of the cells from the water bath.

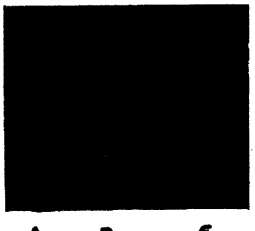

Fig. 4. A: *0.3 % chondroitin polysulfuric acid (K-74) after 7 minutes electrophoresis, tan. α = 0.5 (T-251).*
B: *0.3 % chondroitin sulfuric acid after 7 minutes, tan. α = 0.5 (T-252).*
C: *A solution containing 0.2 % K-74 and 0.2 % chondroitin sulfuric acid after 15 minutes, tan. α = 0.5 (T-265).*

The apparatus has been used for orientating investigations of various kinds, thus in a study of polysaccharide sulfuric acids where it has been found very convenient.

The cellulose-, chitin- and starch polysulfuric acids, prepared according to Astrup, Galsmar and Volkert [13] were investigated. These substances are all highly charged colloidal electrolytes with a high mobility; they were used as sodium salts and 15—45 mg were dissolved in 15 ml of phosphate buffer of pH = 6.7 and ionic strength 0.1. The electrophoresis were carried out with 0.018 ampere during 7—15 minutes. Examples are shown in the paper mentioned. In this manner it is easy to get information about the contents of high molecular electrolytes in the preparation. It was expected, that also the purity of the preparation could be determined in this way, but it was not found possible to differentiate between the cellulose sulfuric acid ester *insoluble* in concentrated salt solutions and the *soluble* ester prepared according to Astrup and Piper,[14] although the soluble substance yields less viscous solutions and inhibits the clotting of blood to a lesser degree than the insoluble substance does. This probably may be the case if the charge per unit of cellulose is the same, while the number of units per molecule is smaller for the soluble substances. Still more unexpected it was, that also a mixture of chondroitin sulfuric acid with a chondroitin polysulfuric acid (active as inhibitor for the blood-clotting) (K-74) did not separate during the electrophoresis (fig. 4).

These results mean that in some cases the results of electrophoresis experiments must be judged with caution. Probably experiments under more varied conditions would yield the information wanted. Compare similar results in electrophoresis of insulin preparations by Hall.[15] Also the boundary anomalies studied by Svensson [16] interfere with the results.

Information about the details of electrophoresis investigations may be found in the comprehensive papers of Longsworth [17, 18] and Svensson.[19]

SUMMARY

1. A method using an elliptical mirror in the optical arrangement of the Tiselius electrophoresis apparatus is described.

2. Electrode vessels of the closed type allowing rapid handling of the apparatus are designed.

3. Experiences in the use of the electrophoresis apparatus are mentioned.

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Di-O-alkylmonothiophosphates and Di-O-alkylmonoselenophosphates and the Corresponding Pseudohalogens

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The present paper contains a study on a new type of thio and seleno pseudohalides and pseudohalogens, *viz.*, di-O-alkylmonothiophosphates $(RO)_2OPS^-$ and di-O-alkylmonoselenophosphates $(RO)_2OPSe^-$, and their dimeric oxidation products $((RO)_2OPS)_2$ and $((RO)_2OPSe)_2$. Some of the salts have been prepared by earlier workers (see p. 11), but their pseudohalide nature was not yet recognized. It is demonstrated in the present work (pp. 17—22) that measurable equilibria exist between the said salts and iodine:

$$2\ (RO)_2OPS^- + I_3^- \rightleftarrows ((RO)_2OPS)_2 + 3\ I^-$$
$$2\ (RO)_2OPSe^- + I_3^- \rightleftarrows ((RO)_2OPSe)_2 + 3\ I^-$$

The redox potentials of the systems

$$2\ (RO)_2OPS^- \rightleftarrows ((RO)_2OPS)_2 + 2\ e$$

as calculated from equilibrium constants, differ but — 0.02 to + 0.06 volts (in dependence of the nature of the alkyl group R) from that of iodine-iodide. Those of the systems

$$2\ (RO)_2OPSe^- \rightleftarrows ((RO)_2OPSe)_2 + 2\ e$$

are 0.19 volts less negative than those of the corresponding thio systems.

The term pseudohalogen was introduced 1925 by Birchenbach and Kellermann[1] as characteristic for some inorganic radicals, the physical and chemical

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properties of which resemble those of the real halogens. The principal radicals coming within this category are those of cyanide, cyanate, thiocyanate, selenocyanate, tellurocyanate, azide, and azidodithiocarbonate.

A general account of pseudohalogens is by Walden and Audrieth.[2]

The term may be extended to include all types of compounds built up by combination in pairs of radicals formed by loss of one electron from a stable, electronegatively charged group.

The reactive bond of halogens and pseudohalogens is a single covalent bond between atoms of the same kind. In reactions, the pseudohalogens, like the halogens, should be capable of undergoing radical fission (electron-pairing) as well as polar fission (electron-sharing).

Thus the following types of compounds may be included in the pseudohalogen list:

1. $((RO)_2OPS)_2$ and $((RO)_2OPSe)_2$. Various oxidation reactions, presumably proceeding by a radical mechanism, are demonstrated pp. 22—29. Polar fission, in the sense

$$((RO)_2OPS^+)\ (SPO(OR)_2^-) \text{ and } ((RO)_2OPSe^+)\ (SePO(OR)_2^-)$$

takes place in the hydrolysis reactions, and in the reactions of the thio compounds with *assym. bis*-(*p*-dimethylaminophenyl)-ethylene.

2. $(RSO_2S)_2$. It was found[3,4] that ethanethiosulphonate and *p*-toluenethiosulphonate give equilibria with iodine:

$$2\ RSO_2S^- + I_3^- \rightleftarrows (RSO_2S)_2 + 3\ I^-$$

From the preliminary experiments made (*l. c.*) the redox potentials of the systems

$$2\ RSO_2S^- \rightleftarrows (RSO_2S)_2 + 2\ e$$

seem to be but 0.03 — 0.05 volts less negative than that of iodide-iodine. Aromatic compounds of the type $(RSO_2S)_2$ were studied by Otto and Troeger,[5] Troeger and Hornung,[6] and Christiansen.[7] They have a tendency to undergo rearrangement as follows:

$$2\ (RSO_2S)_2 = S(SO_2R)_2 + S(S_2O_2R)_2$$

In the aliphatic series they have not yet been isolated, though the existence of $(C_2H_5SO_2S)_2$ is indicated through the said equilibrium with iodide-iodine.

An appropriate nomenclature for the pseudohalogens $((RO)_2OPS)_2$, $((RO)_2OPSe)_2$ and $(RSO_2S)_2$ may be dialkylthiophosphatogens, dialkylselenophosphatogens, and thiosulphonatogens, respectively.

3. Tetrathionate. As demonstrated in a previous paper,[4] tetrathionate in reactions with cyanide ion, sulphite ion, sulphinate ions, and small amounts of ethylxanthate ion undergoes polar fission in the sense

$$(O_3S_2)\ (S_2O_3^{--})$$

thus showing halogen characteristics. A radical mechanism, to a predominant extent, may be assumed to apply in the oxidation by tetrathionate of mercaptides,[8] dithiocarbamates,[9] xanthates,[10] salts of monothio [11] and dithio [10] carboxylic acids, and sulphide.[12]

4. Organic disulphides (including carbonic acid derivatives, such as xanthyldisulphides, thiocarbamyldisulphides, and aroyldisulphides) and diselenides. Disulphides are capable of oxidizing sulphide ion,[13, 14, 15] and react with metals to give mercaptides.[16, 17, 18] Other radical reactions are those with triphenylmethyl:[17, 19]

$$(RS)_2 + 2\ (C_6H_5)_3C = 2\ (C_6H_5)_3CSR$$

Typical examples of polar fission are the reactions with hydroxyl ion (for literature references see Schöberl [20]):

$$(RS)_2 + OH^- = RSOH + RS^-$$

alkali phenate and triphenylmethide: [19]

$$(RS)_2 + R'^- = RSR' + RS^-$$

amines: [21]

$$(RS)_2 + 2\ R_2NH = RSNR_2 + R_2NH_2^+ + RS^-$$

and cyanide ion (first step): [4, 11]

$$(ROCS_2)_2 + CN^- = ROC(S)SCN + ROCS_2^-$$
$$(R_2NCS_2)_2 + CN^- = R_2NC(S)SCN + R_2NCS_2^-$$

Radical fission of diselenides is encountered in reactions with mercury: [22]

$$(RSe)_2 + Hg = (RSe)_2Hg$$

polar fission in their hydrolysis reactions [23] and in their reactions with alkali-metallorganic compounds.[19]

It appears that the thio pseudohalogens may be arranged into three classes with regard to oxidizing properties:

I. Those which give measurable equilibria with iodide, *viz.*, thiocyanogen, dialkylthiophosphatogens, and thiosulphonatogens The potential of the thiocyanogen-thiocyanate electrode is — 0.77 volts.[24] The equilibrium of thiocyanate with iodine has been demonstrated by the present author.[3, 4]

II. Tetrathionate. According to Zimmermann and Latimer [25] the potential of the tetrathionate-thiosulphate electrode is —0.10 volts. The value reported by Scheffer and Böhm,[26] *viz.*, —0.4 volts, is obviously incorrect, since thiosulphate is able to reduce di-*iso*-propylselenophosphatogen (see p. 28), the potential of which is —0.31 volts.

III. Organic disulphides. They may be obtained from the respective anions (thiocarbonyl anions and mercaptides) by oxidation with tetrathionate (see p. 10). The redox potentials of the systems are low, *e. g.*, that of cysteine-cystine + 0.390 ± 0.043 volts.[27, 28a]

A preliminary report on the results of the present investigation has been published.[29] The new method employed for the preparation of di-O-alkyl-monothiophosphates and selenophosphates (pp. 12—16) was discovered 1942—43.[4, 30] The equilibrium of di-O-methylmonothiophosphate with iodine was first investigated 1943.[3, 4]

DI-O-ALKYLMONOTHIOPHOSPHATES AND DI-O-ALKYLMONOSELENOPHOSPHATES

Esters of thiophosphoric acids were first studied by Carius.[31] He prepared di-O-ethylmonothiophosphoric acid $(C_2H_5O)_2OPSH$ and its salts

(1) by the action of ethanol on phosphorus pentasulphide, and

(2) by saponification of the triethyl ester $(C_2H_5O)_3PS$.

Pistschimuka [32] prepared various di-O-alkylmonothiophosphates by means of the method (2) of Carius. Emmet and Jones [33] prepared sodium di-O-methylmonothiophosphate and studied its properties in aqueous solutions.

Pistschimuka (*l. c.*) prepared sodium di-O-ethylmonoselenophosphate $(C_2H_5O)_2OPSeNa$ by saponification of the triethyl ester $(C_2H_5O)_3PSe$.

Carius (*l. c.*) said that di-O-ethylmonothiophosphoric acid is a strong acid, its salts being perfectly stable in the solid state. According to Emmet and Jones (*l. c.*) sodium di-O-methylmonothiophosphate is very stable to hydrolysis, its aqueous solutions being neutral to litmus.

A new and convenient method has been worked out for the preparation of di-O-alkylmonothiophosphates and di-O-alkylmonoselenophosphates, *viz.*, by adding sulphur or selenium to the requisite sodium or potassium phosphites in alcohol-ether solutions:

$$(RO)_2OP^- + S = (RO)_2OPS^-$$
$$(RO)_2OP^- + Se = (RO)_2OPSe^-$$

The reactions are rapid and quantitative.

Solutions of alkali dialkylphosphites are readily obtainable from dialkyl hydrogen phosphites $(RO)_2OPH$ and alcoholic solutions of alkali alkoxide.[34] Dialkyl hydrogen phosphites are formed in excellent yields by the action of phosphorus trichloride on alcohol: [35, 36, 37, 38]

$$3\ ROH + PCl_3 = (RO)_2OPH + 2\ HCl + RCl$$

The alkali dialkyl phosphites are rapidly hydrolysed by water, in anhydrous solvents, however, they are quite stable.

The procedures for preparing sodium and potassium di-O-alkylmonothiophosphates and di-O-alkylmonoselenophosphates are as follows:

0.2 mole sodium or potassium are dissolved in 30—75 ml alcohol (the alcohol corresponding to the dialkyl phosphate to be prepared), and a slight excess (about 0.5 %) of dialkyl hydrogen phosphite, and 60—100 ml ether are added. The ether is essential in effecting a smooth and quantitative process. Sulphur or selenium is added in portions. The reaction takes place vigorously with boiling of the ether, the end being indicated through a rapid change of phenolphtalein to colourless (a crystal added just before the end). Excess of sulphur or selenium is filtered off.

In this way the sodium and potassium methyl, ethyl, *n*-propyl and *iso*-propyl salts have been prepared, and also the potassium *n*-butyl, *iso*-butyl, *sec*-butyl, *iso*-amyl, and *sec*-butylcarbinyl salts. The salts are colourless. From most solvents they separate as needles, which in the case of the sodium salts are very long and thin and distinctly flexible. From petroleum smaller crystals were obtained (sodium propyl and potassium *sec*-butyl salts). Most of them are hygroscopic. The thio salts are perfectly stable in the solid state and in solutions. The seleno salts, if exposed to moist atmosphere and particularly to bright day-light, will assume a red colour on the surface. From aqueous solutions (0.01 *M*) of the seleno salts selenium is sometimes deposited after two or three months.

The salts are readily soluble in water (Pistschimuka [32] said that they were insoluble).

The solubilities of the salts in organic solvents increase with increasing number of carbon atoms in the alkyl groups, however, the *iso*-propyl and *sec*-butyl salts are considerably more soluble than the *n*-propyl and the primary butyl salts, respectively. The sodium salts are more soluble than the corresponding potassium salts, and the seleno salts more soluble than the corresponding thio salts (except for the methyl salts).

These differences in solubility may, partly at least, be explained through a smaller or greater partial covalent character (resonance between purely ionic and purely covalent forms) of the (Na, K) — (S, Se) bonds, increasing covalent character being connected with a higher solubility in non-polar solvents. The percentages of ionic and covalent character are in each case determined by the difference in electronegativity (K<Na<Se<S) of the atoms regarded, a small difference corresponding to a high degree of covalence.[39a] As shown pp. 17—22, the secondary propyl and butyl groups effect a smaller electronegativity of the thio and seleno atoms than do the corresponding primary groups.

The anions probably are resonance hybrids of several electronic structures, as is the phosphate ion.[39b]

In the case of the amyl compounds the free acids are insoluble in water and separate as oil drops if mineral acids are added. They are apparently quite stable.

For the analysis of the salts, accurate and convenient iodometric methods have been worked out. The thiophosphates are oxidized by iodine in presence of sodium hydroxide:

$$(RO)_2OPS^- + 4\ I_2 + 10\ OH^- = (RO)_2PO_2^- + SO_4^{--} + 8\ I^- + 5\ H_2O$$

excess iodine being back-titrated with thiosulphate after addition of sulphuric acid.

The following procedures were adopted:

(1) *0.1* N *iodine*. To 25 ml 0.01 *M* di-O-alkylmonothiophosphate (in the case of the amyl compounds 20 ml) are added 10 ml 1 *N* sodium hydroxide and then 25 ml 0.1 *N* iodine. After standing for 5 minutes, 10 ml 4 *N* sulphuric acid are added, and the excess of iodine is back-titrated with 0.1 *N* thiosulphate.

(2) *0.01* N *iodine*. To 25 ml 0.001 *M* di-O-alkylmonothiophosphate are added 10 ml 0.1 *N* sodium hydroxide and then 25 ml 0.01 *N* iodine. After standing for 5 minutes, 10 ml 2 *N* sulphuric acid are added, and the solution titrated with 0.01 *N* thiosulphate.

The selenophosphates are oxidized by iodine in sodium hydrogen carbonate buffer:

$$(RO)_2OPSe^- + 3\ I_2 + 8\ OH^- = (RO)_2PO_2^- + SeO_3^{--} + 6\ I^- + 4\ H_2O$$

The selenium is oxidized to selenite. For back-titration of the iodine, acetic acid is added, selenite being indifferent to iodide in acetate buffers.

The following procedures have proved their values:

(1) *0.1* N *iodine.* Methyl, ethyl and propyl compounds: To 25 ml 0.01 *M* di-O-alkylmonoselenophosphate are added 2 g sodium hydrogen carbonate and then 20 ml 0.1 *N* iodine. After standing for 5 minutes (stoppered flask), 20 ml 10 per cent acetic acid are added, and the excess of iodine is back-titrated with 0.1 *N* thiosulphate.

In the case of the butyl and amyl compounds a greater excess of iodine must be employed (25 ml 0.1 *N* iodine are added), and the time of standing should be 10 minutes. Moreover, in the case of the amyl compounds 10 ml 96 % ethanol were added prior to iodine, in order to increase the rate of resolution of insoluble intermediates.

(2) *0.01* N *iodine.* To 25 ml 0.001 *M* di-O-alkylmonoselenophosphate are added 2 g sodium hydrogen carbonate and 25 ml 0.01 *N* iodine. After standing for 15 minutes, 20 ml 10 per cent acetic acid are added, and the excess of iodine is back-titrated with 0.01 *N* thiosulphate.

The methods give accurate and self-consistent results.

Experimental

The dialkyl hydrogen phosphites were prepared from phosphorus trichloride and the anhydrous alcohols by the method used by Nylén in the case of the ethyl compound.[38] Three new compounds were included: *sec*-Butyl $(C_4H_9O)_2OPH$, b. p.$_{12}$ 101°C, *iso*-amyl $(C_5H_{11}O)_2OPH$, b. p.$_{10}$ 133°C, *sec*-butylcarbinyl $(C_5H_{11}O)_2OPH$, b. p.$_{15}$ 142°C. Yields 40—50 % methyl compound, 80—90 % ethyl and higher primary alkyl compounds, and 60—70 % *iso*-propyl and *sec*-butyl compounds.

The methyl and ethyl alcohol used in preparing the di-O-alkylmonothiophosphates and selenophosphates had been dried by distilling over magnesium, and in the case of the other alcohols carefully rectified products were employed. In order to avoid rearrangement the same alcohol should be employed as that corresponding to the dialkyl phosphate which is to be prepared.

The sulphur (flowers or rhombic) or selenium (preferably red) were added in portions so as to keep the ether gently boiling during the reaction.

The selenium reacted somewhat smoother than did sulphur, and it is advisable to warm the solution gently before the first portion of selenium is added. When reaction has ceased the excess sulphur or selenium is filtered off (crystals of thiophosphate or

selenophosphate which have separated out may be dissolved by heating). In most cases the products were isolated by evaporation of the alcohol-ether (partly or completely) *in vacuo*. This was effectively and rapidly achieved through attaching the flask directly to the vacuum pipe, omitting the capillary and the condenser, and swirling the flask gently in a water or calcium chloride bath so as to prevent splashing.

The various solvents employed for isolation and recrystallization had all been carefully dried. The petroleum boiled at 80—100° C. Chloroform Ph. N. (containing ethanol) was employed in the case of the ethyl compounds. For all other purposes the ethanol was removed by means of concentrated sulphuric acid, and the chloroform stabilized by addition of two parts per thousand of petroleum.[40]

The general procedure (see p. 12) was employed. The amounts of alcohol and ether reported beneath refer to 0.2 mole sodium (4.6 g) or potassium (7.8 g), and 0.201 mole dialkyl hydrogen phosphite.

Sodium di-O-methylmonothiophosphate and di-O-methylmonoselenophosphate. 50—60 ml methanol, 60 ml ether. The products will crystallize on cooling, and may be more completely isolated through partial evaporation and addition of ether. Recrystallization: 5 g from 50 ml methylacetate and 3 ml methanol. 50 ml ether may be added. Or 5 g from 120—150 ml pure methylacetate. Insoluble in ether and chloroform, readily soluble in pyridine.

Sodium di-O-ethylmonothiophosphate and di-O-ethylmonoselenophosphate. 60 ml ethanol, 60 ml ether. Isolated as above. Recrystallization: 5 g thio salt are dissolved in 50 ml chloroform (Ph. N.), 5 g seleno salt in 30 ml chloroform (Ph. N.). 50 ml ether are added. Practically insoluble in ether, slightly soluble in pure chloroform (0.01 *M* solutions of the thio salt may be prepared).

*Sodium di-O-*n*-propylmonothiophosphate and di-O-*n*-propylmonoselenophosphate.* 60—80 ml *n*-propanol, 60 ml ether. Evaporated to dryness, dissolved in benzene, and petroleum added. Recrystallization: 5 g thio salt from 10 ml benzene (20 ml petroleum are added) or from 80 ml petroleum. 5 g seleno salt from 25 ml petroleum (soluble in 10 ml hot petroleum). Readily soluble in ether.

*Sodium di-O-*iso*-propylmonothiophosphate and di-O-*iso*-propylmonoselenophosphate.* 60—80 ml *iso*-propanol, 60 ml ether. Evaporated to dryness, dissolved in petroleum, and cooled. Recrystallized from petroleum (5 g from 25 ml). Readily soluble in ether and benzene.

Potassium di-O-methylmonothiophosphate and di-O-methylmonoselenophosphate. 30 ml methanol, 80 ml ether. The seleno salt crystallized on cooling, and 100 ml ether were added. The thio salt was evaporated nearly to dryness, and ether added. Recrystallization (thio and seleno): 5 g are dissolved in 15 ml pyridine, and 30 ml ether are added. Readily soluble in acetone, sparingly soluble in methylacetate.

Potassium di-O-ethylmonothiophosphate and di-O-ethylmonoselenophosphate. 50 ml ethanol, 80 ml ether. The salts crystallize excellently. Isolated by cooling and addition of 100 ml ether. Recrystallized from ethylacetate (5 g thio salt dissolved in 50 ml, 5 g seleno salt in 20 ml. 50 ml ether are added). Practically insoluble in ether.

*Potassium di-O-*n*-propylmonothiophosphate and di-O-*n*-propylmonoselenophosphate.* 60 ml *n*-propanol, 100 ml ether. Only the thio salt crystallized on cooling. The seleno salt was isolated through evaporation nearly to dryness, and addition of ether. Recrystallization: 5 g thio salt from 50 ml chloroform and 2 ml *n*-propanol. 50 ml ether are added. Or from 15 ml *n*-propanol (30 ml ether added). 5 g seleno salt from 50 ml chloroform (50 ml ether added).

*Potassium di-O-*iso*-propylmonothiophosphate and di-O-*iso*-propylmonoselenophosphate.* 60 ml *iso*-propanol, 100 ml ether. Evaporated to dryness, dissolved in chloroform, and ether added. Recrystallized from chloroform (5 g thio salt in 20—25 ml, 50 ml ether are added. The seleno salt is more soluble).

*Potassium di-O-*n*-butylmonothiophosphate, di-O-*n*-butylmonoselenophosphate, di-O-*iso*-butylmonothiophosphate, and di-O-*iso*-butylmonoselenophosphate.* 60 ml *n*-butanol and *iso*-butanol, respectively, 100 ml ether. The thio salts crystallized on cooling and were further precipitated by addition of 200 ml ether. Recrystallized from chloroform (5 g dissolved in 50 ml, 50 ml ether added). Seleno salts: Evaporated to dryness, dissolved in benzene, and petroleum added. Recrystallized from benzene-petroleum or from chloroform-ether (5 g dissolved in 15 ml chloroform, 30 ml ether added). Sparingly soluble in ether.

*Potassium di-O-*sec*-butylmonothiophosphate and di-O-*sec*-butylmonoselenophosphate.* 60 ml *sec*-butanol, 100 ml ether. Evaporated to dryness, dissolved in petroleum, and cooled. Recrystallized from petroleum (5 g thio salt from 50 ml, 5 g seleno salt from 25 ml). Readily soluble in ether, benzene, carbon tetrachloride.

*Potassium di-O-*iso*-amylmonothiophosphate, di-O-*iso*-amylmonoselenophosphate, di-O-sec-butylcarbinylmonothiophosphate, and di-O-*sec*-butylcarbinylmonoselenophosphate.* 75 ml *iso*-butylcarbinol and *sec*-butylcarbinol, respectively, 100 ml ether. The thio salts crystallized on cooling. 200 ml ether were further added. Seleno salts: Evaporated to dryness, dissolved in chloroform, and ether added. Recrystallized from chloroform (5 g thio salt dissolved in 30 ml, 5 g seleno salt in 15 ml. 50 ml ether added).

In order to save space the figures for the iodometric analyses of the salts are not included. All the salts were analyzed by the said methods, the found percentages of sulphur and selenium ordinarily differing but 0.1—0.2 relative per cent from the theoretical values. The titration values for equal samples of separate salts usually agreed within less than 0.02 ml.

In the case of the seleno salts 2 g potassium iodide and 20 ml 4 *N* sulphuric acid may be added to the titrated solutions, the following reaction taking place:

$$SeO_3^{--} + 4\ I^- + 6\ H^+ = Se + 2\ I_2 + 3\ H_2O$$

A second titration with thiosulphate may thus be carried out. The results, however, are sometimes too low,[41] and the end point is difficult to observe because of the liberation of finely divided selenium.

Phosphorus analyses were made volumetrically by means of the method of Neumann (for references see Nylén[38]). Selenium was determined gravimetrically according to Fredga,[22] by heating the substance with concentrated sulphuric acid and nitric acid,

and precipitating the selenium by means of hydrazine sulphate. For phosphorus analysis in the filtrate it was diluted to 500 ml, and 100 ml pipetted out, 5 ml concentrated nitric acid added, and excess hydrazine destroyed by means of potassium nitrite. After boiling for some time the phosphate was determined according to Neumann.

0.08388 g $(C_2H_5O)_2OPSK$: 23.19 ml 0.4845 *N* NaOH.
Calc. P 14.9. Found P 14.8.

0.4829 g $(C_2H_5O)_2OPSeK$: 0.1496 g Se, 5 · 21.82 ml 0.4845 *N* NaOH.
Calc. Se 30.9, P 12.1. Found Se 31.0, P 12.1.

THE EQUILIBRIA OF DI-O-ALKYLMONOTHIOPHOSPHATES AND DI-O-ALKYLMONOSELENOPHOSPHATES WITH IODINE. INDUCTIVE EFFECTS OF ALKYL GROUPS

This section contains a study on the reversible equilibria:

$$2\ (RO)_2OPS^- + I_3^- \rightleftarrows ((RO)_2OPS)_2 + 3\ I^- \quad (1)$$

$$2\ (RO)_2OPSe^- + I_3^- \rightleftarrows ((RO)_2OPSe)_2 + 3\ I^- \quad (2)$$

The equilibria are established in neutral and in acid aqueous solutions. The di-O-alkylmonoselenophosphates are more readily oxidized by iodine than are the corresponding thio salts, the latter being indifferent to iodine in presence of iodide. In absence of iodide the di-O-alkylmonoselenophosphates are almost quantitatively oxidized by iodine, in presence of iodide only partly.

The alkyl groups R were found to exert a marked influence on the position of the above equilibria. In the case of (1) it is progressively displaced to the right in the order R = methyl, ethyl, *n*-propyl, primary butyl, *iso*-propyl, *sec*-butyl, and primary amyl. The same sequence was found to hold for the methyl, ethyl, *n*-propyl, and *iso*-propyl seleno compounds.

Within the primary alkyl groups the magnitude of the effect thus depends on the total number of carbon atoms, branching of the chains (butyl and amyl) having no observable influence. Secondary alkyl groups exert a stronger influence than do the primary groups.

The observed effect of the alkyl groups is due to their electron-repelling (electron-release) effect, in terms of the electronic theory of the English school [28b] the $+ I_S$ effect. This is a static effect characteristic of a molecule in equilibrium with its environments. Except for the non-influence of branching of the primary butyl and amyl groups the sequence observed is in accordance with the order usually assigned to the $+ I_S$ effect of alkyl groups: [42] Methyl < Ethyl < *n*-Propyl < *n*-Butyl < *iso*-Propyl.

The case provides an example of $+ I_S$ effect of alkyl groups investigated by a purely static method.

The stoichiometric validity of Eq. (1) has been confirmed at three different concentrations of di-O-alkylmonothiophosphate for R = methyl, ethyl, *n*-propyl, and *iso*-propyl, and hence it may be assumed to apply also for the butyl and amyl compounds. For the equilibrium constants at room temperature of the equilibria (1)

$$K^S = \frac{[((RO)_2OPS)_2]\ [I^-]^3}{[(RO)_2OPS^-]^2\ [I_3^-]}$$

see table 1.

Table 1. Di-O-alkylmonothiophosphate systems.

R	K^S	E_O^S	$\Delta\ E_R^S$
Methyl	0.2	— 0.56	0
Ethyl	1.5	— 0.53	0.03
n-Propyl	3.6	— 0.52	0.04
n-Butyl, *iso*-Butyl	7	— 0.51	0.05
iso-Propyl	24	— 0.50	0.06
sec-Butyl	56	— 0.49	0.07
iso-Amyl, *sec*-Butylcarbinyl	110	— 0.48	0.08

The redox potentials E_O^S of the systems

$$2\ (RO)_2OPS^- \rightleftarrows ((RO)_2OPS)_2 + 2\ e$$

were calculated from the equilibrium constants K^S by means of the equation:

$$E_O^S = E_O^I - \frac{RT}{2F}\ \ln\ K' / K^S$$

Here E_O^I is the standard potential of the iodine-iodide electrode (at unit activity), *viz.*, —0,62 volts, and K' is the dissociation constant of the triiodide ion, *viz.*, 0.0014 at 25° C.[43] Omitting corrections for temperature, we have:

$$E_O^S = -0.54 + 0.029\ \log\ K^S$$

The values so obtained are listed in the third column of table 1.

The figures of the fourth column are the relative potentials

$$\Delta\ E_R^S = E_{O(R)}^S - E_{O(CH_3)}^S$$

They give a measure for the effect of substitution in the methyl groups of di-O-methylmonothiophosphate.

Measurements of the di-O-alkylmonoselenophosphate equilibria (2) at two different concentrations of iodide gave approximately constant values for the equilibrium constants

$$K^{Se} = \frac{[((RO)_2OPSe)_2]\ [I^-]^3}{[(RO)_2OPSe^-]^2\ [I_3^-]}$$

The redox potentials E_O^{Se} of the systems

$$2\ (RO)_2OPSe^- \rightleftarrows ((RO)_2OPSe)_2 + 2\ e$$

calculated as in the case of the thio systems, are listed in the third column of table 2.

Table 2. Di-O-alkylmonoselenophosphate systems.

R	K^{Se}	E_O^{Se}	ΔE_R^{Se}	ΔE_O^{S-Se}
Methyl	$9 \cdot 10^5$	— 0.37	0	0.19
Ethyl	$9 \cdot 10^6$	— 0.34	0.03	0.19
n-Propyl	$2 \cdot 10^7$	— 0.33	0.04	0.19
iso-Propyl	$1 \cdot 10^8$	— 0.31	0.06	0.19

It is interesting to note that the figures for ΔE_K^{Se} $(= E_{O(R)}^{Se} - E_{O(CH_3)}^{Se})$ are identical with those for ΔE_R^S.

Thus the difference between the potentials of the seleno systems and the corresponding thio systems is the same for all the alkyl groups, *viz.*, 0,19 volts.

From the stoichiometric form of Eqq. (1) and (2) it may be concluded that in the case of di-O-alkylmonothiophosphates and di-O-alkylmonoselenophosphates there is no tendency of formation of poly-ions of the type $((RO)_2OPS)_3^-$ and $((RO)_2OPSe)_3^-$.

Experimental

The sodium di-O-methyl, ethyl, *n*-propyl and *iso*-propylmonothiophosphates and selenophosphates were employed, and the potassium salts of the higher alkyl compounds.

Di-O-alkylmonothiophosphates. In examining the equilibrium (1) the following procedure was employed:

To 10 ml aqueous di-O-alkylmonothiophosphate were added 5 ml starch solution (2 parts per thousand), one drop of 10 per cent acetic acid, to prevent hydrolysis, and various amounts of potassium iodide. The volume was adjusted to 25 ml, and the solu-

tion was tested with 0.01 *N* iodine (containing 2 % potassium iodide). The amount of iodide was recorded which had to be present in order that one drop of iodine should produce a stable starch-iodine colour, equal in intensity to that produced by one drop of iodine in 50 ml water containing a few crystals of potassium iodide. The equilibrium constants K^S were calculated from the iodide values so obtained, with the assumption that at this particular intensity the concentration of formed $((RO)_2OPS)_2$ is equal to that of uncombined I_3^-.

The iodine was added from an ordinary buret, the drops having an average volume of 0.03 ml.

Table 3, which gives the amounts of iodine consumed at the said conditions by 10 ml 0.01 *M* thiophosphate, is included in order to illustrate the sensitivity of the method.

Table 3. Amounts of 0.01 N *iodine necessary for equilibrium.*

	Potassium iodide, g								
R	0	0.02	0.06	0.10	0.14	0.20	0.30	0.40	0.50
Methyl		4 drops	1 drop						
Ethyl	1.05 ml	0.45 ml	3 drops	1 drop[b]					
n-Propyl	1.10 ml		6 drops	3 drops	1 drop[b]				
n-Butyl	2.7—9 ml[a]		0.7—8 ml[a]	4 drops	2 drops	1 drop			
iso-Butyl	2.6—8 ml[a]		0.5—6 ml[a]	4 drops	2 drops	1 drop			
iso-Propyl	2.1 ml			0.5 ml		5 drops	1 drop		
sec-Butyl	5.9 ml[a]			0.6 ml[a]		0.4 ml[a]	3 drops	1 drop	
iso-Amyl	6.5—9 ml[a]					3.0—2 ml[a]	9 drops[a]		1 drop
sec-Butyl-carbinyl	6.5—9 ml[a]					3.0—2 ml[a]	9 drops[a]		1 drop

[a]Turbidity [b]Very faint starch-iodine colour

With the methyl, ethyl, *n*-propyl, and *iso*-propyl compounds the solutions remained perfectly clear during the experiments, no insoluble products being formed. In the case of the butyl and amyl compounds, however, the oxidation products $((RO)_2OPS)_2$ are sparingly soluble in water and separated out as a turbidity (butyl compounds after five or six drops of iodine, amyl compounds after four drops). The systems thus became heterogeneous, and more iodine was consumed than would correspond to the true homogeneous equilibria. This is shown by the increase in iodine values when turbidity occurred, and by the fact that if 10 ml 96 % ethanol were added to the turbid reaction mixtures, so as to increase the solubility of the products, iodine was regenerated.

The critical iodide values are given in table 4.

Table 4. Di-O-alkylmonothiophosphate systems. g KI (± 10 %) necessary for equilibrium at one drop of iodine.

R	Molarity of 10 ml di-O-alkylmonothiophosphate			
	0.00354 (= 0.01 · $2^{-3/2}$)	0.01	0.0283 (= 0.01 · $2^{3/2}$)	0.08 (= 0.01 · $4^{3/2}$)
Methyl		0.06	0.12	0.20
Ethyl		0.12	0.22	0.40
n-Propyl		0.16	0.30	0.60
n-Butyl		0.20		
iso-Butyl		0.20		
iso-Propyl	0.15	0.30	0.60	
sec-Butyl		0.40		
iso-Amyl		0.50		
sec-Butylcarbinyl		0.50		

It will be seen that for different concentrations of a single salt the ratio $[I^-]^3 / [(RO)_2 OPS^-]^2$ is nearly constant. There seems to be a slight tendency of higher values at lower concentrations of iodide, perhaps (aside from possible variations in activity coefficients) due to displacement to the right of the equilibrium:

$$I_3^- \rightleftarrows I_2 + I^-$$

which has the equilibrium constant 0.0014 at 25° C.[43] Thus 0.06 g and 0.20 g potassium iodide in a volume of 25 ml give $[I_2] / [I_3^-] = 10$ % and 3 %, respectively. The degree of dissociation is small, and no correction for it has been made in calculating the K^S and K^{Se} values.

The chief source of error in the K^S values is the uncertainty in establishing the exact value of the ratio $[((RO)_2OPS)_2] / [I_3^-]$ at the chosen equilibrium conditions. It may differ ± 50 % from unity, which, together with other probable errors, will give a maximal possible error of ± 60 % in the equilibrium constants, and corresponding errors in the potentials. The relative potentials are more accurate, since the said chief error should remain constant from one measurement to another.

Di-O-alkylmonoselenophosphates. Only the methyl, ethyl, *n*-propyl, and *iso*-propyl systems were examined, these being the only ones that remained homogeneous throughout.

To 10 ml ca 0.01 *M* di-O-alkylmonoselenophosphate (exact concentration determined by iodometric analysis), 5 ml starch solution, and 1 ml 10 % acetic acid, were added 1 g or 2 g potassium iodide, and the solution titrated with 0.01 *N* iodine.

Table 5. Di-O-alkylmonoselenophosphate systems. ml iodine consumed, and calculated equilibrium constants.

R	Molarity of 10 ml	1 g KI		2 g KI	
		ml	*K*	ml	*K*
Methyl	$9.6 \cdot 10^{-3}$	3.0—3.2	$7 \cdot 10^5$	1.0	$10 \cdot 10^5$
Ethyl	$10.2 \cdot 10^{-3}$	7.0	$7 \cdot 10^6$	4.5—5.0	$10 \cdot 10^6$
n-Propyl	$9.9 \cdot 10^{-3}$	7.7—7.9	$1.8 \cdot 10^7$	5.6—5.8	$2.1 \cdot 10^7$
iso-Propyl	$9.8 \cdot 10^{-3}$	8.8—9.0	$1.1 \cdot 10^8$	7.8	$1.4 \cdot 10^8$

In calculating the equilibrium constants the amount of I_3^- present at equilibrium was taken as half a drop of iodine, *viz.*, $\frac{0.03}{2 \cdot 2} \cdot 10^{-5}$ mole.

The maximal possible error in the equilibrium constants and potentials given for the seleno systems should be of the same order of magnitude as those for the thio systems.

The *iso*-propyl seleno salt being the most readily oxidable, its solutions may be accurately titrated with iodine provided that no extra iodide is added. The solution (0.01 *M*) should be about 1 *N* with respect to sulphuric acid. No sharp end points could be obtained in analogeous experiments with the butyl and amyl salts.

DIALKYLTHIOPHOSPHATOGENS AND DIALKYLSELENOPHOSPHATOGENS

In this section the preparation and properties of the dimeric oxidation products $((RO)_2OPS)_2$ and $((RO)_2OPSe)_2$ are described. The terms dialkylthiophosphatogens and dialkylselenophosphatogens, respectively, will be employed (see p. 10). Their structures presumably are:

$$\begin{array}{l} RO \diagdown \quad \diagup OR \\ \quad O = PS - SP = O \\ RO \diagup \quad \diagdown OR \end{array} \quad \text{and} \quad \begin{array}{l} RO \diagdown \quad \diagup OR \\ \quad O = PSe - SeP = O \\ RO \diagup \quad \diagdown OR \end{array}$$

In each series the methyl, ethyl, *iso*-propyl, *iso*-butyl, and *iso*-amyl compounds have been prepared, by the following methods:

Dialkylthiophosphatogens. The methyl compound by the action of bromine on sodium di-O-methylmonothiophosphate in ether suspension, the ethyl compound by the same method from potassium di-O-ethylmonothiophosphate suspended in petrol ether:

$$2\ (RO)_2OPS^- + Br_2 = ((RO)_2OPS)_2 + 2\ Br^-$$

The *iso*-propyl, *iso*-butyl, and *iso*-amyl compounds were prepared from the methyl compound, dissolved in water, and aqueous solutions of the respective potassium di-O-alkylmonothiophosphates:

$$((CH_3O)_2OPS)_2 + 2\ (RO)_2OPS^- \rightleftarrows ((RO)_2OPS)_2 + 2\ (CH_3O)_2OPS^-$$

The equilibrium constants K of these equilibria are related to the differences ΔE_R^S (see p. 18) in redox potentials of the systems

$$2\ (RO)_2OPS^- \rightleftarrows ((RO)_2OPS)_2 + 2\ e$$
$$2\ (CH_3O)_2OPS^- \rightleftarrows ((CH_3O)_2OPS)_2 + 2\ e$$

by the equation:

$$\Delta E_R^S = \frac{RT}{2F} \ln K$$

Furthermore:

$$K = \left(\frac{\alpha}{1-\alpha}\right)^3$$

in which α is the degree of displacement from left to right. For methyl/*iso*-propyl $\Delta E_R^S = 0.06$ and thus $\alpha = 83\ \%$, for methyl/*iso*-butyl $\Delta E_R^S = 0.05$ and $\alpha = 79\ \%$, for methyl/*iso*-amyl $\Delta E_R^S = 0.08$ and $\alpha = 89\ \%$. In all three cases the product $((RO)_2OPS)_2$ is insoluble in water and separates as an oil, which causes further displacement of the equilibrium.

The *iso*-butyl compound was also obtained by the action of iodine, dissolved in ether, on an excess of aqueous potassium di-O-*iso*-butylmonothiophosphate.

The methyl compound $((CH_3O)_2OPS)_2$ forms colourless crystals which melt at 30.5—31° C. It is hygroscopic, and readily soluble in water and in most organic solvents, slightly soluble in petrol ether. The higher alkyl compounds were obtained as colourless oils, being less soluble in water and more soluble in petrol ether the higher the number of carbon atoms in the alkyl groups. The *iso*-butyl and *iso*-amyl compounds are practically insoluble in water.

The compounds are quite stable when kept in a dry atmosphere. The oxidizing power of an 0.01 *N* aqueous solution of the methyl compound is reduced to about half its original value after one hour at room temperature, and after two or three hours sulphur is deposited. Their solutions in inert organic solvents are quite stable.

Dialkylselenophosphatogens. The methyl and ethyl compounds were obtained by the action of iodine, dissolved in ether, on aqueous sodium or potassium di-O-alkylmonoselenophosphate. The *iso*-butyl and *iso*-amyl compounds were prepared from the methyl compound, dissolved in water, and aqueous solutions of the respective di-O-alkylmonoselenophosphates:

$$((CH_3O)_2OPSe)_2 + 2\ (RO)_2OPSe^- \rightleftarrows ((RO)_2OPSe)_2 + 2\ (CH_3O)_2OPSe^-$$

As in the case of the thio compounds the equilibria are further displaced to the right through the insolubility in water of the products.

The dialkylselenophosphatogens may also be obtained from the corresponding thio compounds:

$$((RO)_2OPS)_2 + 2\ (RO)_2OPSe^- = ((RO)_2OPSe)_2 + 2\ (RO)_2OPS^-$$

A difference of 0.19 volt (see p. 19) in the redox potentials of the systems

$$2\ (RO)_2OPS^- \rightleftarrows ((RO)_2OPS)_2 + 2\ e$$
$$2\ (RO)_2OPSe^- \rightleftarrows ((RO)_2OPSe)_2 + 2\ e$$

would give $\alpha = 99\ \%$ for the above change. The ethyl and *iso*-propyl compounds were prepared by this method.

The methyl compound $((CH_3O)_2OPSe)_2$ forms pale greenish crystals with melting point 3—4° C, the higher alkyl compounds are oils. Their solubilities in water and in organic solvents are of the same order of magnitude as those of the corresponding thio compounds. The oils (melts) are green with a brownish tinge, and so are their concentrated solutions (in water and in organic solvents). Dilute solutions have a green colour. In absence of moisture the pure compounds will liberate selenium and thus become red after one or two days. Their solutions in inert organic media are more stable. An 0.01 *N* aqueous solution of the methyl compound liberated selenium after about 10 minutes.

The dialkylthiophosphatogens and dialkylselenophosphatogens are capable of oxidizing iodide (in the case of the seleno compounds only relatively concentrated iodide solutions), thiosulphate (to tetrathionate), and thiocarbonyl salts (to the corresponding disulphides).

With aqueous alkali the thio compounds give a yellow colour, the seleno compounds a brown colour. The hydrolysis probably takes place according to the schemes

$$((RO)_2OPS)_2 + OH^- = (RO)_2OPSOH + (RO)_2OPS^-$$
$$((RO)_2OPSe)_2 + OH^- = (RO)_2OPSeOH + (RO)_2OPSe^-$$

in analogy with the hydrolysis reactions of halogens and organic disulphides.

With *assym. bis*-(*p*-dimethylaminophenyl)-ethylene in acetonitril the thio compounds (methyl and ethyl) give a blue colour, as do iodine and bromine.[44] According to the general scheme [44] the compounds thereby add to the ethylene linkage, with polar fission in the sense $((RO)_2OPS^+)$ $(SPO(OR)_2^-)$.

With aqueous *p*-toluenesulphinate the methyl thio compound immediately gave a semi-solid precipitate, which further solidified on boiling and was dissolved in hot glacial acetic acid. On cooling, crystals of monosulphur di-(*p*-toluenethiosulphonate) $S(S_2O_2C_6H_4CH_3\text{-}p)_2$ separated out. The first step probably is analogeous to the reactions of halogens with sulphinates:

$$((CH_3O)_2OPS)_2 + RSO_2^- = RSO_2SPO(OCH_3)_2 + (CH_3O)_2OPS^-$$

The intermediate $RSO_2SPO(OCH_3)_2$ may be unstable and break up to give thiosulphonatogen $(RSO_2S)_2$, followed by rearrangement [5] into $S(SO_2R)_2$ and $S(S_2O_2R)_2$ (see p. 9).

The methyl seleno compound does apparently not react with aqueous *p*-toluenesulphinate, however, selenium is rapidly liberated. The catalysis may be due to an equilibrium

$$((CH_3O)_2OPSe)_2 + RSO_2^- \rightleftarrows RSO_2SePO(OCH_3)_2 + (CH_3O)_2OPSe^-$$

the compound $RSO_2SePO(OCH_3)_2$ being the unstable intermediate.

Experimental

Ether solutions of dialkylthiophosphatogens and dialkylselenophosphatogens, which in the course of preparation had been in contact with water, were dried over anhydrous sodium sulphate, and the ether subsequently evaporated off *in vacuo*. Before analysis, the products were dried *in vacuo* over sulphuric acid.

Dialkylthiophosphatogens

(1) To 8 g $(CH_3O)_2OPSNa$ suspended in 50 ml abs. ether was added 1 ml bromine. A smooth reaction took place. The solid particles were treated with a glass rod for a few minutes until the bromine colour had vanished, the suspension was then filtered, and the ether evaporated off *in vacuo*. On cooling the remaining oil in a carbon dioxide-acetone freezing mixture it slowly solidified throughout. Crystallization from concentrated ethereal solutions was later effected by cooling and seeding. Colourless crystals, m. p. 30.5—31°C.
0.05061 g subst.: 20.72 ml 0.4845 *N* NaOH (Neumann).
0.08904 g subst.: 0.1469 g $BaSO_4$ (Grote-Krekeler. Phosphate removed as magnesium ammonium phosphate).

$((CH_3O)_2OPS)_2$	(282,2)	Calc.	P 22,0	S 22,7
		Found	» 21,9	» 22,7

In moist air the crystals liquify, and sulphur is liberated. Stability of aqueous solutions: 20 ml 0.009061 *N* thiosulphate to which had been added 10 ml 4 per cent potassium iodide solution and 5 ml starch solution were titrated with
(a) 0.004421 *M* $((CH_3O)_2OPS)_2$ (0.3118 g per 250 ml) in water
(b) 0.004494 *M* $((CH_3O)_2OPS)_2$ (0.3170 g per 250 ml) in 1 *N* aqueous sulphuric acid.

Table 6. Stability of aqueous dimethylthiophosphatogen.

Age of solutions in minutes	ml consumed (a)	(b)
3	21.98	21.65
10	23.03	22.52
20	23.91	23.90
30	25.02	25.31
60	29.53	29.49

Sulphuric acid thus has no influence on the stability. The thiosulphate is evidently oxidized to tetrathionate:

$$((CH_3O)_2OPS)_2 + 2\ S_2O_3^{--} = 2\ (CH_3O)_2OPS^- + (S_2O_3)_2^{--}$$

(2) The suspension of 9 g $(C_2H_5O)_2OPSK$ in 50 ml petrol ether was cooled in an ice-sodium chloride freezing mixture, and 1 ml bromine in 50 ml petrol ether was added. The mixture was treated as decribed in the case of the methyl compound. The residual colourless oil did not crystallize even on cooling to —70° C. It did not distill even at 100° C at 0.07 mm Hg (at 150° rapid decomposition took place).
0.05593 g subst.: 19.08 ml 0.4845 *N* NaOH. 0.08654 g subst.: 0.1193 g $BaSO_4$.

$((C_2H_5O)_2OPS)_2$	(338.3)	Calc.	P 18.3 S 19.0
		Found	» 18.3 » 18.9

(3) 3 g (*iso*-$C_4H_9O)_2OPSK$ (50 per cent excess) in 10 ml water was treated with 1 g iodine dissolved in 25 ml ether. The layers were separated, and the ether solution treated with a few centigrams (*iso*-$C_4H_9O)_2OPSK$ in 2 ml water. Now colourless, it was dried, and the product isolated as described p. 25.
0.1022 g subst.: 0.1065 g $BaSO_4$. 0.09938 g subst.: 24.51 ml 0.5037 *N* NaOH.

$((C_4H_9O)_2OPS)_2$	(450.5)	Calc.	P 13.8 S 14.2
		Found	» 13.7 » 14.3

(4) 1 g $((CH_3O)_2OPS)_2$ dissolved in 10 ml water was added to 10 ml water containing
(a) 3.4 g (*iso*-$C_3H_7O)_2OPSK$ (100 % excess)
(b) 2.1 g (*iso*-$C_4H_9O)_2OPSK$ (10 % excess)
(c) 2.3 g (*iso*-$C_5H_{11}O)_2OPSK$ (10 % excess).
In all three cases a colourless oil immediately separated out and gathered on the bottom of the flask. Ether was added, and the products isolated as described p. 25.
0.06215 g subst.: 17.49 ml 0.5037 *N* NaOH. 0.1089 g subst.: 0.1287 g $BaSO_4$.

$((C_3H_7O)_2OPS)_2$	(394.4)	Calc.	P 15.7 S 16.3
		Found	» 15.7 » 16.2

0.09581 g subst.: 23.83 ml 0.5037 *N* NaOH. 0.1073 g subst.: 0.1123 g $BaSO_4$.

$((C_4H_9O)_2OPS)_2$	(450.5)	Calc.	P 13.8 S 14.2
		Found	» 13.8 » 14.4

0.08334 g subst.: 18.31 ml 0.5037 *N* NaOH. 0.1139 g subst.: 0.1060 g $BaSO_4$.

$((C_5H_{11}O)_2OPS)_2$	(506.6)	Calc.	P 12.2 S 12.7
		Found	» 12.2 » 12.8

Indicator reactions. To 2 ml 0.005 *M* $((CH_3O)_2OPS)_2$ and $((C_2H_5O)_2OPS)_2$, respectively, in acetonitril (previously distilled over phosphorus pentoxide) were added 2 ml *assym. bis*-(*p*-dimethylaminophenyl)-ethylene (one part per thousand) in the same solvent. In a few seconds a blue colour developed. The colour rapidly faded as 4 ml 0.01 *M* piperidinium *cyclo*-pentamethylenedithiocarbamate (in acetonitril) were added.

The reaction with p-*toluenesulphinate.* To 1 g $((CH_3O)_2OPS)_2$ in 10 ml water was added 1 g p-$CH_3C_6H_4SO_2Na \cdot 2H_2O$ (32 per cent excess) in 10 ml water. A semi-solid liquid at once separated out, which further solidified on boiling. Yield 0.68 g (theor. 0,65 g (p-$CH_3C_6H_4SO_2S)_2$). It was recrystallized from glacial acetic acid, 0.31 g dry product being obtained. Colourless crystals, m. p. 184° C, with the typical tetragonal pyramidal appearance[5] of monosulphur di-(p-toluenethiosulphonate).
0.06113 g subst.: 0.1753 g $BaSO_4$. $S(S_2O_2C_7H_7)_2$ = 406.6. Calc. S 39.4. Found S 39.4.
The mother liquor presumably contained the corresponding trithionic compound, however, no definite products could be isolated.

Dialkylselenophosphatogens

(1) To 5 g $(CH_3O)_2OPSeNa$ in 5 ml water were added 2 g iodine dissolved in 50 ml ether. On stirring, the aqueous and the ethereal layer assumed a brown and a brownish-green colour, respectively. The two layers were separated, and the aqueous layer extracted twice with 25 ml ether. The ether solutions were mixed, thoroughly dried over anhydrous sodium sulphate, and the ether evaporated off *in vacuo* until about 20 ml were left. On cooling in a carbon dioxide-acetone freezing mixture pale greenish crystals separated. The mother liquor was decanted, and the crystals treated twice with cold petrol ether. They were then transferred by means of a cooled metal spatula into a small tube-shaped vacuum exsiccator kept in a carbon dioxide-acetone freezing mixture, and the exsiccator was evacuated. Its lower part contained some phosphorus pentoxide and solid paraffine. The melting point was determined as follows: A metal thread was fixed round the mercury bulb of a short thermometer, and the thermometer cooled to —10° C in a transparent tube-shaped vacuum exsiccator containing some phosphorus pentoxide. Some crystals were placed on the thermometer bulb (the metal thread preventing their falling off), and the thermometer replaced in the exsiccator, which was evacuated. The temperature was allowed to rise through removing the exsiccator from the freezing mixture, and the temperature recorded at which the crystals liquified. M. p. 3—4° C (corr.).

Selenium and phosphorus analyses were made as described pp. 16—17.
0.2815 g subst.: 0.1183 g Se, 5 · 17.23 ml 0.4845 N NaOH.

$((CH_3O)_2OPSe)_2$	(376.0)	Calc.	Se 42.0	P 16.5
		Found	» 42.0	» 16.4

An 0.005 M aqueous solution liberated selenium (red) after 10 minutes. 10 ml to which had been added 1 ml 0.01 M p-toluenesulphinate became red after about 1 minute.

(2) To 5 g $(C_2H_5O)_2OPSeK$ in 5 ml water were added 2 g iodine in 50 ml ether, and the mixture was agitated for some minutes. The aqueous layer remained colourless, evidently due to the smaller solubility in water and higher solubility in ether of the ethyl compound as compared with the methyl compound. The ethereal layer was treated in the usual way. The residual oil did not crystallize even when cooled to —70° C.
0.4095 g subst.: 0.1496 g Se, 5 · 22.28 ml 0.4845 N NaOH.

$((C_2H_5O)_2OPSe)_2$	(432.1)	Calc.	Se 36.6	P 14.4
		Found	» 36.5	» 14.6

(3) 1 g $((CH_3O)_2OPSe)_2$ dissolved in 20 ml water was added to 10 ml water containing
(a) 1,8 g (*iso*-$C_4H_9O)_2OPSeK$ (10 per cent excess)
(b) 2,0 g (*iso*-$C_5H_{11}O)_2OPSeK$ (10 per cent excess).
In both cases brownish green oils immediately separated. Ether was added, and the products isolated as described p. 25.

0.4407 g subst.: 0.1266 g Se, 5 · 18.17 ml 0.5037 N NaOH.

$((C_4H_9O)_2OPSe)_2$	(544.3)	Calc.	Se 29.0	P 11.4
		Found	» 28.7	» 11.5

0.4389 g subst.: 0.1153 g Se, 5 · 16.21 ml 0.5037 *N* NaOH.

$((C_5H_{11}O)_2OPSe)_2$	(600.4)	Calc.	Se 26.3	P 10.3
		Found	» 26.3	, 10.3

(4) 2 g $((C_2H_5O)_2OPS)_2$ in 25 ml ether were treated with 3.1 g $(C_2H_5O)_2OPSeNa$ (10 per cent excess) in 10 ml water. The ethereal layer rapidly assumed a brownish green colour. The layers were separated, and the product isolated as described p. 25.

0.4323 g subst.: 0.1576 g Se, 5 · 22.26 ml 0.5037 *N* NaOH.

$((C_2H_5O)_2OPSe)_2$	(432.1)	Calc.	Se 36.6	P 14.4
		Found	» 36.5	» 14.3

(5) 2 g (*iso*-$C_3H_7O)_2OPS)_2$ in 25 ml ether were treated with 3.2 g (*iso*-$C_3H_7O)_2OPSeK$ (10 per cent excess) in 10 ml water.
The reaction took place and the product was isolated as in (4).

0.4166 g subst.: 0.1343 g Se, 5 · 18.85 ml 0.5037 *N* NaOH.

$((C_3H_7O)_2OPSe)_2$	(488.2)	Calc.	Se 32.3	P 12.7
		Found	» 32,2	» 12.6

Solutions of dialkylselenophosphatogens in ether are rapidly decoloured by the action of aqueous sodium thiosulphate. Also piperidine causes a rapid fading of the colour.

The reactions of dialkylthiophosphatogens and dialkylselenophosphatogens with thiocarbonyl salts

The methyl and ethyl compounds were brought into reaction with piperidinium *cyclo*-pentamethylenedithiocarbamate, the *iso*-amyl compounds with potassium ethylxanthate:

$$((RO)_2OPS)_2 + C_5H_{10}NCS_2^- = 2\ (RO)_2OPS^- + (C_5H_{10}NCS_2)_2$$
$$((RO)_2OPSe)_2 + C_5H_{10}NCS_2^- = 2\ (RO)_2OPSe^- + (C_5H_{10}NCS_2)_2$$
$$((RO)_2OPS)_2 + C_2H_5OCS_2^- = 2\ (RO)_2OPS^- + (C_2H_5OCS_2)_2$$
$$((RO)_2OPSe)_2 + C_2H_5OCS_2^- = 2\ (RO)_2OPSe^- + (C_2H_5OCS_2)_2$$

1. To 25 ml 0.22 *M* aqueous $[C_5H_{10}NCS_2]$ $[C_5H_{10}NH_2]$ (1.35 g) in a beaker provided with a mechanical stirrer were added 0,0025 mole phosphatogen, dissolved in water. The reaction product rapidly separated out as volumineous crystals. They were filtered

off, dried, and recrystallized from alcohol. The melting point of *cyclo*-pentamethylene-thiocarbamyldisulphide is 130° C.[45]

(a) 0.71 g $((CH_3O)_2OPS)_2$ (in 25 ml water) yielded 0.78 g crude product (theor. 0.81 g). M. p. (recrystallized) 130° C.

(b) 0.84 g $((C_2H_5O)_2OPS)_2$ (in 100 ml water) yielded 0.80 g crude product (theor. 0.80 g). M. p. (recrystallized) 131° C.

(c) 0.84 g $((CH_3O)_2OPSe)_2$ (in 25 ml water) yielded 0.68 g crude product (theor. 0.72 g). M. p. (recrystallized) 130° C.

(d) 0.99 g $((C_2H_5O)_2OPSe)_2$ (in 100 ml water) yielded 0.73 g crude product (theor. 0.74 g). M. p. (recrystallized) 129—130° C.

2. 25 ml 0.22 M aqueous $C_2H_5OCS_2K$ (0.88 g) were treated for some minutes with 0.0025 mole phosphatogen dissolved in 25 ml ether. The layers were separated, the ethereal layer dried over anhydrous sodium sulphate, and the ether evaporated off *in vacuo*. The residual crystals were recrystallized from alcohol. The melting point of ethylxanthyl-disulphide is 32,5° C.[10]

(a) 1.26 g $((iso\text{-}C_5H_{11}O)_2OPS)_2$. M. p. of product 31° C.
0.03388 g subst.: 0.1302 g $BaSO_4$.
$(C_2H_5OCS_2)_2$ = 242.4. Calc. S 52.9. Found S 52.8.

(b) 1.50 g $((iso\text{-}C_5H_{11}O)_2OPSe)_2$. M. p. of product 31—32° C.
0.03008 g subst.: 0,1157 g $BaSO_4$. Found S 52.8.

SUMMARY

A new method has been worked out for the preparation of di-O-alkyl-monothiophosphates and di-O-alkylmonoselenophosphates, *viz.*, by adding sulphur or selenium to the corresponding sodium or potassium phosphites in alcohol-ether solution.

Iodometric methods are described for the analysis of the salts.

The equilibria

$$2\ (RO)_2OPS^- + I_3^- \rightleftarrows ((RO)_2OPS)_2 + 3\ I^-$$
$$2\ (RO)_2OPSe^- + I_3^- \rightleftarrows ((RO)_2OPSe)_2 + 3\ I^-$$

have been investigated in aqueous solutions, and the redox potentials of the systems

$$2\ (RO)_2OPS^- \rightleftarrows ((RO)_2OPS)_2 + 2\ e \qquad (E_O^S)$$
$$2\ (RO)_2OPSe^- \rightleftarrows ((RO)_2OPSe)_2 + 2\ e \qquad (E_O^{Se})$$

calculated from the obtained equilibrium constants. The potentials, in dependence of the nature of the alkyl groups, decrease in the order methyl, ethyl, *n*-propyl, primary butyl, *iso*-propyl, *sec*-butyl, and primary amyl compounds.

The pseudohalogens $((RO)_2OPS)_2$ and $((RO)_2OPSe)_2$ are prepared, and their properties studied. Reactions are demonstrated, of the type:

$$((CH_3O)_2OPS)_2 + 2\ (RO)_2OPS^- = ((RO)_2OPS)_2 + 2\ (CH_3O)_2OPS^-$$
$$((CH_3O)_2OPSe)_2 + 2\ (RO)_2OPSe^- = ((RO)_2OPSe)_2 + 2\ (CH_3O)_2OPSe^-$$
$$((RO)_2OPS)_2 + 2\ (RO)_2OPSe^- = ((RO)_2OPSe)_2 + 2\ (RO)_2OPS^-$$

The theoretical degrees of reaction are calculated from the redox potentials E_0^S and E_0^{Se}.

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On the Determination of Reducing Sugars

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For a number of years one of us (J. B.) has been working on the enzymatic decomposition of starch. Gradually as the work progressed it was found that the methods usually employed for the determination of the reducing sugars were inadequate. Though the prospects of getting better results seemed doubtful, we nevertheless felt compelled to undertake a testing of known methods, perhaps developing a new one better suitable for our purpose. When looking back on it now we feel almost tempted to say with Jessen-Hansen [1] that »if we had foreseen the difficulties which later appeared in this investigation it is perhaps doubtful that we should ever have undertaken it».

DETERMINATION OF ALDOSES BY MEANS OF IODINE

In alkaline solution aldoses are oxidized by iodine to carboxylic acids. Since the reaction is not sufficiently rapid to make a direct titration possible, the sugar solution is mixed with an excess of iodine and alkali. After standing for some time the solution is acidified and the unconsumed iodine determined. One molecule aldose consumes 2 equivalents of iodine:

$$C_6H_{12}O_6 + I_2 + 3\ OH^- \longrightarrow C_6H_{11}O_7^- + 2\ I^- + 2\ H_2O \qquad (1)$$

This method is originally described by Romijn [2] who realized that the reaction has to be carried out in a weakly alkaline medium. Twenty years later Bougault [3] developed a method that proved better in practice. As base he

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used partly mixtures of Na_2CO_3 and $NaHCO_3$, partly Na_2CO_3 alone. The oxidation of the aldoses proceeded in the presence of the buffer quantitatively in 1 hour, with soda in 30 minutes. Auerbach and Bodländer [4] investigated the effect of the hydrogen ion concentration more systematically. The best results were obtained with equimolecular parts of $NaHCO_3$ and Na_2CO_3. Linderstrøm-Lang and Holter [5] arrived at the result that a more basic carbonate buffer was preferable. Finally, we shall mention Willstätter and Schudel's method [6], which employs NaOH as base. The sugar solution is mixed with about twice the necessary amount of 0.1 *N* iodine; drop by drop there is added 1 ½ time the theoretical amount of 0.1 *N* NaOH; acidifying after 12—15 minutes, and titration of the excess of iodine. Goebel [7] demonstrated that this method, owing to the formation of iodate, only gives good results if the base is added very slowly. This fact is not sufficiently emphasized in the original description and is undoubtedly the reason why satisfactory results have not always been obtained. Myrbäck and Örtenblad [8] prescribe the dropwise addition of 1 $^1/_2$ time the theoretical amount of 0.1 *N* NaOH in the course of not less than 4 minutes.

Table 1. Reagents.

		g/l	mol/l	ml/ analysis	molarity during reaction	pH in blank
I	Iodine 0.1 *N*			10.00	0.033	
II a	$NaHCO_3$	42.0	0.50	10.0	0.167	8.3
II b	Na_2CO_3	10.6	0.10	10.0	0.033	9.3
	$NaHCO_3$	33.6	0.40		0.133	
II c	Na_2CO_3	26.5	0.25	10.0	0.083	9.7
	$NaHCO_3$	21.0	0.25		0.083	
II d	Na_2CO_3	42.4	0.40	10.0	0.133	10.2
	$NaHCO_3$	8.4	0.10		0.033	
II e	Na_2CO_3	53.0	0.50	10.0	0.167	10.6
III	H_2SO_4		1.0	10.0		
IV	$Na_2S_2O_3$ 0.1 *N*					
V	Centrifuged solution of soluble starch					

Method

The reagents employed are listed in table 1. For the measurement of the iodine solution a 10 ml automatic pipette is used, and for titration a 10 ml burette, graduated in 1/50 ml divisions. The reaction flasks are 100 ml flasks as used in the determination of the iodine number. The sugar or the sugar solution is weighed in or pipetted into the

flasks — at the most 0.35 millimole in not more than 10 ml; then follows the addition of 10.00 ml of iodine solution, 10 ml buffer solution, and water, to a total of 30 ml. For the blanks a mixture of 10.00 ml of iodine and 10 ml of buffer solution are mixed with 10 ml of water. The flasks are equipped with glass stopper, shaken and put away, protected against direct sunlight. After 30 minutes 10 ml of sulphuric acid are added, and the excess of iodine titrated with thiosulphate and starch as indicator. The difference: blank experiment — main experiment gives the amount of iodine consumed, and 1 ml of 0.1 *N* iodine corresponds to 9.005 mg of monosaccharide (anhydrous), resp. 17.11 mg of disaccharide (anhydrous). When nothing else is said, the experiments are carried out with 0.25 millimole aldose, which corresponds to an iodine excess of 100 % as well as buffer IId.

Results

Table 2. Influence of pH on oxidation of glucose.

pH in blank	8.3	9.3	9.7	10.2	10.6
Reaction time, min	%	%	%	%	%
15	13.4	78.5	98.7	100.7	99.8
30	20.0	89.9	100.5	100.9	101.0
45	24.6	94.1	100.9	101.0	100.7
60	28.6	96.3	101.3	101.2	101.3

The influence of the acidity on the oxidation of glucose is shown in table 2. The reaction velocity rises with increasing pH. pH is measured in the blank. pH must be about 10 before the reaction proceeds quantitatively in 30 minutes. Since the determinations most frequently involve sugar in weakly acid solution, and since OH^- ions are consumed during the reaction itself (1), one safeguards against a drop of pH below 10 by using a more basic buffer. Like Linderstrøm-Lang and Holter we use in the following method as well as in the definitive method buffer IId. With this buffer pH decreases during the oxidation of the aldose from 10.2 to 10.0. Na_2CO_3 (IIe) — which we have previously used in innumerable experiments — gives also good results, in complete agreement with Bougault. As table 3 shows, galactose, maltose and lactose can be determined with the same accuracy as glucose. In the case of all

Table 3. Influence of reaction time on oxidation of various sugars.

Reaction time, min	Glucose, %	Galactose, %	Maltose, %	Lactose, %
15	100.7	99.8	100.3	99.5
30	100.9	100.0	100.6	100.3
45	101.0	100.2	100.9	101.1
60	101.2	100.3	101.0	101.0

sugars the oxidation must be regarded as completed in 30 minutes. There is no distinct difference between the velocities at which mono- and disaccharides are oxidized. The reaction between aldose and iodine is not altogether confined to the stoichiometric oxidation of the aldehyde group to carboxylic acid, but is accompanied by a secondary reaction, though an insignificant one, which according to Bougault should consist of a slow oxidation of the alcohol groups. Auerbach and Bodländer try to limit this reaction by using a buffer with low pH and by working in a substantially more dilute reaction medium. In order

Table 4. Influence of reaction volume on oxidation of glucose.

Reaction time, min	Reaction volume 30 ml	Reaction volume 60 ml
30	100.9	98.9
45	101.0	—
60	101.2	100.4
120	—	101.2

to limit the reaction time to 2 hours, however, they reduce pH only to 10.1, corresponding to equivalent amounts of Na_2CO_3 and $NaHCO_3$. Our investigations on the influence of the reaction volume on the oxidation af glucose (table 4) show that the secondary reaction is not suppressed by dilution, but that this dilution only slows up the process.

The following experimental series include also dextrins. Since the equivalent weight of dextrins is unknown we shall in the following tables put the iodine consumption under standard conditions (30 min. buffer II d) for each sugar at 100.0 %. While the oxidation of glucose, maltose and maltodextrin already is complete in about 15 minutes, the same (table 5) is not true of the residual- and amylodextrin which are of substantially higher molecular weight. Whether this is due to a slower oxidation of the aldehyde groups or is caused

Table 5. Influence of reaction time on oxidation of glucose, maltose and various dextrins.

Reaction time, min	Glucose, %	Maltose, %	Maltodextrin, %	Residual dextrin, %	Amylo-dextrin, %
15	99.8	99.7	99.3	93.3	75.8
30	100.0	100.0	100.0	100.0	100.0
45	100.1	100.3	100.8	104.8	108.4
60	100.3	100.4	101.9	108.6	114.6
Equivalent weight, calculated (30 min.)	90	171	420	abt. 1600	abt. 2800

by an oxidative attack on the other groups can not be decided. When considering that the residual dextrin contains about 20, the amylodextrin about 35 glucose residues for each free aldehyde group, it is understood that there is ample opportunity for oxidation of alcohol groups or rupture of glucoside bonds. The amylodextrin belongs to the so-called erythrodextrins, *i. e.*, dextrins containing groups or bonds which add iodine under red colour reaction. It is perhaps these which are attacked oxidatively. Dilution to twice the original volume does not (table 6) have any substantial effect on the

Table 6. Influence of reaction volume on oxidation of glucose, maltose and various dextrins.

Reaction volume, ml	Glucose, %	Maltose, %	Maltodextrin, %	Residual dextrin, %	Amylo-dextrin, %
30	100.0	100.0	100.0	100.0	100.0
60	98.0	97.2	98.6	99.2	113.7

iodine consumption, except in the case of amylodextrin where dilution causes an increase in the consumption of iodine. This can be explained by assuming that the above mentioned bonds or groups in the dilute solution occupy less iodine than in the strong solution, so that in the dilute solution there is more iodine available for oxidation of the aldehyde group. The ratio: iodine/sugar (table 7) is without any essential influence on the oxidation of glucose, maltose and maltodextrin. But it is of decisive importance to the oxidation of residual dextrin and amylodextrin. The just mentioned phenomena

Table 7. Influence of quantity of sugar on oxidation of glucose, maltose and various dextrins.

Quantity of sugar % of standard	Glucose, %	Maltose, %	Malto-dextrin, %	Residual dextrin, %	Amylo-dextrin, %
50	100.5	100.4	100.4	107.0	133.1
100	100.0	100.0	100.0	100.0	100.0
150	99.0	98.7	98.4	90.1	69.3

may give rise to difficulties in the determination of the activity of amylase preparations. In the very first stages of the hydrolysis of the starch the reaction mixture still contains so many dextrins that bind iodine so powerfully (the solution turning blue or red) that the oxidation of the free aldehyde groups in the presence of an insufficient excess of iodine does not proceed quantitatively. On the other hand, iodine in alkaline solution is so strong an oxidizing agent that certain bonds in the degradation products of the starch can be split oxidatively.

The Mechanism of the Reaction

By hydrolysis of iodine, hypo-iodous acid and iodine ion are formed:

$$I_3^- + OH^- \rightarrow HIO + 2\ I^- \qquad (2)$$

Hypo-iodous acid is an extremely weak acid. Appreciable amounts of IO^- are not formed until higher $[OH^-]$:

$$HIO + OH^- \rightleftharpoons IO^- + H_2O \qquad (3)$$

Hypo-iodous acid is a very unstable compound which rapidly is decomposed to iodide and iodate. According to Förster and Gyr [9] this reaction depends on the simultaneous presence of free acid and ion, and may summarily be described by the following equation:

$$IO^- + 2\,HIO + 2\,OH^- \rightarrow IO_3^- + 2\,I^- + 2\,H_2O \qquad (4)$$

More recent investigations by Bray [10] have shown, however, that the course of the reaction is much more complicated.

It is well known that the oxidative properties of inorganic, oxygen-containing compounds like chlorate, bromate, iodate, nitrate, nitrite, do not appear in alkaline or neutral solution. The oxidative ability is not displayed until the solution has been acidified, *i. e.*, until the formation of the free acids. Assuming that the same is true of hypo-iodite then it is only the free acid that possesses oxidative properties. The formation of iodate is then explained by oxidation of IO^- with HIO to IO_3^- (4), while the oxidation of an aldose proceeds analogously:

$$R{-}CHO + HIO + 2\ OH^- \rightarrow R{-}COO^- + I^- + 2\ H_2O \qquad (5)$$

If we now look at table 8, with these points of view in mind, the displacement of the pH of the buffer by mixing with iodine potassium iodide is explained by reaction (2). With $NaHCO_3$ the displacement is much smaller than with Na_2CO_3, owing to the formation of increasing amounts of hypo-iodous acid with increasing pH (2). Rising concentration of hypo-iodous acid is accompanied by an increase in the rate at which the aldoses are oxidized, table 2, (5). In the oxidation of aldoses OH^- is consumed; this is distinctly seen from the pH-measurements in the blanks and in the analyses, *i. e.*, before and after the reaction has occurred (5). That the fall in pH during the

Table 8. pH in buffer, blank and analysis after reaction.

	Buffer	Blank	Analysis
ml buffer	10	10	10
ml water	20	10	—
ml 0.1 *N* iodine	—	10	10
ml sugar solution	—	—	10
II a	8.33	8.29	7.50
II b	9.32	9.26	8.60
II c	10.0	9.7	9.4
II d	10.6	10.2	10.0
II e	11.9	10.6	10.5

reaction is much smaller at pH = 10.6 than at pH = 9.3 is due to the circumstance that in the first instance we approach the optimal buffer capacity of the Na_2CO_3—$NaHCO_3$ buffer (pH = about 10.1) while the buffer II b only has a relatively low capacity.

In the soda-iodine-potassium iodide mixture there is still only relatively little IO^- and relatively much HIO, (3). The rate at which the sugar is oxidized is therefore so much greater than the rate at which hypo-iodous acid is converted into iodate that the oxidation of the aldoses proceeds quantitatively. Upon complete oxidation it is without importance whether the iodate formation continues, since the same amount of iodine as that used for this purpose again is formed by acidifying before titration:

$$IO_3^- + 8\ I^- + 6\ H^+ \rightarrow 3\ I_3^- + 3\ H_2O \qquad (6)$$

In the mixture of sodium hydroxide, iodine and potassium iodide the amount of the IO^- is increased relatively to HIO, and the velocity of the two competing reactions is displaced so much in favour of the iodate formation that the oxidation of the aldoses may be incomplete.

When using NaOH one may by slow addition of this base achieve quantitative oxidation of the aldose. Measurement with glass electrode showed that the oxidation, and hence the OH^- consumption, is so rapid that pH up to the time when the theoretical amount has been added only exceeds the value 9—10 immediately after the addition of the base. If the base is added rapidly, however, or too much of it is used, the formation of iodate will get so favourable conditions that the iodine only suffices for a partial oxidation of the aldose. These difficulties are avoided when using carbonate or carbonate-bicarbonate buffer, for which reason this procedure is preferable.

DETERMINATION OF REDUCING SUGARS BY MEANS OF FERRICYANIDE

According to Hagedorn and Jensen [11] the sugar solution is heated with an excess of a weakly alkaline solution of potassium ferricyanide whereby the sugar reduces $Fe(CN)_6^{---}$ to $Fe(CN)_6^{----}$. The excess of ferricyanide is determined by titration with thiosulphate, according to the reaction:

$$2\ Fe(CN)_6^{---} + 2\ I^- \rightarrow 2\ Fe(CN)_6^{----} + I_2 \qquad (7)$$

To complete the conversion, $Fe(CN)_6^{----}$ is precipitated by Zn^{++} and K^+ as $K_2Zn_3\ [Fe(CN)_6]_2$. Since the reaction between sugar and ferricyanide does not proceed stoichiometrically, the amount of sugar is found in empirically established tables or calculated by means of factors.

Method

Table 9. Reagents.

		g/l	mol/l		ml/analysis	molarity during reaction		pH in blank
I	K_3FeCy_6	32.9	0.1		10.00	0.04		
II a	Na_2CO_3	53.0	0.5		10.0	0.2		9.8
	$NaHCO_3$	42.0	0.5			0.2		
II b	Na_2CO_3	53.0	0.4	CO_3^{--}	10.0	0.16	CO_3^{--}	10.5
	HCl 1 *N*	100 ml	0.1	HCO_3^-		0.04	HCO_3^-	
II c	Na_2HPO_4, 12 H_2O	179.1	0.25	HPO_4^{--}	10,0	0.10	HPO_4^{--}	11.9
	NaOH 2.5 *N*	100 ml	0.25	PO_4^{---}		0.10	PO_4^{---}	
III	KI	83.0	0.5		5.0			
	Na_2CO_3	0.5						
IV	$ZnSO_4$, $7H_2O$	72.0	0.25		10.0			
	H_2SO_4, conc.	98.0	1.0					
V	$Na_2S_2O_3$	0.1 *N*						
VI	Centrifuged solution of soluble starch							

The reagents employed are listed in table 9. The ferricyanide solution is measured by a 10 ml automatic pipette, and the titration is carried out with a 10 ml burette graduated in 1/50 ml divisions. The reaction flask is a 25 ml volumetric flask, the titration flask a 150 ml Erlenmeyer. Solutions I and II may be mixed. The sugar solution is weighed in or pipetted off into the volumetric flask. I and II are added. The solution is made up with water to the mark and shaken. The flasks are placed in a boiling water bath. When

the heating is completed they are cooled in running water. The contents of the flasks are washed quantitatively over into the titration flasks. III and IV are added, and the liberated iodine titrated with thiosulphate. Volumetric flasks are chosen as reaction flasks because this procedure reduces the deviations in double determinations. The reason may be a more uniform heating or exclusion of atmospheric oxygen (*vide* copper methods).

Results

Since we have found in experiments with copper methods that the pH of the reaction mixture has a decisive influence on the reduction equivalents (RE) of the sugars, buffers are added to fix the pH of the reaction mixture. We choose buffers IIa, IIb and IIc mentioned above. In the experiments on the influence of the time of heating (table 10), the sugar concentration is kept constant, corresponding to a consumption of about one-half of the amount of ferricyanide added. Blank determinations were made for each time of heating,

Table 10. Influence of time of heating at various pH on the RE of glucose and maltose. Constant sugar concentration.

pH	9.8			10.5			11.9		
Time of heating, min	Glucose 0.067 mmol	Maltose 0.059 mmol	Maltose / Glucose	Glucose 0.073 mmol	Maltose 0.070 mmol	Maltose / Glucose	Glucose 0.077 mmol	Maltose 0.074 mmol	Maltose / Glucose
10	6.94			6.69			6.02		
15	7.73	10.77	1.39	6.72	10.39	1.55	6.03	9.47	1.57
20	7.93			6.97			6.40		
30	8.07	11.53	1.43						
45	8.18	11.96	1.46						

though it was found that this time does not affect the value of the blank. The individual determination is encumbered with considerable uncertainty. In the case of both sugars, RE increases with the heating time and decreases with increasing pH. Under the same conditions RE of the maltose is substantially higher than that of the glucose. The reason may be that »maltose by heating with the alkaline ferricyanide is broken down to glucose», as assumed by Linderstrøm-Lang and Holter [5]. In favour of this idea is also the fact that the quotient: RE-maltose/RE-glucose increases with increasing pH. Since the rate at which sugar is oxidized increases with increasing pH we have in the investigation on the influence of the sugar concentration, (table 11) at pH = 9.8 used the time of heating 45 minutes, at pH = 10.5 and 11.9 15 minutes which is the heating time usually employed. At pH = 9.8 and 11.9

Table 11. Influence of sugar concentration at various pH on the RE of glucose and maltose. Constant time of heating.

pH	9.8		10.5		11.9	
Time of heating, min	45		15		15	
abt. mmol	Glucose	Maltose	Glucose	Maltose	Glucose	Maltose
0.02	9.41	12.80		10.29		
.03					7.47	10.93
.04			6.60			
.05	8.53	12.06		10.28		9.86
.06					6.44	
.07	8.26	11.93	6.72	10.39		9.47
.08					6.17	
.10	8.21		6.74		6.08	
.12					5.87	

the RE of the glucose and of the maltose decreases strongly with increasing sugar concentration. In the case of both sugars RE varies the least at pH = 10.5, and less for maltose than for glucose. This is in agreement with Hanes [13] who, with Na_2CO_3 and 15 minutes of heating, reaches constant RE for maltose and with increasing amount of sugar only slightly decreasing RE for glucose.

Table 12. Modifications of the method of Hagedorn, Jensen.

	The reaction mixture Molarities $FeCy_6$---	Alkali	pH abt.	Time of heating, min	Glucose RE	Maltose RE	Maltose in % of glucose
Hagedorn, Jensen [11]	0.0007	0.014 Na_2CO_3	11.7	15	5.08		
Pucher, Finch [12]	0.0007	0.014 »	11.7	15	5.08 (calc.)	7.65 (calc.)	151
Hanes [13]	0.0125	0.05 »	11.7	15	5.24	8.26 K	158
Sobotka, Reiner [14]	0.0125	0.05 »	11.7	15	5.30	8.73	165
Nørby [15]	0.006	0.024 »	11.7	20		7.94 K	
Blisch [16]	0.0333	0.13 »	11.7	20		8.50 K	
Fujita, Iwatake [17]	0.003	0.13 K_3PO_4 0.53 K_2HPO_4	11	15	5.98 K		
Blom, Rosted	0.04	0.2 Na_2CO_3 0.2 $NaHCO_3$	9.8	15	7.73	10.77	139
» »	0.04	0.16 Na_2CO_3 0.04 $NaHCO_3$	10.5	15	6.72	10.39	155
» »	0.04	0.1 Na_3PO_4 0.1 Na_2HPO_4	11.9	15	6.03	9.47	157

»K» indicates that RE is constant. Calculation by means of a factor.
In all other cases RE is not constant. Calculation by means of a table.

Since Hagedorn and Jensen published the ferricyanide method in 1923 various modifications have been introduced, some of which are listed in table 12. In most instances Na_2CO_3 is employed. Fujita and Iwatake [17] use a phosphate buffer with pH about 11. In the different methods the RE of the glucose lies between 5 and 7.7 and the RE of the maltose between 7.5 and 10.8, which is about 1 $^1/_2$ times as high as the RE of the glucose. As was to be expected, the RE of the maltose as well as that of the glucose increase with increasing $[Fe(CN)_6^{---}]$ under the same conditions, *i. e.*, same pH and same time of heating. Our ferricyanide concentration of the reaction mixture is the highest, which must account for the strong oxidation of the sugar. However, the ratio between the RE of the maltose and the RE of the glucose is not substantially displaced from 3 : 2. Experiments with dextrins are mentioned in the last chapter.

DETERMINATION OF REDUCING SUGARS BY MEANS OF COPPER TARTRATE

When heated, alkaline solutions of cupric salts oxidize reducing sugars with precipitation of Cu_2O. In the alkaline medium Cu^{++} can only be kept in solution through complex formation. Most frequently oxy-acids are used for this purpose, in most instances tartaric acid as in Fehling's solution. Alkaline copper tartrate solutions are not stable, owing to their large contents of organic substance in a strongly alkaline medium. Hence Fehling's solution is divided into two components which are not mixed until the agent is to be used: I, $CuSO_4$, and II, Seignette's salt + NaOH. Even solution II is not really stable and may, when stored for a longer period of time, be a cause of error, Josephson [18]. Nor is a solution of Seignette's salt stable, being a good substrate for microorganisms. Hence Kjeldahl [19] mixes the components immediately before the analysis, adding Seignette's salt in solid form.

According to Kjeldahl, the sugar solution is boiled on a water bath with an excess of Fehling's solution for 20 minutes while hydrogen is led through. The precipitated Cu_2O is sucked off, reduced to Cu and weighed. The amount of sugar is found in empirically prepared tables. This method is undoubtedly more carefully worked out and gives more exact results than any other method employing Fehling's solution. But being rather elaborate it has not been widely used.

Bertrand's [20] method is a very popular copper method. The sugar solution is boiled over open fire with an excess of Fehling's solution for 3 minutes. Cu_2O is sucked off and determined after dissolving in sulphuric acid containing Fe^{+++} by titration with $KMnO_4$ of the Fe^{++} formed. This method is more

rapid when using a special filter stick, Blom [21]. In this form we have applied Bertrand's method to thousands of analyses.

In the direct methods Cu_2O is sucked off. Since Cu^+ can remain in solution by complex formation with NH_3, amino acids, peptones, or the like, Neuberg [22], and thus not be included in the determination, direct methods can give too low values. This source of error is avoided in the indirect methods. In these the sugar solution is boiled with an excess of alkaline copper tartrate solution and the amount of unconsumed Cu^{++} determined by titration. Such methods are encumbered with the fundamental errors of a difference determination, but since the methods are very rapid and especially suited for serial analyses they are widely used.

In order to temper the conditions for the reaction, and thereby reduce the hydrolytic cleavage of oligo- and polysaccharides, we have replaced the strong alkali by a Na_2CO_3—$NaHCO_3$ buffer in the proportion 1 : 1. In spite of very comprehensive experiments (table 13) we failed, however, in developing a

Table 13. The reduction equivalent (RE) of various sugars with alkaline solutions of copper tartrate.

Method	Molarity of Cu^{++} in mixture	Alkali	pH	Time of heating, min	Glucose	Galactose	Maltose	Lactose
Bertrand	0.053	NaOH	>14	3	5.4	5.2	5.9	7.4
Kjeldahl	0.083	NaOH	>14	20	6.1	5.5	6.9	8.3
Blom, Rosted	0.080	Na_2CO_3 $NaHCO_3$	9.9	45	8.2	—	8.2	—

method of analysis on this basis that gave results which were reproducible with the desired accuracy. Within each individual series of experiments it was possible to reproduce the results satisfactorily, but when a new reagent was prepared the results might show a displacement of 2 %. Even when the individual components of the reagent were mixed immediately before use it was not possible to eliminate these fluctuations, just as we could not find their cause. Similar difficulties led Kjeldahl to start his well known investigations. Maquenne [23] and Jessen-Hansen [1] encountered the same problem, which also made us, at last abandon the copper tartrate methods in favour of the copper method described in the following chapter.

DETERMINATION OF REDUCING SUGARS BY MEANS OF POTASSIUM COPPER CARBONATE

Concentrated solutions of potassium bicarbonate, potassium carbonate or mixtures of these salts dissolve freshly precipitated copper carbonate under the formation of complex potassium copper carbonate. Soldaini [24] was the first to recommend potassium copper carbonate as reagent for reducing sugars, and Ost [25] was the first to use it in quantitative determinations. While Soldaini's reagent contains only bicarbonate, Ost's contains also carbonate. According to Ost, solutions containing only the bicarbonate react so slowly that they are not suitable for sugar determinations. In his »Investigations on the reaction of the sugars to alkaline copper solutions», Kjeldahl [19] presents a comprehensive experimental material on the determination of different sugars by means of Ost's reagent, without however giving definite sugar tables and without any final appraisal of the method.

Our reason for starting the present investigations was that potassium copper carbonate presents several obvious advantages in relation to Fehling's solution. Cu^{++} remains complexly in a solution of well defined inorganic compounds which at the same time constitute components of a weakly alkaline buffer. Because of the lower pH the reagent does not decompose oligo- and polysaccharides to the same extent as does Fehling's solution.

Method

The sugar solution is heated on a boiling water bath with an excess of potassium copper carbonate. Cu^{++} is reduced by the sugar to Cu^{+} which is precipitated as Cu_2O. The excess is determined iodometrically. A blank experiment is carried out under the same conditions. From the difference between the blank and the main experiment the amount of sugar is calculated by multiplication by a factor.

Reagents: I: The copper sulphate is dissolved in a 1 liter beaker in 200 ml of boiling water. After cooling K_2CO_3 I is added cautiously while stirring. When CO_2 has escaped, which is accelerated by stirring, there are added 400 ml of water, pulverized $KHCO_3$ and K_2CO_3 II. When the main part of the salts is dissolved, the total contents are washed over into a 1 liter volumetric flask and diluted with water to the neck of the flask. K_2CO_3 is easily dissolved, $KHCO_3$ with difficulty. Large crystals of $KHCO_3$ must therefore be pulverized very carefully. Gradually everything dissolves when the flask is shaken, but at the same time K_2SO_4 appears in fine crystals. After dilution to the mark and shaking, the flask is left overnight for continued precipitation of K_2SO_4, whereupon the content is filtered. The solution does not keep indefinitely, as there slowly separate bluish salts, probably consisting of copper carbonates.

II: Na_2CO_3 stabilizes the solution.

III: The lead acetate is dissolved in about 20 ml of water, to which a few drops of glacial acetic acid are added, before addition of the hydrochloric acid.

Table 14. Reagents.

		g/l	mol/l	ml/ analysis	mmol/ analysis
I	$CuSO_4$, $5H_2O$	41.6	0.167	15.00	2.5
	K_2CO_3 I	23.0	0.167		—
	$KHCO_3$	166.9	1.67		25.0
	K_2CO_3 II	230.3	1.67		25.0
II	KI	33.2	0.2	5.0	1.0
	Na_2CO_3	1.0	—		
III	HCl, conc.	333 ml	4.0	25.0	100.0
	Pb-acetate	1.0	—		
	Glacial acetic acid	4—5 drops	—		
IV	KCNS	194	2.0	5.0	10.0
V	$Na_2S_2O_3$		0.1		
VI	Centrifuged solution of soluble starch				

V: The solution of 2 % of soluble starch is centrifuged in order to remove particles which blur the end-point.

Apparatus: Volumetric flasks, 25 ml, marked with the weight in decigrams, width of neck 8—9 mm, surface about 0.6 cm^2. Small rings with numbers. A wire basket with compartments for the flasks. A pot, the water level of which is kept above the marks of the measuring flasks by regulating with the aid of a Mariotte bottle. Burette, 25 ml, graduated in 1/20 ml divisions.

Procedure: The sugar solution is weighed in or pipetted into the flasks which are equipped with rings. The solution must not occupy more than 10.0 ml. 15.00 ml of reagent I are added. Into 2—3 volumetric flasks there are pipetted off 15.00 ml for blank determinations. The flasks are filled to the mark, shaken and placed in the boiling water bath which must not cease boiling during the operation. Splashing must be avoided. The time is noted. After 45 minutes the flasks are cooled in running water. 300 ml Erlenmeyer flasks are used for the titration. 5 ml of reagent II are measured off in the flask. The reaction mixture is transferred and the measuring flask rinsed 3 times with distilled water. No attention is paid to any Cu_2O that may have stuck in the volumetric flask. In the blanks one will often find a ring of CuO in the neck of the flask. The cupric oxide is dissolved in a few ml of reagent III and washed over into the titration flask. 25 ml of reagent III are cautiously added. Effervescence! The flask is shaken while CO_2 escapes. Then 5 ml of reagent IV are added. Titration with $Na_2S_2O_3$ follows immediately. The starch solution is not added until the titration is almost finished. The colour shift is from blue to white-yellow for small amounts of Cu^{++} and from blue to pink for larger amounts. The end-point is rather sharp. When the sun is not shining, the end-point is best observed in the light of a daylight lamp.

This procedure was adopted for the following reasons: In common with all methods employing Cu^{++} as oxidizing agent, the potassium copper carbonate method has the drawback that the amount of precipitated Cu_2O is dependent on the extent to which air has access to the mixture during the heating. Kjeldahl, who was the first to call attention to this fact, drove out the oxygen by leading hydrogen through the reaction

mixture. According to Kjeldahl, the influence of the atmospheric oxygen depends on the size of the surface of the solution, and can, practically speaking, be eliminated by using flasks of suitable size with narrow necks, filling the flasks clear to the neck. For practical reasons this was the procedure adopted. The heating must be carried out in boiling water, which at the same time makes it possible to obtain a better control of the conditions of the reaction than in the case of direct heating. Just as in the copper tartrate methods, the analysis itself may involve a determination of the Cu^+ formed or the Cu^{++} not consumed. We have chosen the indirect method because it is much more rapid and relatively accurate. The excess of Cu^{++} is determined according to a modified iodometric method with thiocyanate and iodide (Bruhns [26]). A disadvantage of this method is that one has to use many different reagents. According to Kühnel Hagen [27], an addition of a little Pb^{++} causes the end-point to be sharp, which enables one to make a copper determination with a standard error of 0.2—0.3 % (relatively).

Results

The stability of the potassium copper carbonate complex is dependent on the total as well as the relative carbonate- and bicarbonate concentrations. Potassium copper carbonate is stable only at very high concentrations of carbonate and/or bicarbonate. Upon dilution, basic copper carbonates are precipitated. Potassium copper carbonate in K_2CO_3-solutions is unstable when heated; in $KHCO_3$-solutions it can stand boiling. Solutions like Soldaini's reagent, which contains only bicarbonate, are, according to Ost, not suitable for quantitative sugar determinations since they react too slowly. The composition of a suitable reagent is therefore limited to mixtures of $KHCO_3$ and K_2CO_3, and even in that case only within a relatively narrow range. Experiments with buffer mixtures (table 15) show that pH has a very great influence on the reduction equivalent (RE) of the different sugars. The definitive choice

Table 15. Influence of pH and time of heating on RE, pH and molarity of salts in reaction mixture (25 ml).

$CuSO_4$ mol/l		0.1		0.1		0.1
K_2CO_3 »		0.5		1.0		1.0
$KHCO_3$ »		1.0		1.0		0.5
pH		9.4		9.9		10.2
Time of	RE		RE		RE	
heating, min	Glucose	Maltose	Glucose	Maltose	Glucose	Maltose
10	8.61		9.43			
15	9.20		10.05			
20	10.11	8.79	10.17	9.45	9.21	8.64
30	10.42	9.58	10.24	9.99	9.16	8.85
40	10.54	9.92	10.31	10.22	9.28	9.06
50	10.53	10.27	10.31	10.43	9.33	9.05
60	10.62	10.53	10.34	10.61	9.35	9.45

of a reagent was therefore a solution containing equimolecular amounts of $KHCO_3$ and K_2CO_3. This solution has maximal buffer capacity and thus is the best one for fixing pH and the reduction equivalent. By selecting 25 ml volumetric flasks and mixing 15 ml of reagent with 10 ml of sugar solution the reaction mixture obtains the composition:

Total Cu^{++} : 0.1 molar
» HCO_3^- : 1 »
» CO_3^{--} : 1 »

The blank proved to be constant. This was a great advantage in comparison with Fehling's solution, the tartrate content of which causes the autoreduction to increase with the time of heating.

For the same heating time the RE of the glucose decreases with increasing pH, while RE of the maltose has a maximum at pH = 9.9 (table 15). At this pH the RE of the glucose and that of the maltose reach the same value after 40—50 minutes of heating. This is yet another essential reason for the final choice of the composition of the reagent as well as of the heating time. In investigations of mixtures of glucose and maltose, as they occur, for example, after enzymatic degradation of starch, it is a great advantage to have an analytical method in which the two sugars have the same RE.

All experimental series dealing with the effect of the heating time on the reduction equivalents of different sugars (table 16) show that the increase in

Table 16. Influence of time of heating on RE at constant pH. K_2CO_3 1.0 M, $KHCO_3$ 1.0 M, pH = 9.9.

Time of heating, min	Galactose	Invert sugar	Glucose	Maltose	Lactose
10	7.94	9.63	9.43	—	—
15	8.44	10.17	10.05	—	—
20	8.71	10.23	10.17	9.45	8.69
30	8.76	10.30	10.24	9.99	9.25
40	8.86	10.39	10.31	10.22	9.67
50	8.89	10.48	10.31	10.43	9.84
60	—	—	10.34	10.61	10.05

RE becomes slower and slower with the time of heating, although RE does not reach a constant value. Galactose has a lower RE than glucose. The same is observed in oxidation with Fehling'ss olution. This can be deduced from Kjeldahl's and Bertrand's tables. Sobotka and Reiner [14] found similar results for ferricyanide.

The most suitable heating time is that at which $\triangle$ RE/$\triangle$t is so small that unavoidable variations in the time of heating have but an insignificant effect on the definitive result. From the experiments in table 16 it is seen that this time, practically speaking, is reached for the sugars investigated in the course of 40—50 minutes. A very large number of determinations showed that RE within certain limits is independent of the amount of sugar. If the determinations are limited to this range it is thus unnecessary to prepare any tables. The calculations are then based on the factor given in table 17 for each sugar (molecular weight / reduction equivalent).

Table 17. Range, RE and factors for calculation.

Sugar	Determinations	Range mg sugar	RE average	Factor $\frac{MW}{RE}$
Galactose	21	10—33	8.86	20.33
Invert sugar	17	10—25	10.48	17.18
Glucose	98	10—33	10.34	17.48
Maltose	37	25—60	10.31	33.19

$$\text{mg sugar} = (\text{blank} - \text{main experiment}) \times n \times \text{factor}.$$

The factors are calculated for the anhydrous sugars. The relative standard error is 0.8 % (based on 240 determinations). The RE of the lactose is not constant, but increases with increasing excess of copper. Hence we have omitted both factor and table.

COMPARATIVE VALUATION OF THE DIFFERENT METHODS

The determination of sugar in pure solutions presents many difficulties. In the case of determinations in solutions that contain other oxidizable organic compounds — and these are the ones to be dealt with in practice — the difficulties increase.

Methods for the determination of reducing sugars are generally based on non-specific oxidation reactions. Since the total consumption of oxidizing agent is computed as sugar one must in principle prefer the method in which the reaction with non-sugar substances is the least. The oxidizing agent which is just capable of oxidizing the reducing carbohydrates must be regarded as the ideal one. And even in that case the non-sugar substances which are more easily oxidized than sugar will enter into the calculations as sugar.

A measure of the strength of the oxidizing agent is to be found in the oxidation-reduction potential. Oxidation-reduction measurements in the reaction mixtures employed in the iodine-, ferricyanide- and potassium copper carbonate methods (table 18) show that iodine is the most powerful oxidizing

Table 18. Oxidation-reduction potentials of various reaction mixtures. $t = 20.0°$.

Method	[Ox]		[Red]		$[HCO_3^-]$	$[CO_3^{--}]$	pH	E_h Volt
Iodine	I_2	0.03	I^-	0.05	0.03	0.13	10.2	+ 0.57
Ferricyanide	$Fe(CN)_6^{---}$	0.02	$Fe(CN)_6^{----}$	0.02	0.20	0.20	9.8	+ 0.49
Potassium copper carbonate	Cu^{++}	0.05	Cu^+	?	1.0	1.0	9.9	+ 0.01

agent. Ferricyanide is almost as strong, but potassium copper carbonate is much weaker. Measurements were carried out with the electrode arrangement of Jørgensen [28]. A saturated calomel electrode was used as comparison electrode. In the case of potassium copper carbonate one-half of Cu^{++} is, by heating with the estimated amount of glucose, converted into Cu_2O. The potentials are stable and reproducible. Since hydrogen ions do not enter into the equilibrium, the potentials become independent of pH. Displacements in the dissociation, salt error, formation of complexes, sparing solubility of one of the components, etc., do not, however, make the potentials much different from the normal ones for the particular oxidation-reduction systems: $I^- \rightleftharpoons I$, $Fe(CN)_6^{----} \rightleftharpoons Fe(CN)_6^{---}$, and $Cu^+ \rightleftharpoons Cu^{++}$.

Iodine is so active that the oxidation of aldoses proceeds at room temperature. This is an advantage, but the readiness to react will at the same time mean that the presence of reactive non-sugar substances makes the determinations problematic, so much more so as the equivalent weight of the sugar according to the iodine method is very high. Even though ferricyanide is almost as powerful an oxidizing agent as iodine, it nevertheless requires heating before the reaction with the sugar proceeds at a reasonable rate. Copper methods have the same drawback. In the choice between ferricyanide and copper methods the latter must, in principle, be preferable since ferricyanide oxidizes many non-sugar substances that copper does not attack at all. On the basis of experiments on »Bestimmung des echten Blutzuckers ohne Hefe», Fujita and Iwatake [17] arrived at the same conclusion: »Das Reaktionsvermögen der Nichtzuckersubstanzen ist bei der Ferricyanidmethode bedeutend grösser als bei der Cuprimethode».

But non-sugar substances are not alone in causing trouble. Both »reducing» and »non-reducing» oligo- and polysaccharides may make the determination

unreliable. This is probably due to a partial, oxidative rupture of the glucosidic bonds and a substantial oxidation of the cleavage products. This reaction is dependent on the oxidation-reduction potential of the oxidizing agent (table 19). The experiments are carried out with solutions containing mixtures

Table 19. Comparative determinations by various methods.

	Potassium copper carbonate	Copper tartrate	Iodine	Ferricyanide
E_h Volt	0.01	—	0.57	0.49
% T.M.	23.4	24.1	26.1	29.2
% T.M.	34.3	34.9	36.8	42.0

of mono-, oligo- and polysaccharides, as formed by partial hydrolysis of starch by means of α-malt-amylase. %T.M. denotes the degree of hydrolysis in % of the theoretically possible amount of maltose from total hydrolysis of starch. The values obtained with iodine is about 10 %, with ferricyanide about 25 % higher than those obtained with the copper methods. In this connection it should be remembered, however, that the oxidation with iodine is carried out at room temperature. Other experiments, which will not be mentioned in detail, show that the difference almost solely is due to the dextrins. In practice these facts play a part in the determination of diastatic power in grain, malt and enzymatic malt preparations where the value often is determined by the enzymatic activity. Concordant results cannot be obtained by the different methods. Hence it is necessary along with the result to state the method employed. In principle, however, one can say that the method employing the oxidizing agent with the lowest oxidation-reduction potential must give the most correct, if not *the* correct, result. The copper methods must therefore be preferable.

The best one of the different copper methods must be the one the reaction mixture of which has the lowest pH. At the lowest $[OH^-]$ the rate at which the hydrolytic cleavages, for instance, of glucoside bonds, proceeds will be reduced to a minimum. Add to this that the lowering of pH is connected with a more thoroughgoing oxidation of the sugars whereby their equivalent weights become smaller. This means that the oxidation of the non-sugar substances has less influence on the result. However, since the reaction velocity declines strongly with decreasing pH it is not expedient to make pH substantially less than 10.

The use of Cu^{++} in alkaline medium presupposes that Cu^{++} is kept complexly in solution. For this purpose tartaric acid, as in Fehling's solution, is

most frequently used. This means poor keeping qualities, an autoreduction dependent on the heating time, and dependence on the degree of purity of the Seignette's salt. These drawbacks are avoided by the use of potassium copper carbonate which contains only inorganic components. But potassium copper carbonate too has its defects. It cannot be produced in solutions stronger than about 0.167 molar with respect to Cu^{++}, and the reagent cannot be substantially diluted without hydrolysis of the copper complex. The boiling time of 45 minutes is an inconvenience in the case of single analyses, but is partly offset in serial determinations because the waiting time here can be employed with titration of preceding analyses.

SUMMARY

The methods most generally employed in quantitative determinations of reducing sugars have been tested and a new method has been developed.

The determination of aldoses by means of *iodine* gives reliable results when using a buffer that ensures a pH of about 10.2. The method should only be used in the case of pure or almost pure sugar solutions, because of the high reactivity of iodine with many organic substances. An explanation is given of the pH-dependence of the reaction. HIO must be regarded as the actual oxidizing agent.

The determination of reducing sugars by means of *ferricyanide* is rapid. The titration is accurate, but the oxidation of the sugar varies somewhat.

The determination of reducing sugars by means of *copper tartrate* gives serially well reproducible results. With new chemicals, displacements occur in the results, the cause of which is not yet understood. It is to be assumed, that a fundamental reason for this phenomenon is the high content of organic substances of the reagent.

A new method has been developed for the determination of reducing sugars by means of *potassium copper carbonate*. The reagent consists of nothing but inorganic salts which can be procured in a highly purified state. The reagent has a well defined pH and a very high buffer capacity. The procedure involves heating on water bath in filled-up volumetric flasks, practially eliminating the influence of the air. The actual determination is based on iodometric titration. The method is particularly suitable for serial determinations. The relative uncertainty of a single determination is 0.8 %.

The advantages and the drawbacks of the methods are discussed. Owing to the low oxidation-reduction potential of the system $Cu^{+} \rightleftharpoons Cu^{++}$ relative to $Fe(CN)_6^{----} \rightleftharpoons Fe(CN)_6^{---}$, copper methods are, in principle, the better ones.

APPENDIX

Preparation of the sugars employed

Glucose, galactose, sucrose and *lactose* were purified by recrystallization of the purest commercial products. The recrystallization was continued to constant $[\alpha]_D$.

Glucose	$[\alpha]_D^{20} = 53.07°$	(p = 17.34)
Tollens [29]	$[\alpha]_D^{17} = 53.01°$	(p = 17.3)
Galactose	$[\alpha]_D^{2)} = 80.3°$	(p = 12.22)
Riiber, Minsaas, Lyche [30]	$[\alpha]_D^{20} = 80.5°$	
Sucrose	$[\alpha]_D^{20} = 66.42°$	(p = 16.70)
Frühling, Spengler [31]	$[\alpha]_D^{20} = 66.50°$	(p = 17)
Lactose hydrate	$[\alpha]_D^{20} = 52.59°$	(p = 12.16)
Kjeldahl [19]	$[\alpha]_D = 52.50°$	(p = 12—13)
Schmoeger [32]	$[\alpha]_D = 52.53°$	

Invert sugar. Since reversion products are formed by inversion of cane sugar, the inversion was carried out in highly diluted solution.

Maltose was prepared by enzymatic hydrolysis of starch. The action of α-amylase yields, besides the maltose, low-molecular dextrins, trisaccharides and glucose, which only with great difficulty can be separated from the maltose. β-amylase gives only maltose and a high-molecular dextrin which easily is separated from the maltose. Inasmuch as commercial preparations of maltose ordinarily are produced with malt extracts which contain both α- and β-amylase, we preferred, like Baker and Day [33], the more elaborate procedure with β-amylase from barley.

Maltose hydrate	$[\alpha]_D^{20} = 129.8°$	(p = 4.78)
Hudson, Yanovski [34]	$[\alpha]_D = 129.5°$	

Maltodextrin was produced by hydrolysis of potato starch by means of amylase from bacteria, and repeated fractionation with alcohol.

Residual dextrin was produced by hydrolysis of potato starch by means of α- + β-amylase from malt to about 80 % T.M. After fermentation, fractionation with alcohol.

Amylodextrin (Nägeli) was prepared by treatment of non-gelatinized potato starch with about 12 % HCl for 4 months, Brown, Morris [35]. The undissolved part was filtered off and dissolved in water from which it crystallized out as spherical crystals. The »recrystallization» was repeated.

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On the Application of p-Carboxyphenylhydrazones in the Identification of Carbonyl Compounds

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Among the different substituted hydrazines proposed for use in the identification of carbonyl compounds no doubt 2,4-dinitrophenylhydrazine has been most widely applied on account of the very slight solubility and the excellent crystallisability of the 2,4-dinitrophenylhydrazones. Allen and Richmond,[1] however, have called attention to some inconveniences in the application, namely the facts that cis-trans-isomerism often most disturbingly has to be dealt with, that dimorphism, too, may produce difficulties in the determination of melting points, and that the 2,4-dinitrophenylhydrazones of different carbonyl compounds are often able to form mixed crystals, thus preventing the isolation by crystallisation of the pure dinitrophenylhydrazones from a mixture of carbonyl compounds.

Moreover, the 2,4-dinitrophenylhydrazones are useful in the *qualitative* determination of the carbonyl compounds only, their applicability in the *quantitative* determination being restricted to nitrogen estimation, *e. g.* after Dumas, but not after Kjeldahl. The present author, therefore, some years ago [2] proposed the use of p-carboxyphenylhydrazine for identification purposes as it proved possible to use the p-carboxyphenylhydrazones for the quantitative estimation of the molecular weight of carbonyl compounds simply by titrating the ethanolic solution of the p-carboxyphenylhydrazone with standard sodium- or barium hydroxyde solution. Only some few examples were given in the paper mentioned, but later some papers dealing with the applicability of the proposed method have been published in a Danish journal.[3, 4, 5] The following is a survey of the results given in these papers and the presentation of some results so far unpublished.

The preparation of the reagent p-carboxyphenylhydrazine hydrochloride (I) has previously [2] been described. In order to prepare the p-carboxyphenylhydrazones the carbonyl compounds are added to 4 % aqueous solutions of (I). If the carbonyl compound is slightly soluble in water, ethanol may be added. Generally the precipitation of the p-carboxyphenylhydrazone starts at once. If no precipitation takes place the solution may be heated on the steam-bath for 1 hour. The precipitation is completed by cooling the mixture in ice water.

It may be added that when amino-groups are present in the carbonyl compound it is necessary to add 1 equivalent of NaOH to the mixture in order to prevent the contamination of the p-carboxyphenylhydrazone with its hydrochloride.

The p-carboxyphenylhydrazones are recrystallised from ethanol or diluted ethanol. Generally one recrystallisation suffices. It is inconvenient that the melting points of the p-carboxyphenylhydrazones are rather high (about 200°, many of them between 250° and 300°) so that the diagnostic value for the qualitative identification is restricted. Their quantitative estimation, on the other hand, is so convenient that they have proved to be of outstanding value, at all events in high school courses of identification of organic substances.

So far we have only met with difficulties caused by more forms of the p-carboxyphenylhydrazone in one case. Salicylic aldehyde p-carboxyphenylhydrazone exists in two forms, m. p. 241—243° or 254—255°. We do not know if it is a case of cis-trans isomerism or a case of dimorphism.

As mentioned above the method of determination of molecular weight is simply a titration with standard base. The p-carboxyphenylhydrazone is dissolved in ethanol, if necessary in hot ethanol, phenolphthalein is added and the solution titrated until it assumes a faint pink colour. The colour fades very soon on account of the fast absorption of CO_2 from the air, but it is not at all difficult to observe the end point of the titration.

In case of aromatic carbonyl compounds with hydroxy groups in the benzene nucleus difficulties may arise. For instance when salicylic aldehyde p-carboxyphenylhydrazone is titrated the molecular weight is found some 2—3 % too low when phenolphthalein is used as indicator, but the correct value is found if methyl red is used instead of phenolphthalein. Presumably the hydroxy group is partly neutralised before the virage of phenolphthalein is reached.

Still worse is it, that when two or more hydroxy groups in o- or vic. position are present the titration becomes impossible because the solution assumes a strong brown or green colour during the addition of alkali which makes an

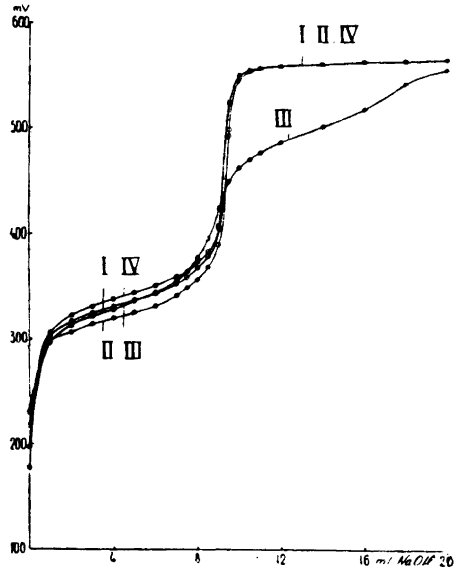


Fig. 1. Potentiometric titration of p-carboxyphenylhydrazones of ketones.
Curve I: Acetophenone-p-carboxyphenylhydrazone.
Curve II: o-Hydroxyacetophenone-p-carboxyphenylhydrazone.
Curve III: Gallacetophenone-p-carboxyphenylhydrazone.
Curve IV: p-Aminoacetophenone-p-carboxyphenylhydrazone.

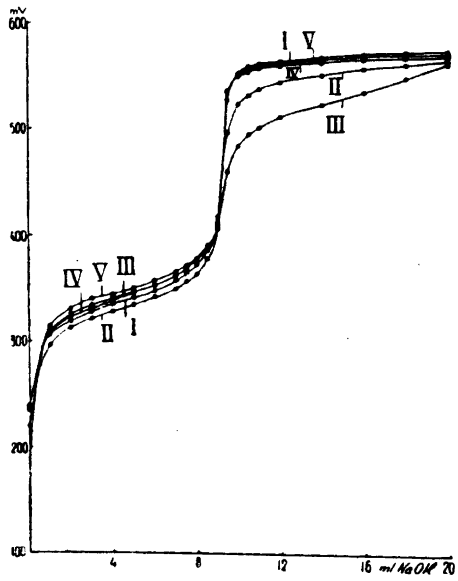


Fig. 2. Potentiometric titration of p-carboxyphenylhydrazones of aldehydes.
Curve I: Benzaldehyde-p-carboxyphenylhydrazone.
Curve II: Salicylic aldehyde-p-carboxyphenylhydrazone.
Curve III: Vanillin-p-carboxyphenylhydrazone.
Curve IV: Veratric aldehyde-p-carboxyphenylhydrazone.
Curve V: p-Dimethylaminobenzaldehyde-p-carboxyphenylhydrazone.

observation of the end point of the titration impossible. In such cases the titration may be carried through potentiometrically, using a glass electrode. From the shape of the titration-curve indications of the presence of phenolic groups in the molecule may be obtained. Fig. 1 and fig. 2 show examples of titration curves of unsubstituted and hydroxy- or amino-substituted aromatic carbonyl compounds.

The influence of the hydroxy-group may most clearly be seen from the branch of the titration-curve beyond the neutralisation of the carboxy-group. In fig. 1 the difference between curve I (acetophenone) and curve II (o-hydroxyacetophenone) is not great, but when 3 hydroxy-groups are present as in gallacetophenone (curve III) the buffering capacity of the substance

becomes quite obvious. An exact determination of the equivalent weight of the p-carboxyphenylhydrazone is no longer possible but a determination with an exactitude of some 1—2 % can be made. From the shape of the titration-curve it may be concluded that besides the carboxy-group further acid groups, weaker than the first, are present in the molecule.

From fig. 2 the conclusion is drawn that a methoxy-group may also be of influence upon the shape of the curve. A comparison of the curves I, II, and III (benzaldehyde, salicylic aldehyde, and vanilline) shows the increasing buffering capacity of the 3 substances examined. Curve IV (veratric aldehyde) shows that the methylation of the free hydroxy-group in vanillin annihilates the buffering capacity.

Curve IV of fig. 1 and curve V of fig. 2 (p-aminoacetophenone and p-dimethylaminobenzaldehyde) show that amino-groups have no significant influence on the shape of the titration-curves. In both substances the acidic branch of the curve is displaced a little towards potentials indicating lesser acidity than the derivatives of the unsubstituted carbonyl compounds, but the effect is not great enough to be of conclusive diagnostic value as some derivatives of the hydroxy-substituted carbonyl compounds also have the titration-curves displaced in the same direction. An explanation of this somewhat puzzling phenomenon cannot be given so far but the investigation is being continued.

In case of substances with other functional groups than a single carbonyl group complications known from other hydrazine compounds arise. In most cases, however, the quantitative determination is not rendered impossible if due regard is paid to the nature of the condensation product. The following survey may be of some assistance.

Alifatic α-diketones form bis-p-carboxyphenylhydrazones (III) as the normal reaction product. Only if the solution of (I) is added to a great excess of the diketone-solution the mono-p-carboxyphenylhydrazone (II) may be isolated.

Aromatic α-diketones form mono-p-carboxyphenylhydrazones (II) as the normal reaction products, but by prolonged boiling with an excess of (I) the formation of the bis-p-carboxyphenylhydrazone may be obtained.

The titration of the reaction products is normal, the mono-p-carboxyphenylhydrazones requiring 1, the bis-hydrazones 2 equivalents of base. The bis-hydrazones are often so sparingly soluble in ethanol that the titration has to be carried out in a nitrogen atmosphere, the base being added very slowly. For details, see experimental part.

β-Diketones, both alifatic and aromatic, yield with (I) as with other hydrazine derivatives pyrazol-derivatives (IV), but these, even when crystallised

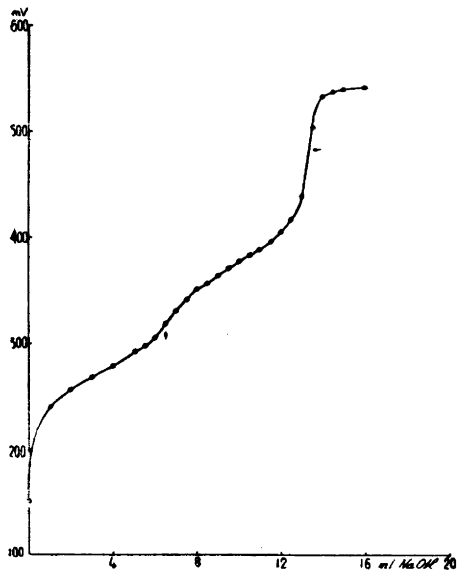


Fig. 3. Potentiometric titration of 1-p-carboxyphenyl-3-methylpyrazolone-(5).

from ethanol, crystallise with 1 mol H_2O. By titration, therefore, a molecular weight corresponding to the p-carboxyphenylhydrazone is found, but by drying the substance at 130° (or in vacuum over P_2O_5 even at ordinary temperature) the anhydrous compounds are obtained.

γ-Diketones give in the cold with an excess of (I) the bis-p-carboxyphenylhydrazone (V), but by recrystallisation from ethanol this compound is disproportionated to p-carboxyphenylhydrazine and a cyclic compound, a pyridazine (VI), which by titration requires 1 equivalent of base.

α-Ketoacids react normally with the reagent, yielding a p-carboxyphenylhydrazone (VII) requiring 2 equivalents of base to its neutralisation as both the carboxyl group of the keto-acid and of the reagent may be titrated.

Esters of β-ketoacids react in the cold with principal formation of p-carboxyphenylhydrazones (VIII) requiring 1 equivalent of base, but if the reaction is carried out on steam-bath, ring-closure to a pyrazolone-derivative (IX) usually occurs. By heating the primary reaction product to 120—130° or by boiling it with glacial acetic acid the ring-closure will take place even in cases where it is not completed on steam-bath.

The resulting 5-pyrazolones require by titration, if unsubstituted or monosubstituted in position 4, 2 equivalents of base as the enolised C:O-group in position 5 is completely neutralised before the colour change of the indicator (phenolphthalein). If the β-ketoester is disubstituted the ring-closure meets

with difficulties, and even if it is obtained, no enolisation can possibly take place so that only 1 equivalent of base is required to complete neutralisation.

The potentiometric titration-curve of an unsubstituted 5-pyrazolone (fig. 3) shows that 2 inflexion-points really may be discerned, the one indicating the neutralisation of the carboxy-group allowing only a very approximative determination of the molecular weight, the other indicating the neutralisation of the hydroxy-group formed by enolisation being more pronounced and allowing a fairly exact determination.

Alifatic γ-ketoacids usually form p-carboxyphenylhydrazones (X) requiring 2 equivalents of base to neutralisation, whereas in the case of *aromatic γ-ketoacids* (*e. g.* o-benzoylbenzoic acid) ring-closure takes place with formation of a phthalazone-derivative (XII). By prolonged heating of the p-carboxyphenylhydrazones of the alifatic γ-ketoacids during their recrystallisation ring-closure to pyridazinone-derivatives (XI) may occur, and by boiling the hydrazones with glacial acetic acid the ring-closure may be obtained even in cases where no ring-closure takes place spontaneously.

The phthalazones and the pyridazinones require only 1 equivalent of base for their neutralisation.

Alifatic α-hydroxyaldehydes or -ketones and *alifatic α-halogenoaldehydes or -ketones* yield bis-p-carboxyphenylhydrazones of the corresponding α-ketoaldehyde or α-diketone (III), whereas the corresponding *aromatic* compounds in the cold react only with 1 mol (I), presumably with formation of a mono-p-carboxyphenylhydrazone (XIII) which then looses 1 mol H_2O or HX (X = the halogen present), giving the substance (XIV) or (XV), *cf.* Bodforss [6]. and van Alphen.[6a] If the reaction is carried out on steam-bath the bis-p-carboxyphenylhydrazone of the corresponding di-carbonyl compound is formed.

β-Ketoles form at room temperature p-carboxyphenylhydrazones (XVI) which on heating loose 1 mol H_2O, yielding hydrazones of unsaturated compounds (XVIII) or pyrazoline derivatives (XVII). Both the p-carboxyphenylhydrazones and the pyrazolines require 1 equivalent of base.

α-β unsaturated aldehydes and *ketones.* The purely alifatic or mixed alifatic-aromatic compounds (*e. g.* crotonic aldehyde or benzylidene acetone) usually yield the normal p-carboxyphenylhydrazone (XVIII) whereas the p-carboxyphenylhydrazones formed by aromatic compounds (*e. g.* benzalacetophenone) spontaneously will be transformed into pyrazoline-derivatives (XIX) by ring-closure (*cf.* v. Auwers and co-workers.[7, 8, 9] In cases where the ring-closure does not take place spontaneously it may be provoked by boiling the p-carboxyphenylhydrazone with glacial acetic acid.

The solutions of the pyrazolines in ethanol show a brilliant bluish fluorescence which is not shown by the ethanolic solutions of the p-carboxyphenylhydrazones. Both types of derivatives require 1 equivalent of base for their neutralisation, and as the ring-closure in this instance is not accompanied by loss of water but only by the displacement of a hydrogen atom from nitrogen to carbon, the molecular weights of both types are identical.

(I) may react also with other compounds than carbonyl compounds, *e. g.* with *mustard oils* [4]. The reaction product is a thiosemicarbazide (XX) which in ethanolic solution may be titrated with standard base, requiring 1 equivalent of base for its neutralisation (indicator phenolphthalein). When prepared by boiling solutions of (I) and mustard oil on steam bath 1-p-carboxyphenyl-4-alkyl (aryl)-thiosemicarbazides are obtained.

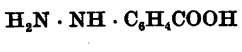
That it is the 1-p-carboxyphenyl-substituted and not the isomeric 2-substituted thiosemicarbazide *(cf.* the papers of Marckwald,[10] of Busch and Holzmann,[11] and of Busch, Oppermann and Walther [12]) which is formed at boiling temperature may be seen by treating its ethanolic solution with an ethanolic solution of nickel chloride. Jensen and Rancke Madsen [13] have shown that 2-substituted thiosemicarbazides but not the 1-substituted ones form intensely coloured complex nickel salts. The thiosemicarbazides prepared by the method mentioned do not give coloured solutions with nickel chloride and consequently they are 1-substituted.

From the *aromatic mustard oils* the 2-substituted thiosemicarbazides may be prepared by reaction between the sodium salt of p-carboxyphenylhydrazine and mustard oil in diluted ethanol at room temperature. After 1—2 hours the 2-p-carboxy-4-aryl-thiosemicarbazide (XXI) is precipitated by addition of hydrochloric acid, filtered off, washed with diluted ethanol and dried at room temperature.

These substances too may be titrated with standard base. Their ethanolic solutions assume by addition of a solution of nickel chloride a deep red colour which with ammonia shifts to dark blue. As the colour of the solution is the same with 3 as with 2 mol thiosemicarbazide per mol $NiCl_2$ it is to be assumed that the complex is formed of only 2 mol thiosemicarbazide per mol $NiCl_2$. It has not been possible to isolate the complex salt.

From *alifatic mustard oils* only the 1-p-carboxyphenylthiosemicarbazides are formed, even by reaction at room temperature between the sodium salt of p-carboxyphenylhydrazine and mustard oils.

The aromatic 2-p-carboxyphenyl-thiosemicarbazides are transformed into the 1-substituted isomers by recrystallisation from ethanol.



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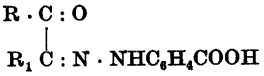

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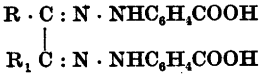

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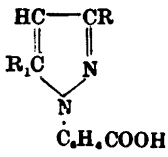

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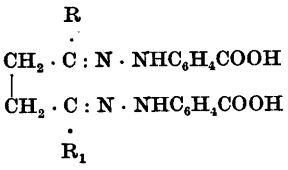

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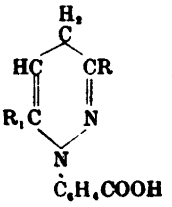

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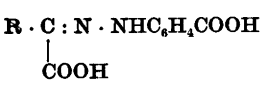

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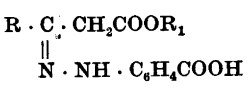

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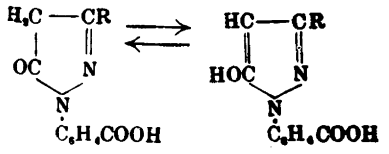

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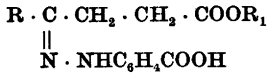
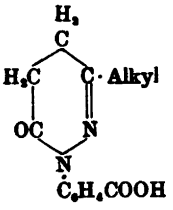


X



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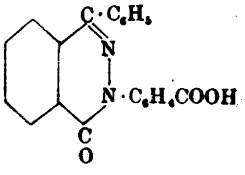

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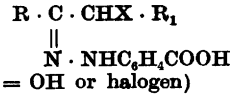
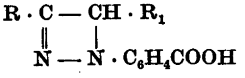


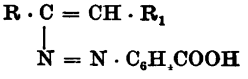
(X = OH or halogen)

XIII



XIV

or



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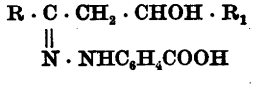

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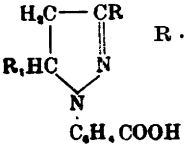

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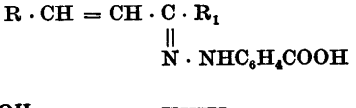

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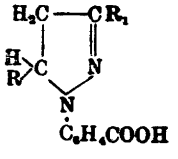

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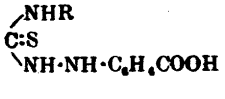

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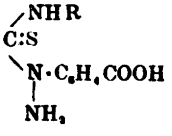

XXI

Table 1. p-Carboxyphenylhydrazones of carbonyl compounds

Carbonyl compound	M. p.	Equivalent weight found	Molecular weight calc.
Formaldehyde	ca. 285°	—	164.2
Acetaldehyde	210—211°	179.2	178.2
Propionaldehyde	174—175°	192.2	192.2
n-Butyraldehyde	164—165°	207.8	206.2
Benzaldehyde	234—235°	239.0	240.2
p-Toluylaldehyde	240—242°	253.3	254.3
Hydrocinnamic aldehyde	173—174°	269.6	268.3
Cinnamic aldehyde	236—238°	264.6	266.3
Salicylic aldehyde	241—243°	256.4 [1]	256.3
Salicylic aldehyde	254—255°	257.2 [1]	256.3
Protocatechualdehyde	ca. 265°	—	272.3
Vanillin	244—246°	284.6 [2]	286.3
Anisaldehyde	250—251°	271.3	270.3
2.4-Dimethoxybenzaldehyde	211—213°	299.8	300.3
3.4-Dimethoxybenzaldehyde	225—227°	299.6	300.3
Piperonal	244—246°	282.8	284.3
m-Nitrobenzaldehyde	260—262°	283.6	285.3
p-Dimethylaminobenzaldehyde	251—252°	280.9	283.3
o-Carboxybenzaldehyde [3]	280—282°	264.9	266.3
Furfural	210—212°	229.4	230.2
Acetone	230—231°	190.8	192.2
Methylethylketone	173—174°	206.8	206.2
Methylpropylketone	149—150°	219.1	220.2
Diethylketone	179—180°	220.4	220.2
Methylisopropylketone	190—192°	219.0	220.2
Methylbutylketone	148—149°	232.8	234.2
Pinacolone	220—222°	233.5	234.2
Methylamylketone	148—150°	248.2	248.3
Methylhexylketone	145—146°	260.9	262.3
Di-n-butylketone	115—116°	278.2	276.3
Cyclopentanone	250—251°	218.6	218.2
Cyclohexanone	240—241°	230.7	232.3
Acetophenone	250—251°	253.5	254.2
p-Methylacetophenone	245—246°	268.6	268.3
o-Hydroxyacetophenone	290—292°	271.3 [2]	270.3
p-Methoxyacetophenone	246—248°	282.1	284.3
p-Aminoacetophenone	237—238°	268.0	269.3
Propiophenone	193—194°	267.3	268.3
p-Hydroxypropiophenone	143—145°	—	284.3

[1] Indicator methyl red. [2] Potentiometrical titration. [3] Phthalazone (XII). [4] Pyrazoline (XIX). [5] Pyridazinone (XI). [6] Pyrazole (IV). [7] Pyridazine (VI). [8] Pyrazolone (IX). [9] Pyrazoline (XVII). [10] Cyclic or azo-compound (XIV or XV).

Carbonyl compound	M. p.	Equivalent weight found	Molecular weight calc.
Valerophenone	156—157°	295.3	296.3
Gallacetophenone	274—277°	301.3 [2]	302.3
Methyl-α-naphthylketone	230—232°	302.7	304.3
Methyl-β-naphthylketone	279—280°	303.1	304.3
Benzophenone	248—249°	317.9	316.3
Benzalacetophenone [4]	220—221°	339.4	342.3
p-Bromacetophenone	260—261°	332.2	333.3
Benzalacetone	193—194°	279.0	280.3
Benzalacetone [4]	249—250°	279.3	280.3
Pyruvic acid	265—266°	111.7	222.2
Levulinic acid	212—213°	125.9	250.3
Levulinic acid [5]	157—158°	231.0	232.2
β-Benzoylpropionic acid	205—207°	157.2	312.3
β-Benzoylpropionic acid [5]	194—195°	291.7	294.3
o-Benzoylbenzoic acid [3]	273—274°	341.1	342.3
p-Toluyl-o-benzoic acid [3]	269—270°	355.8	356.3
Diacetyl	> 350°	179.0	354.3
Benzil mono-carboxyphenylhydrazone	213—215°	344.6	344.4
Benzil bis-carboxyphenylhydrazone	ca. 320°	237.3	474.6
Acetylacetone [6]	158—160°	216.0	216.2
Benzoylacetone [6]	153—154°	276.4	278.3
Dibenzoylmethane [6]	213—214°	340.7	340.3
Acetonylacetone [7]	234—235°	228.5	230.3
Ethyl acetoacetate [8]	297—300°	108.9	218.2
Ethyl ethylacetoacetate [8]	246—247°	122.9	246.3
Ethyl benzoylacetate [8]	278—280°	139.8	280.3
Acetylpyruvic acid [6]	288—291°	125.0	246.2
Ethyl acetylpyruvate [6]	181—182°	272.1	274.3
Form-isobutyraldol [9]	173—174°	218.9	218.2
Benzoin [10]	151—153°	329.1	328.3

Table 2. Thiosemicarbazides from mustard oils

Mustard oil	M. p.	Equivalent weight found	calc.
Methyl	248—250°	224.5	225.3
Allyl	212—214°	250.0	251.3
Phenyl	221—223°	287.7	287.4
p-Tolyl	213—215°	299.8	301.4

EXPERIMENTAL PART

As an example of the preparation of a p-carboxyphenylhydrazone without complications may be quoted methyl-β-naphthylketone-p-carboxyphenylhydrazone. 1.70 g methyl-β-naphthylketone are dissolved in 25 ml ethanol. The solution is added to a solution of

2 g (I) in 50 ml water, the mixture is refluxed for 1 hour on the steam bath and then cooled. The precipitate is filtered off and dried, weight 2 g, m. p. 277—279°. Recrystallised from ethanol the m. p. is raised to 279—280°.
0.2136 g dissolved in 50 ml ethanol require 7.53 ml 0.0936 *N* $Ba(OH)_2$. Equivalent weight calc. 304.3, found 303.1.
As an example of the potentiometrical titration may be quoted acetophenone-p-carboxyphenylhydrazone (m. p. 251—252°). Titrated with $Ba(OH)_2$ with phenolphthalein as indicator 0.1978 g require 7.91 ml. 0.0986 *N* base. Equivalent weight calc. 254.2, found 253.5.
0.2292 g in 50 ml ethanol were titrated potentiometrically with 0.0971 *N* NaOH. An electronic-tubepotentiometer, type »Radiometer PHM 3 f» and a glass electrode were used, the other half element being a calomel electrode. Curve I on fig. 1 shows the result. 9.30 ml were required for neutralisation. Equivalent weight calc. 254.2, found 253.8.
From all the p-carboxyphenylhydrazones, the titration-curves of which are shovn in fig. 1 and fig. 2, 0.090 mol are dissolved in 50 ml ethanol, and in all cases 9.3 ml 0.0971 *N* NaOH are required for neutralisation, *i. e.* within the limits of the experiment the amount calculated.

Alifatic α-diketones. 4 g (I) are dissolved in 100 ml water and a solutionof 0.85 g diacetyl in 50 ml ethanol is added. The p-carboxyphenylhydrazone is precipitated spontaneously but in order to complete the formation the mixture is refluxed for 2 hours on the steam-bath. The yellow precipitate is filtered off. As it is practically insoluble in all solvents it is purified by boiling it repeatedly with ethanol. It has a m. p. over 350°.
0.1145 g are dredged in 50 ml ethanol in a conical flask. Phenolphthalein is added and the flask is closed with a cork stopper equipped with a glas tube through which a stream of CO_2-free N_2 is conducted into the solution, and another glass tube through which $Ba(OH)_2$ may be added from a burette. The $Ba(OH)_2$-solution is added in small quantities, 0.1—0.2 ml, no further amount being added until the red colour has vanished. The titration is very tedious, 6.90 ml being added during some 6 hours. The red colour then persists for many hours. Equivalent weight calc. for diacetyl-bis-p-carboxyphenylhydrazone 177.2, found 179.0. By nitrogen determination (micro-Dumas) was found 15.2 % N, calc. 15.8 % N.
If, on the other hand, a solution of (I) in water is added to a solution of excess diacetyl in ethanol at room temperature a yellow precipitate is formed which by recrystallisation from ethanol becomes colourles. M. p. 273—274°. 0.2666 g require 13.66 ml 0.0893 *N* $Ba(OH)_2$, *i. e.* the substance is diacetyl-mono-p-carboxyphenylhydrazone, equivalent weight calc. 220.2, found 219.3.

Aromatic α-diketones. Here the mono-p-carboxyphenylhydrazones are the normal reaction products. To a solution of 2 g (I) in 15 ml water + 35 ml ethanol is added a solution of 2 g benzil in 25 ml ethanol. The mixture is refluxed for 1 hour and then cooled. The precipitate is filtered off, recrystallised from ethanol and shows then the m. p. 213—215°. v. Auwers and Clos[14] indicate for the monohydrazone m. p. 212°. 0.2084 g require 6.13 ml 0.0986 N $Ba(OH)_2$. Equivalent weight calc. 344.4, found 344.6. By prolonged refluxing the bis-hydrazone may be formed. M. p. 320°.

β-Diketones. A hot solution of 4.6 g dibenzoylmethane in 200 ml ethanol is added to a hot solution of 4 g (I) in 100 ml of water. The mixture is refluxed on the steam-bath

for 4 hours and then cooled. A precipitate is filtered off and recrystallised from ethanol. It shows m. p. 214° and equivalent weight 359.2. For the mono-carboxyphenylhydrazone the calc. equivalent weight is 358.3.
Heated to about 140° for 3 hours or dried *in vacuo* over P_2O_5 for a night the substance looses 1 mol of water. The anhydrous substance shows unaltered m. p. 214° but by titration the equivalent weight is found to 340.7 (0.3327 g, 10.08 ml 0.0969 *N* $Ba(OH)_2$), calc. for 1-p-carboxyphenyl-3.5-diphenyl-pyrazole (IV) 340.3.
When the pyrazole-derivative is recrystallised from ethanol the m. p. is not altered but the equivalent weight of the recrystallised product is 359, *i. e.* 1 mol of water is retained, which is lost before the m. p. since identical m. p.'s are found for the anhydrous substance and for the hydrate.
Also with benzoylacetone and with acetylacetone pyrazole-derivatives are formed which heated to 130—140° or dried *in vacuo* over P_2O_5 loose 1 mol of water. It has not yet been established if it is the 3-methyl-5-phenyl- or the 3-phenyl-5-methyl-pyrazole which is formed from benzoylacetone.

γ-Diketones. If a cold solution of (I) in 100 ml of water is added to a solution of 1.1 g acetonylacetone in 25 ml of water a precipitate is formed which after 1 hour is filtered off, washed with water and ethanol and air-dried, Yield 2 g. Heated slowly the substance begins to decompose at 180°. On bloc Maquenne the instantaneous m. p. (destruction point) is 277—280°. The substance is practically insoluble in cold ethanol. 0.1594 g were dissolved in 50 ml ethanol by addition of 10.05 ml 0.1005 *N* $Ba(OH)_2$. The solution is alcaline towards phenolphthalein, the excess base corresponding to 1.82 ml 0.1060 *N* HCl. Equivalent weight found 194.8, calc. for acetonylacetone-bis-p-carboxyphenylhydrazone 191.2.
Heated with ethanol for some time the substance is dissolved. Water precipitates a substance with m. p. 235° (bloc Maquenne) and equivalent weight 228.5 (0.1056 g require 4.60 ml 0.1005 *N* $Ba(OH)_2$), calc. for the pyridazine-derivative (VI) 230.3. The filtrate contains p-carboxyphenylhydrazine. By addition of acetone a precipitate of the acetone-p-carboxyphenylhydrazone (m. p. 230—231°) is readily obtained. The bis-p-carboxyphenylhydrazone of acetonylacetone first formed is by heating with ethanol disproportionated to a mixture of the pyridazine-derivative and p-carboxyphenylhydrazine.
If the reaction is carried out on the steam-bath only the pyridazine-derivative is formed.

α-Ketoacids. No complications have been met so far. The p-carboxyphenylhydrazones are readily formed and both carboxy-groups are titrated, *e. g.* the p-carboxyphenylhydrazone of pyruvic acid: 0.1167 g require 10.60 ml 0.0986 *N* $Ba(OH)_2$ equivalent weight found 111.7, molecular weight calc. 222.2.

Esters of β-ketoacids. If the usual procedure for the preparation of p-carboxyphenylhydrazones is followed, a mixture of p-carboxyphenylhydrazone (VIII) and pyrazolone (IX) is formed. When the precipitation, with ethyl acetoacetate for example, is carried out at room temperature and the product purified by dissolving it in cold ethanol and reprecipitating it by addition of water and refrigerating the mixture in ice, approximately pure (VIII) may be obtained. 0.3832 g require 14.95 ml 0.1048 *N* $Ba(OH)_2$. Equivalent weight found 244.5, molecular weight calc. 264.2, *i. e.* the product is a mixture of (VIII) and (IX) with some 13 % (IX). If, on the other hand, the mixture of (I) and ethyl acetoacetate is refluxed for some time, the precipitate filtered off, dissolved by refluxing

it with ethanol and reprecipitated by addition of water after filtration, the pure pyrazolone (IX) is obtained. 0.1764 g require 15.46 ml 0.1048 *N* $Ba(OH)_2$. Equivalent weight found 108.9, molecular weight calc. 218.2. Obviously, the pyrazolone is titrated as a dibasic acid.
This may be seen too from the potentiometric titration-curve shown in fig. 3. 0.1432 g were titrated with 0.0871 *N* NaOH. The first inflexionpoint is at 6.70 ml, the second at 13.48 ml, the corresponding equivalent weights 220.1 and 109.4. The m. p. of (IX) is 297—300°. For (VIII) no m. p. can be determined. On bloc Maquenne an instantaneous m. p. may be observed at 168—170°, but this only indicates the temperature at which ring-closure to (IX) occurs momentaneously.

γ-Ketoacids. If levulinic acid is treated with (I) a p-carboxyphenylhydrazine (X) with m. p. 212—213° and equivalent weight 125.9 is obtained. 0.1446 g require 12.30 ml 0.0936 *N* $Ba(OH)_2$. Molecular weight calc. 250.3. Evidently the p-carboxyphenylhydrazone is titrated as a dibasic acid. If (X) is heated to 150° for 1—2 hours ring-closure occurs with loss of water. The product (XI) is recrystallised from diluted ethanol and shows the m. p. 157—158° and equivalent weight 231.0. 0.2238 g require 10.35 ml 0.0936 *N* $Ba(OH)_2$. The molecular weight calc. for (XI) is 232.2, *i. e.* the pyridazinone is titrated as a monobasic acid.
Refluxing of (X) with glacial acetic acid for 15 minutes does not result in ring-closure. (X) may be recovered unaltered.
From *β*-benzoylpropionic acid a p-carboxyphenylhydrazone with m. p. 205—207° is formed. 0.2065 g require 14.03 g 0.0936 *N* $Ba(OH)_2$. Equivalent weight found 157.2, molecular weight calc. 312.3. If this substance is refluxed with glacial acetic acid for 5 minutes or heated to some 160° for 6 hours, ring-closure occurs. The m. p. of the recrystallised product is 194—195°, the equivalent weight found 291.7 (0.2919 g require 10.69 ml 0.0936 *N* $Ba(OH)_2$). The calc. molecular weight is 294.3. Even boiling of (X) with ethanol for some hours may in this instance provoke ring-closure.
From o-benzoylbenzoic acid (and other *γ*-ketoacids of this type) only the pyridazinones (XII), which are also named phthalazones, are formed. M. p. of the phthalazone from o-benzoylbenzoic acid 273—274°. 0.1619 g require 5.07 ml 0.0936 *N* $Ba(OH)_2$. Equivalent weight found 341.1, molecular weight calc. 342.3.
That really a phthalazone is formed may be proved by heating the substance over free flame to the boiling- (or destruction-) point. After cooling, the reaction-product may be separated into an acid and a neutral part. From the acid part the original substance may be recovered, from the neutral part a substance with m. p. 168°, concordant with the melting-point of 1,3-diphenylphthalazone-(4) formed from the original phthalazone by decarboxylation.

α-Hydroxy-aldehydes or -ketones and α-halogeno-aldehydes or -ketones. When *alifatic* compounds of these types are treated with (I) only the bis-p-carboxyphenylhydrazones of the corresponding diketones (III) are formed, at room temperature as well as on the steam-bath.
The corresponding *aromatic* compounds, too, will on the steam-bath give rise to the bis-p-carboxyphenylhydrazones of the diketones. At room temperature, however, substances of the type (XIV) or (XV) are formed by a very slow reaction (some days), even if an excess of (I) is used. When the reaction mixture is diluted with water an oil is formed which by treatment with benzene is brought to crystallisation. From benzoin and de-

sylchloride identical reaction products, free from chlorine and with m. p. 151—153° when recrystallised from ethanol, were formed. By titration equivalent weights of 328.3 or 329.1 respectively were found. (0.2232 or 0.2451 g require 7.60 or 8.34 ml 0.0893 *N* $Ba(OH)_2$). The calc. molecular weight is 328.3. When boiled with (I) in diluted ethanol the substance (XIV) or (XV) is transformed into the bis-p-carboxyphenylhydrazone of the corresponding diketone.

β-Ketoles. 1.5 g form-isobutyraldol, $HCHOH \cdot C(CH_3)_2 \cdot CHO$, in 25 ml ethanol and 3 g (I) in 75 ml water were mixed at room temperature and kept over night in the ice-box. Yield 2.5 g of a substance with an instantaneous m. p. on bloc Maquenne about 160°. Slowly heated a m. p. of 173° was found. When boiled for a few minutes with glacial acetic acid, precipitated by 5 volumes of water and recrystallised from ethanol a substance with m. p. 174° without a preceeding instantaneous m. p. is obtained. The substance prepared at room temperature has an equivalent weight of 237.4. (0.2189 g require 9.50 ml 0.0971 *N* NaOH), the transformation product an equivalent weight of 218.9. (0.1938 g require 9.12 ml 0.0971 *N* NaOH). The calc. equivalent weights are for the hydrazone (XVI) 236.2, for (XVIII) and the pyrazoline (XVII) 218.2.

α -β unsaturated aldehydes or ketones. When cinnamic aldehyde is treated with (I) a substance with m. p. 236—238° is formed. The equivalent weight found by titration is 264.6. (0.1164 g require 4.70 ml 0.0936*N* $Ba(OH)_2$), calc. 266.3. This substance is either (XVIII) or (XIX) ($R = C_6H_5$, $R_1 = H$), but from the data obtained no decision can be made. When benzylideneacetone is treated in the same manner a substance with m. p. 194—196° is formed. If refluxed for 1 hour with glacial acetic acid the m. p. is raised to 249—250°. Both substances show by titration the calc. equivalent weight 280.3. (0.3753 or 0.2647 g require 11.05 or 10.62 ml 0.0893 *N* $Ba(OH)_2$. Equivalent weight found 279.0 or 279.3). The ethanolic solution of the highest melting substance show a blue fluorescence and is, therefore, presumably the pyrazoline (XIX), the other one being the normal p-carboxyphenylhydrazone (XVIII).
By condensation of (I) with benzylideneacetophenone only one substance is formed. Its alcoholic solution shows a brilliant blue fluorescense, and the substance is consequently of the type (XIX), *cf.* the condensation of benzylideneacetone with phenylhydrazine, v. Auwers and co-workers.[7, 8, 9]

Mustard oils. When alcoholic solutions of mustard oils are refluxed for 1—2 hours with aqueous solutions of (I), thiosemicarbazides (XX) separate on cooling. Recrystallised from ethanol the 1-p-carboxyphenyl-4-alkyl(aryl)-thiosemicarbazides show sharp melting-points. They may be titrated in ethanolic solution with standard base. 1.6 g phenyl mustard oil yield 2.3 g (XX) ($R = C_6H_5$) of m. p. 220—222°, which by recrystallisation is raised to 221—223°. 0.1981 g require 7.71 ml 0.0893 *N* $Ba(OH)_2$. Equivalent weight found 287.7, calc. 287.3.

SUMMARY

The reaction products of p-carboxyphenylhydrazine with different types of carbonyl compounds have been described.

The conditions governing ring-closure of the p-carboxyphenylhydrazones primarily formed to pyrazole- or pyridazine-derivatives have been established.

It has been stated which derivatives require 1 and which 2 equivalents of base for their neutralisation.

It has been shown that the potentiometrical titration of p-carboxyphenylhydrazones is possible even in cases where the ordinary titration cannot be accomplished. From the shape of the titration curve conclusions as to the presence of weakly acidic or basic groups as substituents in the molecule of the carbonyl-compound may be drawn.

With mustard oils substituted thiosemicarbazides are formed.

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A Note on the Growth Promoting Properties of an Enzymatic Hydrolysate of Casein

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In a recent paper [1] it was demonstrated that the catheptic enzyme of spleen acting on proteins at pH 4—5 rather rapidly produced a mixture of amino acids and low molecular peptides. The investigation was carried out to study the proteolytic split products obtained by the digestion of protein known to contain Castle's extrinsic factor. As a preliminary procedure to the clinical application the possible presence of toxic products formed during the hydrolysis was tested on young rats. It was found that the administration of the hydrolysate had no unwarranted influence on the growth curves when compared with the growth curves of the control animals.

In this series of experiments the average daily food consumption was not determined but the general impression obtained was that the food intakes of the rats in the experimental series were less than the intakes of the control animals. A quantitative determination of the possible difference was accordingly carried out in a new series of experiments.

EXPERIMENTAL

The animals were young albino rats with an average weight of about 80 g, of the stock bred in the Department of Hygiene of the University and the diet used was that recently described by Gard.[2] The animals were kept in individual cages. The catheptic casein hydrolysate was obtained by digesting 4 litres of a 3 % casein solution with 0.6 kg of beef spleen at 30° C for 72 hours. The hydrogen ion concentration was adjusted to pH 4.2 after periods of 24 hours. At the end of the digestion period insoluble material

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was centrifuged of and the centrifugate concentrated *in vacuo* at 15° C, so as to contain about 20 mg of nitrogen per ml and then glucose was added to a final concentration of 75 mg per ml. Assuming an average nitrogen content of 16 % in the digested proteins the rats were given a volume of the hydrolysate corresponding to 3 g of protein per kg body weight and day with pipettes. This group of animal, like the control group, was given the stock diet and water *ad libitum*; weighings of the food consumed and of the animals were made each day. The growth data are given in table 1.

Table 1. Growth of rats on a mixed diet with the addition of a catheptic protein hydrolysate.

Group	No. of animals	Average daily weight gain	Average daily food consumption	Weight gain per g food eaten
Experimental	16	2.4 ± 0.11*	11.0 ± 0.24*	0.22 ± 0.010*
Control	15	2.5 ± 0.12	16.3 ± 0.31	0.15 ± 0.012

* The values in these columns are the means and the standard errors.

The average daily weight gain was the same in both groups in accordance with the previously obtained results. On the other side there was a considerable difference in the average daily food consumption. The small amount of catheptic hydrolyzed protein with glucose daily administered (0.36 g of dry substance) was capable of promoting a significant increase in the weight gain per g food eaten. This property of the hydrolysate may possibly be correlated with the presence of enzyme resistant peptides with special growth effects in the extract.[3] The presence of a similar effect in a tryptic digest of casein has previously been demonstrated by Wolley.[4] The chemical and clinical properties of the hydrolysate are being further studied at present.

SUMMARY

The administration of small amounts of a catheptic casein hydrolysate to young rats significantly increased the weight gain per g food eaten.

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Die Konstitution der Harzphenole und ihre biogenetischen Zusammenhänge

X.* Herausspaltung des „Mittelstückes" des Pinoresinols

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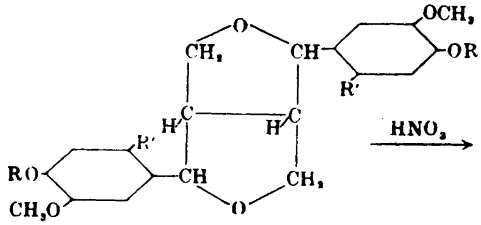
Für das Pinoresinol des Fichtenüberwallungsharzes wurden vor etwa zwölf Jahren von dem einen von uns auf Grund chemischer Untersuchungen und biologischer Überlegungen Formelvorschläge aufgestellt, unter denen I (R = H, R′ = H) vorgezogen wurde[1].



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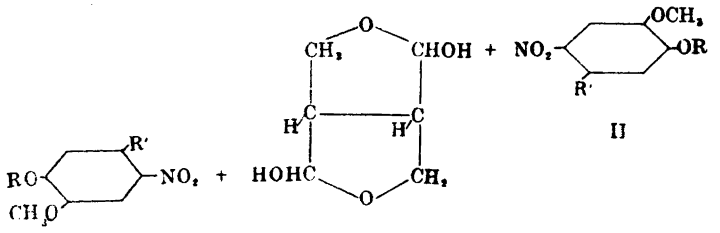


II III

* IX. Mitt.: J. Gripenberg, *Suomen Kemistilehti* 19B (1946) 138.

** Diese Arbeit wurde durch Mittel aus den staatlichen Beiträgen zur Förderung der technischen Forschung 1945 unterstützt. Fräulein A. Kårlin danken wir für geschickte Hilfe.

Der Dimethyläther des Pinoresinols (I; R = CH_3, R′ = H) erwies sich als die optische Antipode des Eudesmins, dessen Struktur schon 1914 von Robinson und Smith [2] diskutiert worden war. Diese Forscher waren zu der Auffassung gekommen, dass das Eudesmin aus zwei Veratrylgruppen besteht, die irgendwie durch ein wahrscheinlich aus zwei verschmolzenen Tetrahydrofuranringen aufgebautes Mittelstück verknüpft sind. Dieses heterocyclische Gebilde könnte das Kohlenstoffgerüst des *n*-Hexans, des 3-Methylpentans oder des 2:4-Dimethylbutans enthalten. Zu jener Zeit war noch kein einziges Mitglied der später als »Lignane» bezeichneten Familie von Naturprodukten aufgeklärt, und die genannten Autoren liessen die Frage nach der Struktur des Mittelstückes sowie nach dem Ort der Verankerung der beiden Veratrylgruppen offen. Die Auffassung der Lignane als 1:4-Diaryl-2:3-dimethylbutanderivate geht auf die Arbeiten von Schroeter, Lichtenstadt und Irineu [3] zurück, welche 1918 die Struktur der Guajakharzsäure aufklärten.

Für Formel I sprach der spätere Befund [4], dass das Molekül des Pinoresinols strukturell und konfigurativ symmetrisch gebaut ist. Dieser Befund wurde neuerdings von dem einen von uns [5] in unabhängiger Weise gestützt und erhärtet. Obwohl die Lignannatur des Pinoresinols eigentlich niemals in Frage gestellt worden ist, fehlte es jedoch an einem bündigen Beweis hierfür. Dagegen war im Laufe der Zeit Formel I mehrfach angezweifelt und besonders die andere Möglichkeit, die eines Olivilanhydrides, von verschiedenen Forschern [6, 7, 8] in den Vordergrund gestellt worden.

Die oxydativen Methoden hatten für die strukturelle Aufklärung des Mittelstückes dieser oxydischen Naturprodukte bisher keine prinzipiell neuen Beiträge geliefert. Auf die offenbare Ähnlichkeit des Pinoresinols mit dem Sesamin wurde von dem einen von uns [1] 1935 hingewiesen. Für diesen Stoff waren damals besonders Strukturformeln aufgestellt, die vom 1:6-Diphenyl-*n*-hexan abgeleitet waren.

Im Jahre 1936 wurde auf Grund der auffälligen Drehungsumkehrungen bei der Nitrierung des eben entdeckten Asarinins der Schluss gezogen, dass das Asarinin strukturell und konfigurativ als ein Methylendioxy-analogon des Eudesmins aufgefasst werden muss [9]. Von den ersten Bearbeitern der Konstitution des Asarinins wurde aber eine olivilanhydridähnliche Struktur bevorzugt. [7, 8]

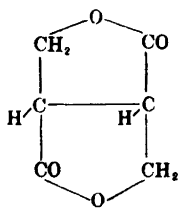
v. Bruchhausen und Gerhard [10] zeigten jedoch 1939, dass Asarinin durch katalytische Hydrierung in Eisessiglösung nach Art der Benzyläther zu einem Diol aufgespalten wird, was nur nach dem Strukturprinzip der Formel I erklärt werden kann.

In derselben Weise spalteten gleichzeitig Haworth und Woodcock [11] Pinoresinoldimethyläther zu dem entsprechenden Diol auf. Aus diesen Arbei-

ten folgten eine Reihe schöner Zusammenhänge zwischen verschiedenen Gliedern der Lignangruppe, und die Struktur des Pinoresinols nach Formel I wurde endgültig bewiesen.

Bei den Arbeiten über die Symmetrie des Pinoresinols war es aufgefallen, wie glatt die Dibromderivate der Pinoresinoldialkyläther (I; R = CH_3 oder C_2H_5, R′ = Br) von Salpetersäure abgebaut werden. Es wurde in vorzüglicher Ausbeute (80—85 % d. Th.) 4-Brom-5-nitro-guajakol-alkyläther, aus Dibrompinoresinol-dimethyläther somit das 5-Brom-4-nitroveratrol (II; R = CH_3, R′ = Br) gebildet[12]. Bei dieser Reaktion konnte das Mittelstück des Pinoresinolmoleküls entweder zugrunde gehen oder aber nach Art des 2:4:5:2′:4′:5′-Hexamethoxydiphenylmethans, welches glatt zu 5-Nitro-oxyhydrochinon-trimethyläther und *Benzaldehyd* gespalten wird[12], in ein Halbacetal von der Struktur III übergeführt werden.

Es ist zu erwarten, dass diese Verbindung wenig charakteristische Eigenschaften besitzt und deshalb als solche schwer isolierbar ist. Bei den Versuchen, sie zu erfassen, wurde die Reaktionslösung mit 2:4-Dinitrophenylhydrazin gefällt und gefunden, dass das tatsächlich gebildete Hydrazinderivat für Charakterisierungszwecke wenig geeignet war. Unter Umständen wurden auch methoxylhaltige Produkte erhalten, was darauf hindeutet, dass der Dibrompinoresinol-dimethyläther teilweise nur halbseitig gespalten worden war. Über ähnliche wenig ermunternde Erfahrungen berichtete zu gleicher Zeit Cohen[13], der die Spaltung des Dibromsesamins studierte. Wir haben deshalb Dibrompinoresinol-dimethyläther unter etwas schärferen Bedingungen mit Salpetersäure behandelt in der Absicht, eine vollständige Spaltung des Moleküls zu bewirken unter gleichzeitiger Oxydation des Dihalbacetals III zu dem Dilacton IV, welches voraussichtlich leichter charakterisierbar wäre.



IV

Die Durchführung dieser Reaktion ist geglückt, und das Dilacton konnte als schön krystallisierender Stoff isoliert werden. Es erwies sich, wie erwartet, als optisch aktiv, $[\alpha]_D^{21} = +206°$. Aus der Struktur I für Pinoresinol, seiner optischen Aktivität und der Tatsache, dass sein Molekül symmetrisch ist, folgt nämlich, dass die Wasserstoffatome, die an den beiden den Tetrahydro-

furankernen gemeinsamen Kohlenstoffatomen gebunden sind, in *cis*-Stellung zu einander stehen müssen. Die spannungstheoretisch unwahrscheinliche *trans*-Konfiguration würde zu einer inaktiven, nicht spaltbaren Form leiten.

Als nun *d,l*-Eudesmin in ähnlicher Weise gespalten wurde, konnte ein inaktives Lacton erhalten werden, welches sich als identisch erwies mit dem synthetischen Dilacton derselben Struktur, welches Michael und Ross [14] durch Kondensation von Äthantetracarbonsäure mit Formaldehyd und nachfolgende Decarboxylierung der entstandenen Dilactoncarbonsäure gewonnen haben. Durch diesen Abbau ist also in einer glatten Reaktion das Molekül des Pinoresinols in drei charakteristische Bruchstücke aufgespalten worden, wobei sämtliche Kohlenstoffatome in ihrer ursprünglichen Anordnung gefasst worden sind. Voraussichtlich lässt sich diese Reaktion auf ähnlich gebaute Lignane, z. B. Sesamin, Asarinin, Phillygenol (Forsythigenol) und vielleicht auch Gmelinol übertragen.

Nur mit der Struktur I, nicht aber mit der Struktur eines Olivilanhydrides, die schon aus Spannungsgründen unwahrscheinlich ist, ist die Tatsache vereinbar, dass es wiederholt geglückt ist, aus Pinoresinol-dimethyläther bzw. Asarinin durch Einwirkung von Säuren mehr als ein Epimerisierungsprodukt zu erhalten (Waldensche Umkehrung an einem der die aromatischen Kerne tragenden Kohlenstoffatome, bzw. an beiden). Wir haben Gründe dafür, anzunehmen, dass die gewöhnlichen, unter milden Bedingungen erhaltenen Epimeren mit relativ niedrigen Drehungen unsymmetrisch (einseitige Waldensche Umkehrung), und dass die »überzähligen» höher drehenden Epimerisierungsprodukte symmetrisch gebaut sind und mit Phillygenol in Beziehung stehen. Wir werden hierauf bald zurückkommen.

VERSUCHSTEIL

Abbau von Dibrom-pinoresinol-dimethyläther mit Salpetersäure. Dibrom-pinoresinol-dimethyläther (1,5 g) wurde in kleinen Portionen zu konzentrierter Salpetersäure (20 ml, sp. Gew. 1,41) gegeben und gut zerrieben. Unter Entwicklung nitröser Gase ging der Äther allmählich in Lösung. Nun wurde Wasser hinzugefügt, solange ein Niederschlag entstand. Dieser (0,52 g) erwies sich als 5-Brom-4-nitroveratrol. Die Lösung wurde mit Natriumbicarbonat genau neutralisiert und eingedampft. Die getrocknete Masse wurde im Soxhletapparat mit Äther erschöpfend extrahiert. Nach Abdestillieren des Äthers wurde der Rückstand in warmem Wasser gelöst, filtriert und eingedampft. Das zurückgebliebene Material wurde mit Benzol ausgekocht. Aus der Benzollösung krystallisierte, besonders bei Zusatz von etwas Äther, das Lacton aus. Die Ausbeute betrug 0,26 g (65% d. Th.). Aus Alkohol umkrystallisiert, wurden borsäureähnliche Krystallschuppen erhalten, die bei 160—161° schmolzen.

$[\alpha]_D^{21} = +\ 206°$ ($\alpha = +\ 1{,}20°$; $l = 0{,}55$ dm; $c = 1{,}06$ in Wasser).

67,0 mg Substanz verbrauchten (beim Erwärmen) 9,33 ml 0,1000 *N* NaOH.

$C_6H_6O_4$	Ber.	C 50,7	H 4,3	Äquiv.-Gew.	142,1.
	Gef.	» 50,9	» 4,5	» »	143,6.

Abbau von d,l-*Dibromeudesmin mit Salpetersäure.* *d,l*-Dibromeudesmin (1,25 g) wurde in Salpetersäure (20 ml, sp. Gew. 1,41) gelöst und die Lösung wie im vorigen Versuch aufgearbeitet. Es wurden 20 mg des gesuchten Lactons erhalten, welches bei 136—137° schmolz. Es stellte schöne prismatische Nadeln dar.

Synthese des d,l-*Bis-oxymethyl-bernsteinsäure-dilactons.* Die Synthese wurde nach den Angaben von Michael und Ross[14] ausgeführt, nur wurde die als Zwischenprodukt erhaltene Lactonsäure ohne weitere Reinigung durch Erhitzen im Vacuum dekarboxyliert und destilliert. Das Reaktionsprodukt wurde aus einem Gemisch von Benzol und Äthanol umkrystallisiert und schmolz bei 137—138°. Michael und Ross geben 138° an. Der Mischschmelzpunkt mit dem Abbauprodukt aus *d,l*-Dibrom-eudesmin vom Schmelzpunkt 136—137° war 137—138°.

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X-ray Investigation of Gold-Cadmium Alloys Rich in Gold

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This X-ray study of gold-rich gold-cadmium alloys was carried out because of discrepancies in the results of the investigations of this system by Ölander,[1] Köster and Schneider,[2] Owen and Roberts,[3] and Owen and Rees.[4] These previous investigations of the system differ in the methods applied. Ölander investigated the system by measuring the potential of the cell $Cd_{liq.}|electrolyte|(Cd, Au)_{solid}$ and determined its temperature coefficient for different compositions of the alloy electrode. Owen and co-workers, on the other hand, made an X-ray analysis of the system, and Köster and Schneider combined the X-ray analysis with measurements of the modulus of elasticity.

As Ölander states in his paper, there was some difficulty in measuring electrodes of α-alloys. The diffusion in these alloys was rather small and thus the surface composition of the electrodes could easily differ from the gross composition. To avoid this, Ölander measured the α-alloys not against liquid cadmium but against electrodes of $\alpha + \beta$-alloys, thus lowering the cadmium gas pressure considerably. In fig. 1, Ölander's potential measurements for the

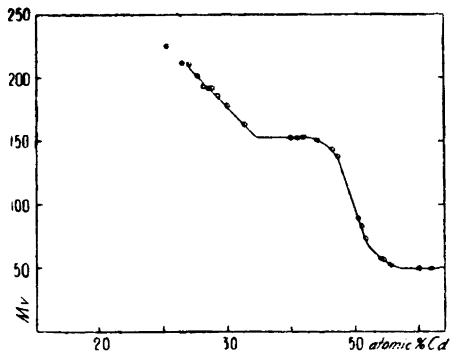

Fig. 1. Potential of the cell Cd/KCl, LiCl, $CdCl_2$/(Cd, Au) at 427° C according to Ölander.

gold-rich gold-cadmium alloys are reproduced. As will be seen from the figure, there may possibly be a narrow two-phase region at about 23 atomic % Cd. This was later established by Owen and Roberts. However, Owen and Roberts not only divided the α-region of Ölander in α, $\alpha + \alpha_2$, and α_2 regions, but also found a new phase at about 45 atomic % Cd, called α_3, and the existence of this phase is certainly not indicated by the potential measurements of Ölander.

EXPERIMENTAL

The alloys were made from 99.98 % gold from the Boliden Mining Company and cadmium *pro anal.* from Kahlbaum with a purity of at least 99.9 %. The following cell dimensions were found for the two metals: Au $a = 4.068 \pm .002$ kX, Cd $a = 2.973 \pm .002$ kX, $c = 5.604 \pm .003$ kX. Within the estimated errors these values agree with the precision determinations by Jette and Foote for Au ($a = 4.0704$ kX) and for Cd ($a = 2.9731$ kX, $c = 5.6069$ kX).[5]

Two types of X-ray cameras were employed. Focussing cameras were used for the quenched alloys, and a high-temperature camera with 72 mm diam. was used when the β-alloys were investigated. With the focussing cameras the precision in the determination of the cell dimensions is about 0.05 %. The temperature measurements were made with Fe-constantan and Pt-PtRh thermo-couples, which were checked during the course of work.

The alloys were prepared from weighed quantities of the metals — these having been sealed in evacuated silica tubes and melted. The ingots were then lump-annealed at 500—600° C and rapidly air-cooled. Afterwards the ingots were weighed and the loss in weight was always sufficiently small to make it possible to conclude that the composition of the ingots was within 0.1 atomic % of that intended. This was confirmed by chemical analyses of some samples chosen at random. For the analyses, the samples were dissolved in a mixture of hydrochloric and nitric acid. The solution was evaporated to dryness, hydrochloric acid was added and evaporated twice. Then the mixture of chlorides was dissolved in dilute hydrochloric acid and the gold reduced with SO_2. The gold was taken on filter paper, ignited in a porcelain crucible and weighed. Sulphuric acid was added to the filtrate, and the solution was evaporated until sulphuric acid fumes appeared. The final evaporation was made in a platinum crucible, in which the solid residue of cadmium sulphate was weighed after being heated to a constant weight at about 550° C.

For the investigation of quenched alloys, samples were prepared by taking filings from the ingots. The filings were placed in small thin-walled silica tubes, which were evacuated and put to anneal at the intended temperature. The temperature of the furnace was controlled by a regulator (Hartmann and Brauns »Kleinregler») and was constant within 5° C.

For the high temperature camera, wires were cast in the following way: The sample was placed in a small bulb of silica. To the mouth of the bulb, a capillary tube was attached, this being bent to a U-form with the shank on the bulb-side very short. The

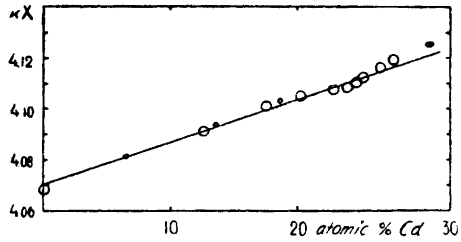

Fig. 2. The parameter-composition curve for the α-phase. The small circles show the measurements of Owen and Roberts.

bulb was evacuated through the capillary tube and filled with hydrogen. Then the sample was heated above the melting point. By the pressure of the hydrogen in the bulb, and by the application of a small suction to the tube, the molten alloy was forced into the capillary tube which after cooling was crushed. In the high-temperature camera the wires were heated by a little electrical furnace. The camera was filled with hydrogen during the exposure.

THE α-, α'-, AND α_2-PHASES

In fig. 2 the parameter-composition curve at room temperature for the cubic α-phase is shown. As will be seen, the cell dimensions determined by Owen and Roberts, and those determined by ourselves, fit rather well on the same curve. The existence of a hexagonal α_2-phase was clearly demonstrated by the powder photographs. For the position of the boundary α — (α + α_2) our results agree well with those of Owen and Roberts. The boundaries of the narrow regions $\alpha + \alpha'$, α', and $\alpha' + \alpha_2$, are rather difficult to determine. In a series of alloys with 20.2, 22.8, 24.6, 25.3, and 25.9 atomic % Cd annealed at 350° C, the first one only gave reflections belonging to the α-phase, and the following two only gave reflections belonging to the tetragonal α'-phase, whereas the last two showed reflections belanging to both the α and the α_2-phase. The lattice constants of the α'-phase were:

Atomic % Cd	a in kX	c in kX	$\frac{c}{a}$	V in kX^3
22.8	4.099	4.130	1.008	69.39
24.6	4.103	4.124	1.005	69.43

The tetragonal deformation is somewhat larger than that found by Owen and Roberts. Judging from these samples the $\alpha + \alpha'$ region is a little narrower and the α'-region a little wider than that shown by the phase diagram of Owen and Roberts. It is evident from these figures that the deformation of the tetragonal cell decreases with increasing cadmium percentage. In the two-phase region $\alpha' + \alpha_2$, the cell seemed to be cubic but the photographs of these alloys were diffuse and a faint tetragonal deformation may exist without having been observed.

In photographs of slowly cooled alloys of the composition Au_3Cd, Köster and Schneider observed super-lattice lines. On the other hand, Owen and Roberts stated that no super-lattice reflections were present in the powder photographs of α'-alloys. According to them, the structure of these alloys was face-centered tetragonal. Saldau,[6] however, found a high electrical conductivity for the α'-phase and this should indicate an ordered distribution of the atoms.

Contrary to Owen and Roberts, we found in the photographs of the α'-phase super-lattice lines showing an ordered distribution of the cadmium and gold atoms. The structure can be described as follows:

Space group D^1_{4h} — P4/mmm

1 Cd in 1(a): 000
1 Au in 1(c): $\frac{1}{2}\ \frac{1}{2}\ 0$
2 Au in 2(e): $0\ \frac{1}{2}\ \frac{1}{2}$, $\frac{1}{2}\ 0\ \frac{1}{2}$

As is seen from table 1, the agreement between observed and calculated intensities is quite satisfactory.

Table 1. Powder photographs of α'-alloy with 22.8 atomic % Cd. Quenched from 350° C. Fe-K radiation. β-reflections are omitted when not coinciding with α-reflections.

hkl	$\sin^2\Theta$ obs.	$\sin^2\Theta$ calc	*I* obs.	*I* calc.	*hkl*	$\sin^2\Theta$ obs.	$\sin^2\Theta$ calc.	*I* obs.	*I* calc.
101	0.1093	0.1103	v.w.	1.0	300	—	.5004	—	0.1
110		.1111		0.5	103	—	.5483	—	0.1
111	.1649	.1659	v.st.	38	301	.5547	.5547	v.w.—	0.1
002	.2184	.2190	m.	6.7	310		.5556		0.1
200	.2218	.2222	st.	13	113	.6040	.6040	m.	7.1
102	.2750	.2746	v.w.	0.3	311	.6104	.6104	st.	14
201	.2794	.2772	v.w.+	0.3	222	.6645	.6634	m.	7.3
210		.2778		0.3	203	—	.7151	—	0.1
112	.3319	.3301	v.w.+	0.2	302	—	.7191	—	0.1
211		.3326		0.4	320	—	.7223	—	0.1
202	.4422	.4412	st.br.	9.3	213	—	.7716	—	0.2
220		.4444		4.6	312	.7764	.7746	v.w.	0.2
003	—	.4928	—	0.0	321		.7770		0.2
212	—	.4968	—	0.3	004	.8771	.8762	w.	2.6
221	.4970	.4992	w.	0.1	400	.8898	.8888	m.	5.5
β 113				1.4					

However, these super-lattice reflections were observed not only in the photographs of the tetragonal α'-phase but also in those of the cubic α-phase of compositions in the vicinity of the formula Au_3Cd. This modification we

call α''. As was expected, the intensity of the super-lattice reflections decreased with increasing annealing temperature but they could still be observed, though very weak, in the photographs of samples annealed at 550° C.

The parameters of the α_2-lattice for different compositions are collected in the table below:

Atomic % Cd	a in kX	c in kX	V in kX^3
25.7	2.902 ± .002	4.774 ± .003	34.83
27.5	2.904	4.777	34.89
28.2	2.904	4.779	34.91
30.0	2.907	4.790	35.07
33.3	2.909	4.802	35.21

The lattice dimensions now given differ rather considerably from those obtained by Owen and Roberts. In spite of this fact, our determinations of the boundaries of the α_2-region agree fairly well with those of Owen and Roberts. We calculated the $\alpha_2 - (\alpha_2 + \beta)$ boundary to be at 33.3 (200° C), 34.8 (400° C), and 35.8 (550° C) atomic % Cd, agreeing very well with the values 33.2 (extrapolated), 35.0 and 35.5 obtained by Owen and Roberts. The other boundary of the α_2-region is more difficult to determine because of the diffuse reflections of the photographs, but the only deviation which perhaps slightly exceeds the accuracy of the measurements is at 550° C, where we found the boundary to be at 28.6 atomic % Cd instead of 29.1.

According to Owen and Roberts the structure of the α_2-phase is close-packed hexagonal. In table 2 the reflections of a sample of the composition Au_2Cd are listed. The intensities are calculated for a random structure, *i. e.* $\frac{2}{3}$ Cd and $\frac{4}{3}$ Au in the position 2 (c): $\frac{1}{3}\ \frac{2}{3}\ \frac{1}{4}$; $\frac{2}{3}\ \frac{1}{3}\ \frac{3}{4}$ of the space-group D_{6h}^4—C6/mmc. The agreement between observed and calculated intensities is good, the observed high intensity of the reflections 002 and 004 certainly being due to a preferred orientation of the crystallites. However, the structure

Table 2. Powder photographs of α_2-alloy with 33.3 atomic % Cd, quenched from 450° C. Fe-K radiation.

hkl	$\sin^2\Theta$ obs.	$\sin^2\Theta$ calc.	I obs.	I calc.	*hkl*	$\sin^2\Theta$ obs.	$\sin^2\Theta$ calc.	I obs.	I calc.
100	0.1457	0.1472	m.	21	112	.6027	.6035	st.	25
002	.1607	.1620	st.	25	201	.6284	.6293	m.	18
101	.1863	.1877	v.st.	88	004	.6480	.6478	m.—	4
102	.3084	.3092	st.	14	202	.7510	.7508	w.	7
110	.4408	.4416	m.	16	104	.7947	.7950	w.	7
103	.5113	.5116	st.	20	203	.9532	.9532	st.	43
200	.5880	.5888	v.w.	3					

Table 3. Comparison between observed intensities in powder photographs of α_2-alloys of different compositions quenched from 450° C.

hkl	25.7 atomic % Cd	29.5 atomic % Cd	33.3 atomic % Cd	*hkl*	25.7 atomic % Cd	29.5 atomic % Cd	33.3 atomic % Cd
100	w.d.	w.d.	m.	103	v.w.d.	w.d.	st.
002	st.	st.	st.	200	—	v.w.d.	w.
101	v.w.d.	w.d.	v.st.	112	v.st.	st.	st.
102	—	v.w.d.	st.	201	—	w.d.	m.
110	m.	m.	m.	004	m.	m.	m.

of this phase is not so simple at lower cadmium percentages. In table 3 the intensities of some reflections are compared at different compositions of the phase. It is obvious that the atomic arrangement of these three preparations must differ considerably.

As will be seen from table 3, there are in the photographs of α_2-alloys with 25.7 and 29.5 atomic % Cd some reflections which are weakened and diffuse while others remain sharp and apparently with about the same intensity as in the photographs of the alloy with 33.3 atomic % Cd. The broadening of these reflections is considerable: in the photographs of a preparation with 28.2 atomic % Cd (quenched from 350° C) the diffuse reflections (100, 101, 102, 103, 200, 201) seem to be 4—6 times broader than the sharp ones (002, 110, 112, 004, 114).

Broadened reflections in photographs often indicate that the crystallites of the preparation are very small. The sharpness of 002 and 004 shows clearly that in the direction of the c-axis the crystallites are at least 10^{-4} cm. Thus the crystallites should be thin in the a-direction, but this is contradicted by the sharpness of 110 and 112, which in this case must be broadened as is for instance 103. From these facts the conclusion must be drawn that the broadening of the reflections must depend on some special feature in the arrangement of atoms.

Now the structure of these alloys cannot differ much from a hexagonal close-packed one, as all reflections to be expected from that structure are present in the photographs of the alloy with 29.5 atomic % Cd and only three of them are missing in those of the alloy with 25.7 atomic % Cd. Of these missing reflections, 200 has the lowest calculated intensity of the listed ones (see table 2). 102 would be expected to appear near the inner end of one of the three photographs which were taken with our focussing cameras to cover the whole range. At this end the general blackening is very marked. 201 would be expected to appear near the other end of the photograph where the special intensity factors of the focussing cameras considerably weaken the intensity of the reflections.

In the photographs of hexagonal cobalt, Edwards and Lipson [7] have observed a similar mixture of sharp and diffuse reflections. This is explained by them as being due to occasional faults in the structure, making a sequence ABABAB ... change over to BCBCBC ... and so on (A, B, and C representing three layers of close-packing with x, y-coordinates 00, $\frac{1}{3}$ $\frac{2}{3}$, and $\frac{2}{3}$ $\frac{1}{3}$ for the atoms respectively). Obviously the reflections 001 are not affected by these faults as the 001 spacing is still perfect. For the other reflections Edwards and Lipson have shown that those with $\frac{1}{3}$ $(h\text{-}k)$ = an integer and l even have normal intensities, whereas those with $\frac{1}{3}$ $(h\text{-}k)$ = an integer $\pm \frac{1}{3}$ would give weakened and (if the faults are repeated at irregular intervals) blurred reflections. Thus, just as we observed them to appear, 002, 110, 112, and 004 should be sharp reflections and the other more or less diffuse.

THE β- AND β'-PHASES

In addition to the electrochemical investigation of the system Au-Cd, Ölander carried out a structure determination for the β and β'-phases.[8] The report of this latter investigation does not seem to have been observed by Owen and Rees. For the β-phase Ölander found the structure to be of the CsCl-type with a cube edge at about 400° C of 3.34 kX for an alloy with 47.5 atomic % Cd. The β'-phase was shown to be orthorhombic and had by the same composition the dimensions at room temperature a = 3.144 kX, b = 4.851 kX, and c = 4.755 kX. The space group is D^2. The two cadmium atoms are situated in 000 and 0 $\frac{5}{8}$ $\frac{1}{2}$ and the two gold atoms in $\frac{1}{2}$ $\frac{1}{2}$ 0 and $\frac{1}{2}$ $\frac{1}{8}$ $\frac{1}{2}$. This atomic arrangement can be looked upon as a deformed CsCl-structure. Ölander found a transition point at 267° C and believed this transition to be $\beta' \rightarrow \beta$. The existence of an orthorhombic modification of AuCd was later denied by Köster and Schneider. They thought that the low-temperature modification was tetragonal with a = 5.074 kX and c = 4.487 kX.

In the gold-rich part of the β-region Owen and Rees believed that they had found a new phase which they denoted α_3. The reflections in the powder photographs were said to fit on a rhombohedral hexagonal chart in a position with $\frac{c}{a} = 2.301$. The hexagonal cell was said to have $a = 5.473$ kX and $c = 12.593$ kX. They divided the rest of Ölanders β-region into a two-phase region $\alpha_3 + \beta$ and a single phase region, β, without the transition $\beta' \rightarrow \beta$. As is seen from the cell dimensions of this α_3-phase its c-axis is 4 times the a-axis of Ölanders β'-phase. There seems, however, to be no relation between the a-axis of α_3 and the b- and c-axis of β'. (The a-axis of α_3 is $\sqrt{3}$ times the a-axis of β').

In order to settle the question concerning the existing phases in this part of the phase diagram, a series of alloys was prepared with compositions ran-

ging from 40.2 to 54.2 atomic % Cd. Filings of these alloys were then annealed at 200° C (14 days), 400° C (3 days), and 550° C (26 hours), and then quenched. The phases that could be identified in the powder photographs of these preparations are listed in the table below.

Atomic % Cd	200° C	400° C	550° C
40.2	$\alpha_2 + \beta'$	$\alpha_2 + \beta' + x_1$	$\alpha_2 + \beta'$
43.0	$\alpha_2 + \beta'$	$\alpha_2 + \beta' + x_1$	$\alpha_2 + \beta'$
46.3	$\beta' + \alpha_2$	$\beta' + x_1$	β'
48.1	$\beta + \beta' + x_2$	$\beta + \beta' + x_2$	$\beta + \beta' + x_2$
50.1	$\beta + x_2$	$\beta + x_2$	$\beta + x_2$
54.2	$\beta + x_2$	$\beta + x_2$	$\beta + x_2$

The photographs of most of the preparations which are listed above show some reflections which we have not been able to identify. These extra reflections seem to belong to two different patterns. One of them appears in the photographs of the β'-phase (x_1 in the table above). In this case there are only a few unidentified reflections (table 4) while in the other case (x_2 in the table) the number of unidentified reflections is rather large (table 5). The

Table 4. Powder photographs of Au-Cd alloy with 46.3 atomic % Cd. Quenched from 400° C. Fe-K radiation.

hkl	R	$\sin^2\Theta$ obs.	$\sin^2\Theta$ calc.	*I* obs.	Phase	*hkl*	R	$\sin^2\Theta$ obs.	$\sin^2\Theta$ calc.	*I* obs.	Phase
100	α	0.0946	0.0958	v.w.	β'	022	α	.3223	.3227	m.+	β'
?		.1102		v.w.	x_1	130	β	.3705		w.	β'
020	β	.1286		w.	β'	200	α	.3826	.3831	m.	β'
002	β	.1344		m.	β'	131	β	.4034		v.w.	β'
111	β	.1442		m.	β'	?		.4098		v.w.	x_1
020	α	.1566	.1574	m.	β'	113	β	.4164		m.	β'
002	α	.1642	.1653	m. +	β'	220	β	.4438		v.w.	β'
?		.1711		w.	x_1	130	α	.4493	.4499	m. +	β'
111	α	.1756	.1764	st.	β'	211	α	.4640	.4637	v.w.	β'
?		.1795		w.	x_1	221	β	.4784		v.w.	β'
021	α	.1979	.1987	m.	β'	131	α	.4913	.4913	m.	β'
102	β	.2141		v.w.	β'	113	α	.5063	.5070	st.	β'
?		.2280		v.w.	x_1	?		.5193		v.w.	x_1
112	β	.2469		v.w.	β'	023	α	.5287	.5293	v.w. +	β'
120	α	.2530	.2532	v.w. +	β'	220	α	.5411	.5405	m.	β'
102	α	.2595	.2611	w.	β'	202	α	.5482	.5484	m.	β'
022	β	.2648		w.	β'	221	α	.5812	.5818	m.	β'
121	α	.2937	.2945	v.w.	β'	132	α	.6157	.6152	m.	β'
112	α	.2996	.3004	w.	β'	004	α	.6605	.6612	m.	β'
?		.3056		v.w.	x_1	041	α	.6723	.6710	m.	β'
200	β	.3138		v.w.	β'	222	α	.7050	.7058	m.	β'

Table 5. Powder photographs of Au-Cd alloy with 50.1 atomic % Cd, quenched from 400° C. Fe-K radiation.

hkl	R	$\sin^2\Theta$ obs.	$\frac{\sin^2\Theta \text{ obs.}}{\Sigma h^2}$	*I* obs.	Phase	*hkl*	R	$\sin^2\Theta$ obs.	$\frac{\sin^2\Theta \text{ obs.}}{\Sigma h^2}$	*I* obs.	Phase
100	α	0.0841	0.0841	m.	β		α	.4652		w.	x_2
	α	.1300		w.	x_2		α	.4737		m.	x_2
	α	.1330		w.	x_2		α	.5037		w.	x_2
	β	.1382		m.	x_2	211	α	.5131	.0855	st.	β
110	β	.1408	.0704	m.	β		α	,5334		w.	x_2
	β	.1540		w.	x_2		β	.5558		v.w.	x_2
	α	.1600		v.w.	x_2	220	β	.5646	.0706	v.w.	β
	α	.1680		v.st.	x_2		α	.5712		v.w.	x_2
110	α	.1710	.0855	v.st.	β		α	.5793		w.	x_2
	α	.1744		v.w.	x_2		α	.5875		m.	x_2
	α	.1779		v.w.	x_2		α	.6166		m.	x_2
	α	.1870		m.+	x_2		α	.6456		v.w.	x_2
	β	.2009		w.—	x_2		α	.6749		w.	x_2
	α	.2444		m.	x_2	220	α	.6866	.0858	m.	β
111	α	.2563	.0854	w.	β		α	.6943		w.	x_2
200	β	.2789	.0697	w.	β	310	β	.7055	.0706	w.	β
	α	.3081		v.w.	x_2		a	.7523		v.w.	x_2
200	α	.3399	.0850	st.	β	300 / 221		.7717	.0857	v.v.w.	β
	α	.3609		v.w.	x_2						
	β	.3848		v.w.	x_2			.7830		v.v.w.	x_2
	β	.3897		v.w.	x_2			.7917		v.v.w.	x_2
	β	.4142		v.w.	x_2			.8083		v.v.w.	x_2
211	β	.4215	.0702	m.	β			.8195		v.v.w.	x_2
210	α	.4284	.0857	v.w.+	β			.8473		w.	x_2
	α	.4457		w.+	x_2	310	α	.8563	.0856	m.	β

appearance of these extra reflections may indicate that the lattices of the β- and β′-phases are more complicated than those assigned to these phases by Ölander, but they may also belong to some other phases. However, the reflections do not fit either in a quadratic form of the α_3-type, or in the tetragonal quadratic form proposed by Köster and Schneider. The x_2-phase is probably one of the γ- or δ-phases of Ölander.

For the β- and β′-phases the lattice parameters at different compositions were (room temperature):

The β-phase

Atomic % Cd	*a* in kX	*V* in kX³
46,3	3.303 ± .002	36.02
48,1	3.304	36.07
48,5	3.305	36.10
50,1	3.305	36.10
54,2	3.308	36.20

The β'-phase

Atomic % Cd	a in kX	b in kX	c in kX	V in kX^3
46.3	3.125 ± .003	4.868 ± .004	4.757 ± .004	72.36
47.6	3.144	4.851	4.755	72.52
48.1	3.158	4.845	4.758	72.80

For the β-alloys Owen and Rees have given parameters that are considerably larger than ours and which decrease with increasing cadmium content. However, the cadmium atom is larger than the gold atom ($r_{Cd} = 1.47$ kX, $r_{Au} = 1.40$ kX) and thus an increase in the cell dimensions seems more probable. Besides, both the α and the α_2 alloys show lattice dimensions which increase with increasing cadmium content. The a-axis of the β'-alloys also increases with the cadmium content, but here the b-axis decreases and the c-axis is constant. The increase of the a-axis dominates and thus even in this case the cell volume increases with the cadmium content.

Some experiments by Owen and Rees show that the photographs of the β-alloys contain only the reflections of the body-centered cube, if the photographs are taken immediately after the quenching operation (that not all the reflections from the simple cube appeared, is probably due to the short exposure of only 30 minutes), but if the exposure was delayed for some time, the photographs contained a number of extra reflections. This fact makes it questionable whether our alloys — the powder photographs of which show unidentified reflections (x_1 and x_2 in the table) — can be in spite of this looked upon as alloys in single phase regions. This seemed to be possible, as they showed variation of the cell dimensions with the composition. The dimensions of the β'-phase in the alloys also containing α_2-phase, together with this variation, were used in attempts to fix the boundary between the $\alpha_2 + \beta$ region and the β region. The position of the boundary was to be compared with Ölander's boundary, which runs from 44.7 (300° C) to 41.2 (550° C) atomic % Cd. It was calculated to be at 47.5 (200° C), 44.8 (400° C) and 44.8 (550° C) atomic % Cd. It is, however, rather uncertain whether the dimensions of the phase in the two-phase alloys correspond to the quenching temperature. The mobility of the atoms is perhaps too great and Ölander's determination of this boundary is probably more reliable. Thus in the equilibrium diagram, (fig. 4) the boundaries of the β- and β'-phase have been taken from Ölander's measurements.

It is obvious that an investigation of the equilibrium diagram with quenched alloys cannot be considered as satisfactory in the β-region. Hence we made some X-ray photographs of wires in a high temperature camera (see p. 77)

at temperatures from 20° C to 500° C. It appeared that at temperatures higher than about 400° C the evaporation of cadmium from the wires was so rapid that changes from β to α_2 or α occured during the exposure. Thus we were not able to investigate the β-region from the solidus curve down to 400° C.

The X-ray photographs of wires cast from β-alloys showed transformation to cubic β-phase at 85° $\pm$ 15° C. Below the transition temperature, complicated patterns were obtained. The small radius and consequently low resolving power of the high-temperature camera made the identification of the reflections rather difficult, but it seems that they belong to the orthorhombic β'-phase. In any case it is certain that they fit in the quadratic form of this phase. The crystallites in the wires were rather large and strongly orientated with the result that the reflections were very spotty and difficult to measure. To avoid the orientation effects we also made X-ray photographs on specimens prepared by mixing heat-treated powder of the alloy with Canada balsam into a thick paste, which was coated on a fine glass fibre. The intensities and spacings of the reflections in a photograph of an alloy with 47.5 atomic % Cd agreed very well with those of the orthorhombic β'-phase previously measured.

Ölander found that wires of β'-phase at room temperature have a very remarkable rubber-like elasticity which is most pronounced at 47.5 atomic % Cd, for which reason he estimates the β'-region at this temperature to extend only between 47 and 48 atomic % Cd (private communication). This rubber-like elasticity, however, disappears when the wire is held in boiling water. It is thus characteristic for the β'-structure.

A specimen of an alloy with 50 atomic % Cd at room temperature gave (as was the case with the quenched alloy of this composition) reflections belonging to the cubic β-phase and the x_2-phase. At higher temperatures (about 70° C) the reflections of x_2 disappeared and those of β remained. Thus this specimen probably passes the two-phase boundary at 70° C. If this is true it is possible to super-cool the β-phase when it after cooling is mixed with x_2 but not in single phase alloys. Our future work on the cadmium-rich AuCd-alloys will perhaps throw light on this question also. It is, however, certain that the stable phases in the β-region are at low temperatures the orthorhombic β'-phase and at higher temperatures the cubic β-phase.

Köster and Schneider's measurements of the variation of the modulus of elasticity with the temperature show a definite transition at 30—60° C, in rather good agreement with our findings. Ölander, on the other hand, believed that the transition occurs at 267° C. With the X-ray method he, however, only showed that the alloy was cubic at 400—450° C, and orthorhombic at room temperature. The transition point was calculated from the temperature coefficient of the cell Cd|electrolyte|Cd, Au for $\beta + \gamma'$ and $\beta' + \gamma'$ alloy elec-

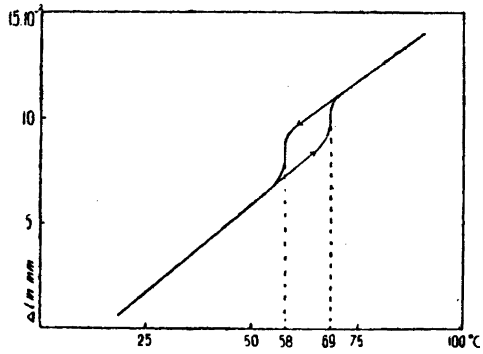

Fig. 3. The dilatation-temperature curve from room temperature to 100° C.

trodes. His measurements, however, were only extended down to 250° C, and it seems that he had found some other transition.

We also determined the transition point dilatometrically. A rod which was 63.3 mm long and 5.7 mm in diameter was made from an alloy with 47.5 atomic % Cd. For the investigation we used a dilatometer constructed by Pyk, Stålhane, and Westberg.[9] In fig. 3, one part of the dilatation-temperature curve is given. Slight super-heatings and super-coolings are difficult to avoid, even if the temperature is raised and lowered very slowly. However, the transition temperature could be estimated to be 64° ± 6° C. There might also be a slight effect at about 290° C (fig. 4), *i. e.* not very far from Ölander's transition at 267° C. Ölander's determination might be affected with rather large errors because of the small differences in temperature coefficient of the two forms β and β'. According to Köster and Schneider there is also an effect on the modulus of elasticity versus temperature curve at about this temperature: they obtained a maximum at 275° C. However, the X-ray photographs show no structural change at this temperature.

In a recent paper, Kubaschewski [10] concludes from calorimetric determinations of the heat content of an alloy of the composition AuCd just below

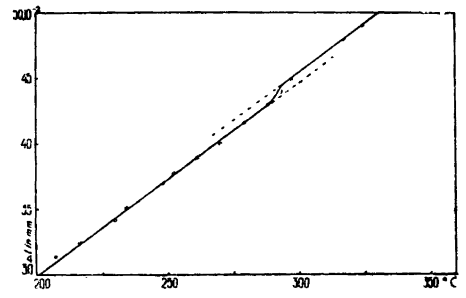

Fig. 4. The dilatation-temperature curve from 200°C to 325°C.

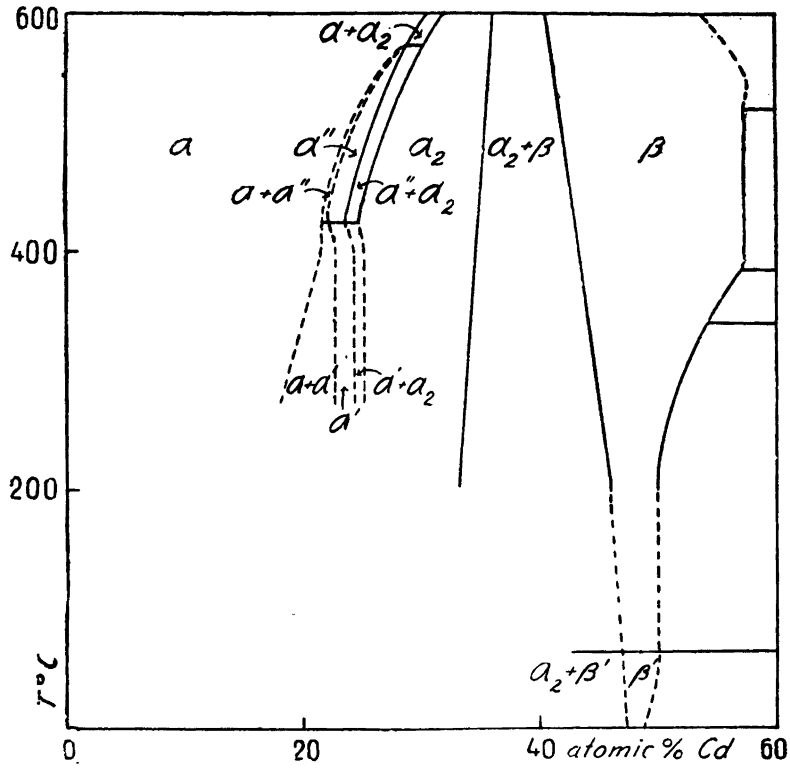

Fig. 5. Phase diagram of the gold-rich part of the gold-cadmium system.

and above the melting point that the β-phase becomes disordered before it melts. Owing to the rapid evaporation of Cd at elevated temperatures we have not been able to check this statement with X-ray photographs. However, Ölander determined the degree of disorder to be 0.5 % at 450° C.[1, 11] As the melting point of the β-alloy at the composition AuCd is as high as 627° C it seems very reasonable that the alloy will be completely disordered before it melts.

SUMMARY

The results of the present studies, together with those of previous investigations, are shown in the equilibrium diagram in fig. 5. It differs from the recently published diagram of Owen and O'Donnell Roberts, and from that of Owen and Rees in the following respects:

1. α' denotes a tetragonally deformed ordered structure.
2. α'' is a cubic structure, which is also ordered, the degree of order decreasing with increasing temperature. The α'' and the $\alpha + \alpha''$ regions (if the latter exists) must both be very narrow. The cell dimensions of ordered and disordered alloys of the same composition were equal as far as we could make out by our measurements, and we could not fix the boundary between the two regions. Together they occupy about two atomic percentages and in the diagram the $\alpha + \alpha''$ region has been drawn very narrow without any experimental evidence.
3. α_3 does not exist.
4. The β-phases have a transition at 64° C $\pm$ 6° C. The structure is changed from orthorhombic β' to cubic β by this transition. Probably, another transition without any structural change occurs at 280—300° C. The β-phase is ordered at low temperatures, but becomes disordered before it melts.

The α_2-phase has a pure hexagonal close-packed structure only when the composition is near to Au_2Cd. In more gold-rich parts of the α_2-region several reflections in the X-ray photographs are broadened and weakened. This effect is probably due to the fact that there are occasional faults in the hexagonal close-packing.

We wish to thank Professor G. Hägg, who placed the high-temperature camera at our disposal, Mr. B. Stålhane, from whom we have borrowed the dilatometer, Professor A. Ölander for his encouraging interest in our work and Nobelstiftelsen for financial support.

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On the Relation between Nitrogen Fixation and Leghaemoglobin Content of Leguminous Root Nodules

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Some years ago certain observations were made in this laboratory which opened new possibilities of investigating the machinery indispensable to nitrogen fixation in the root nodules. Certain strains of legume bacteria were then discovered which truly form nodules on the roots of the host plant, but nodules which do not fix nitrogen.[1] A strain of this kind is, for instance, the pea *Rhizobium* H VIII of our laboratory. Comparison between the nodules formed by this strain and those formed by effective strains showed that a red pigment is absent from the H VIII nodules, whereas it is always present in the effective nodules.[2] In this way a distinct *chemical* difference between the effective and ineffective nodules was for the first time detected. This observation suggested that the red pigment is in some way connected with the nitrogen fixation in the nodules. Many different findings in continued investigations have consistently led to the same result. Nitrogen fixation could never be noted, unless the root nodules contained red pigment. Of particular significance is the observation [2,3] that the colour of the red active nodules changes to green when nitrogen fixation ceases in annual plants at the end of vegetative growth or even at an earlier stage if the plants are removed into the dark for some days.

The haemoglobin nature of the red pigment of the root nodules discovered by Kubo [4] just before the war was confirmed in our laboratory in 1944.[2] The pigment is able to store and carry oxygen. Keilin and Wang [5] also arrived at the same result, whereas Burris and Haas [6] were at first unable to ascertain the haemoglobin nature of the pigment although they found it to be a haemo-

protein. — The pigment can be brought into solution simply by crushing nodules. It can be purified by precipitating with ammonium sulphate. In this laboratory a preparation was first obtained the purity of which was 80—85 % on the basis of the iron and haematin contents [7] (assuming these to be the same as in the blood of the vertebrates). The molecular weight of this preparation was determined by Pedersen and found to be 34 100,[7] which indicates that the molecular weight of the protein is about a half of that of haemoglobin. The experiments so far made thus indicate that the protein of the leghaemoglobin is different from that of the haemoglobin. Later the purification was carried so far that the iron content of the pigment was the same as that of pure haemoglobin.

Leghaemoglobin (the haemoglobin of the leguminous root nodules) differs from the haemoglobin of the blood of vertebrates in that it is autoxidized more easily. While normal blood contains at the most only a few fractions of per cent methaemoglobin, the root nodules, on the other hand, often contain considerably legmethaemoglobin.[3] This may in part be due to the action of oxidases in nodules, but in part at least also to autoxidation of leghaemoglobin which takes place fairly rapidly also in highly purified preparations. Accordingly, it may be assumed that the protein component of leghaemoglobin is linked to the prosthetic group in a somewhat differing way than in haemoglobin of blood.

The green pigment can be isolated from the green nodules in the same way as leghaemoglobin from the red ones. It is a chromoprotein (probably a mixture) and contains still iron. It has no longer the absorption bands of leghaemoglobin or of its O_2 and CO-compounds with distinct maxima between 500 and 600 mμ.[3] The iron is liberated from the green pigment by boiling with dilute hydrochloric acid. It is thus obvious that the porphyrin ring has been broken in the green pigment and simultaneously the iron has become more easily cleavable. Accordingly, the green pigment would be an intermediate stage between the leghaemoglobin and the bile pigments,[3] resembling substances which Barkan *et al.*[8] and Lemberg *et al.*[9] have prepared from haemoglobin. Such a formation of chromoprotein from the leghaemoglobin in the root nodules shows that leghaemoglobin is decomposed in approximately the same way as haemoglobin in animal organism. After all the nodules on the roots have turned green nitrogen fixation has completely ceased.

This paper presents an experimental material a part of which describes our recent findings, a part again is connected with the results published in preliminary communications. A critical review of the biology and chemistry of the nitrogen fixation by legume bacteria has recently been written by Virtanen.[26]

EXPERIMENTS

Detection of the ineffectiveness of H VIII strain

The pea bacterial strain H VIII was isolated in 1942 from the root nodules of pea in a field cultivation. In pot experiments with quartz sand as substrate pea did not grow when inoculated with this organism. All the nodules were white. Under these conditions, however, it is not possible to detect a few milligrams' fixation of nitrogen per plant. In order to prove the ineffectiveness of the strain as accurately as possible two series of experiments were carried out in the sterile culture system used in this laboratory. The results were very similar in both cases. Details of the second one are given in the following.

Seeds of Torsdag pea (of Svalöv) weighing 189—193 mg were selected for the experiments. Ten seeds of the same lot, 190 mg each, contained nitrogen according to the Kjeldahl determination as follows: 6.86, 6.02, 7.14, 6.44, 6.72, 6.44, 7.14, 6.72, 6.86, and 6.86 mg, on the average 6.72 mg. When nitrogen was determined from 5 samples of seeds each containing 10 seeds of equal weight, the analysis of each lot gave the following values for the average nitrogen content of one seed: (seeds weighing 188 mg) 7.14, 6.99, 7.42, 6. 71, 6.50, mean value 6.96 mg; (seeds weighing 194 mg) 7.21, 6.78, 7.49, 7.77, 7.21, mean value 7.25 mg. These figures alone reveal that fixation of about 1—2 mg nitrogen cannot be demonstrated by two parallels.

Sterilization of seeds and the technique of preparing sterile cultures have been described in previous papers.[10] Sterilized seeds were placed in tubes 31. VIII. 1943 and the seedlings were transferred to culture flasks 9. IX. For culture flasks we used 1 litre suction flasks connected with rubber tube to reserve flasks. The experiment comprised 9 flasks with one inoculated pea plant in each and 9 flasks with one uninoculated pea plant in each. The composition of the nutrient solution in the culture flasks was: KCl 250 mg, $Ca_3(PO_4)_2$ 250 mg, $CaSO_4$ 250 mg, $MgSO_4$ 394 mg, 3 drops of 5 % $FeCl_3$ solution, tap water to 1 litre. The reserve flasks contained tap water only. On transfer of the seedlings to the culture flasks the nutrient solution in 9 of these was inoculated with 1 ml of a water suspension of H VIII strain prepared by suspending the bacteria in water from the surface of agar. This amount of bacterial suspension contained 0.01 mg N, an insignificant amount in these experiments.

The peas grew in the greenhouse under natural light conditions. Nodules appeared on the plants inoculated with H VIII strain 17. IX, 8 days after inoculation. The results are given in table 1.

On the basis of the results it can be stated that no nitrogen fixation could be ascribed to H VIII strain. As the nutrient solution was the same in both series and the plants took up during the growth practically the same amounts of tap water the small amount of nitrogen (0.28 mg per litre) has not affected the results. In order to supply the nodules with sufficient oxygen the level of the water was allowed to lower to middle height of the flask thus providing excellent conditions for nitrogen fixation. The experiment included in the table for comparison shows that growth and nitrogen fixation were good in these circumstances when the plant was inoculated with the effective H 6 strain.

In the nodules formed by H VIII strain no traces of red pigment could ever be noted although the nodules could be examined continuously in the culture flask. On the other hand, the nodules formed by H 6 strain turned reddish in a few days after their appearance. Especially the nodules above the surface of the water had a beautiful reddish tinge.

Table 1. Experiment illustrating the nitrogen fixing ability of H VIII strain of pea Rhizobium. Test plant: Torsdag pea. Substrate: nutrient solution without combined nitrogen. Period of growth: 9. IX—6—11. X. 1943. Nodules appeared 17. IX.

	Tops			Roots			Nodules			Total dry weight, mg	Total N, mg	Tap water per plant, litres
No. of expt.	Dry weight, mg	N, mg	N, %	Dry weight, mg	N, mg	N, %	Dry weight, mg	N, mg	N, %			
Inoculated												
1	181	5.0	2.7	85	1.7	2.0	12	0.8	6.7	278	7.5	1.340
2	210	4.7	2.2	69	1.1	1.6	18	1.5	8.3	297	7.3	1.350
3	218	6.8	3.1	67	1.6	2.3	18	0.6	3.3	303	9.0	1.305
4	183	4.4	2.4	84	1.7	2.0	11	0.3	2.8	278	6.4	1.310
5	182	4.7	2.6	63	1.5	2.3	16	0.8	5.0	261	7.0	1.330
6	233	5.8	2.5	104	1.9	1.8	25	0.7	2.8	362	8.4	1.320
7	208	4.9	2.3	68	1.5	2.2	27	0.8	3.0	303	7.2	1.320
8	218	5.1	2.3	89	1.7	1.9	24	0.6	2.5	331	7.4	1.180
9	215	4.9	2.3	114	2.1	1.8	8	0.3	3.7	337	7.3	1.320
Average	205	5.1	2.5	83	1.6	2.0	18	0.7	4.2	306	7.5±0.77	1.308
Uninoculated												
1	219	6.4	2.9	111	2.3	2.1				303	8.7	1.300
2	185	5.1	2.7	119	2.4	2.0				304	7.6	1.340
3	175	4.7	2.7	120	2.1	1.8				295	6.8	1.330
4	189	4.9	2.5	130	2.4	1.8				319	7.3	1.270
5	178	4.4	2.4	115	2.4	2.1				293	6.8	1.350
6	188	4.6	2.4	124	2.3	1.9				312	6.9	1.100
7	186	5.0	2.7	127	2.5	2.0				313	7.5	1.100
8	182	4.3	2.3	119	2.5	2.1				301	6.8	1.330
9	188	4.4	2.3	140	2.7	1.9				328	7.1	1.340
Average	188	4.9	2.5	123	2.4	2.0				308	7.3±0.62	1.273
Inoculated with strain H 6												
	3430	94.9	2.8	247	5.0	2.0	98	4.6	4.7	3775	104.5	

Deeper under the water the colour was paler. The indispensability of sufficient supply of oxygen to the growth of inoculated legumes, which has been previously shown in this laboratory[11] is probably associated with the formation and action of leghaemoglobin in the nodules. — Leghaemoglobin is found in the interior of the nodule as well as the active bacterial mass. The easily accomplished passing of the pigment to solution on crushing of nodules implies that it is not in bacterial cells. In microscopic examination the H VIII nodules were found to contain exclusively rods surrounded by a slimy layer. In nodules formed by effective strains, such as H 6, only bacteroid forms[2] were found when the nitrogen fixation was at highest whereas in green nodules rods again were noted.

Through the courtesy of Prof. A. Wilska we were offered the possibility of examining the bacteroids in living state by the new micro-optics invented by him. By means of his microscope the differences in the refraction index are reflected in the darkness of the colour. The drawing in fig. 6 shows the bacteroid types observed. The large black granules are characteristic of bacteroids. The spaces between them seem to be optically almost empty.

The colour photographs (figs. 1—3) on the plate show nodules formed by the ineffective strain H VIII, red active nodules formed by the effective strain H 6, and nodules of the same strain which have changed to green and inactive in the dark. Fig. 4 on the same plate illustrates different shapes of bacteria in white, red, and green nodules, drawn according to the microscopic dyed preparation. Fig. 5 is a drawing of the living bacteroids visible under the microscope of Prof. Wilska.

Influence of nitrate on nitrogen fixation by peas

The much discussed question as to whether the root nodules fix nitrogen if the host plant is supplied with nitrate or with some other suitable form of combined nitrogen has not been solved. The experiments so far made had failed to give any solution owing to inadequate methods.[12] In connection with the experiments which we undertook to elucidate the indispensability of leghaemoglobin to nitrogen fixation solution was also found to the open question.

The experiments were made in the sterile culture system by using the so-called branched tube system which has been previously described in detail.[13] In this system the roots are divided into two culture flasks (fig. 7). This arrangement makes it possible to supply both branches with different nutrient solutions.

The experiment lasted from 25. V. to 25. VI. 1946. One culture flask contained 119 mg NO_3-N (in the form of $Ca(NO_3)_2$), the other was left without combined nitrogen, but inoculated with effective strain H 7. The composition of the nutrient solution was otherwise the same in both flasks.

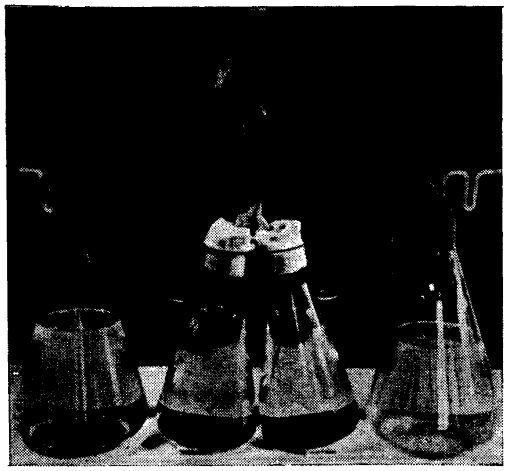


Fig. 6. The sterile branched tube system for investigation of nitrogen fixation and nutrient uptake in general.

The seedling was transferred to the system 25. V. The first nodules appeared on the inoculated root branch 1. VI and the nodules began to turn red after 4 days. The nodulation thus occurred as rapidly as in the experiments where the whole root system grew in the nutrient solution without combined nitrogen. The experiment succeeded in so far that the root branches grown into both flasks were approximately as well developed. The shape of the inoculated root system differed from that of the nitrate roots. In another paper we shall describe the effect of different nitrogen nutritions on the shape of roots. The experiment was interrupted 25. VI, at the start of flowering. The inoculated root system had then 68 red nodules, 22 green nodules, and 5 white nodules; a total of 95 nodules. The results of the analyses are summarized in table 2.

Table 2. Experiment with Torsdag pea in sterile branched tube system. Period of growth: 25. V— 25. VI. 1946. One culture flask contained 119 mg NO_3-N, the solution in the other was without combined nitrogen, but inoculated with the effective H 7 strain. Red nodules formed on this branch. Nitrogen balance at the end of the experiment appears from the figures in the table.

	Dry weight, mg	N, mg		mg
Nodules	22	1.1	Initial N of the nutrient solution	119.0
Roots, inoculated branch [1]	217	6.6	N in seed [2]	7.5
Roots, nitrate branch	180	7.0	N in 2 litres of tap water [2]	1.0
Green parts	2 055	74.9		
Total	2 474	89.6	Total initial N	127.5
Left in the nutrient solution		56.7	**Increase of N in the system**	**18.8**
Total final N		146.3		

The experiment proves convincingly that even when the pea was supplied with sufficient nitrate, red active nodules were formed in the roots and nearly 20 mg nitrogen were fixed. On the other hand, nitrate has evidently caused a marked decrease in the number and size of nodules compared to the plants grown without nitrate, and the amount of fixed nitrogen represents therefore only perhaps one third of that which would have been fixed had even the uninoculated branch grown in nitrate-free solution. Such an experiment was not carried out as parallel here but on the basis of the corresponding experiments previously made the influence of nitrate can be approximately estimated.

In another experiment where the ordinary sterile culture system was employed and the whole root system grew in the same culture flask, the following was noted. If the concentration of nitrate in the nutrient solution is comparatively low, less than 25 mg NO_3-N per litre, red normal-sized nodules are formed in the upper part of the roots, though they are few in number, and fixation of nitrogen takes place. If the concentration

[1] Nodules separated.

[2] Estimated by the control.

of nitrate is high, e. g. 100 mg NO_3-N per litre, in most cases no nodulation occurs. Exceptionally a few very small nodules are formed. No nitrogen is then fixed. In some experiments in which the nutrient solution contained 30—50 mg NO_3-N per litre a few quite small nodules were at first formed in the upper part of the root system, a part of them showing red colour. No nitrogen fixation was detected. Whether fixation has occurred in minute quantities can be demonstrated only by isotope method. It is possible that a compound of nitric oxide-leghaemoglobin is then formed preventing the action of leghaemoglobin. This has, however, not yet been experimentally proved.

When ammonium sulphate forms the source of nitrogen large red nodules are formed in concentrations where the nitrate nitrogen reduces the size of nodules to quite small and prevents nitrogen fixation. For example, in an experiment where the nutrient solution contained in the beginning 50 mg and at the end 31 mg NH_4-N per litre, the pea took up from the solution 42.4 mg NH_4-N (total amount in the solution 100 mg N) and received almost an equal amount of nitrogen fixed in the root nodules, since the whole plant contained 87.4 mg N. There seems thus to exist a great difference between the nitrate and ammonium nitrogens in their relation to the nitrogen fixation. The result obtained in the branched tube system indicates that the NO_3-concentration in the roots has an inhibiting effect on the action of nodules.

Purification of leghaemoglobin

As a starting material we used samples of 200—500 g of the root nodules of soya grown in large wooden boxes of sand or in the experimental field of our laboratory. The nodules were detached from the roots of the test plants when in best growth. In trying to obtain as pure preparation of leghaemoglobin as possible we collected the fraction which precipitates when ammonium sulphate is added up to 66—75 % saturation,[7] the solution neutralized and kept overnight in cold. The nodules were crushed in a mortar,. a large volume of water was added, and the suspension was centrifuged. To the red solution ammonium sulphate was first added to 60 % saturation. After addition of ammonium sulphate the solution was always neutralized with sodium hydroxide. After standing overnight in cold, the precipitate was separated by centrifuging, and ammonium sulphate was added to the solution to make the saturation 80 %. After a further standing overnight in cold the precipitate was again separated by centrifuging, dissolved in water, and precipitated first by adding ammonium sulphate to 66 % saturation and then after removal of the precipitate up to 66—75 % saturation. The first purifications in 1945 of the leghaemoglobin from the nodules of soya led after five successive precipitations (66—75 % saturation) to a preparation which was chiefly legmethaemoglobin and which after dialysis against running water until free from ammonium sulphate contained 0.27 % Fe in the dry matter. The haematin content of the preparation was determined as pyridine haemochromogen. The extinction was determined by filter S 53. Haemochromogen prepared in the same way from the haemoglobin of blood was used as control. The haematin content of the preparation was 3.8 %. On the basis of these values the purity of the preparation was 80—85 %[7] presuming that the iron and haematin contents are the same as those of the haemoglobin of blood.

With this preparation K. O. Pedersen in Uppsala determined in November 1945 the sedimentation and diffusion constants. We are greatly indebted to Dr. Pedersen for his valuable determinations. On the basis of his values the molecular weight of the leghaemo-

globin would be 34 100 or a half of that of the haemoglobin of blood. Leghaemoglobin will accordingly contain two haem groups in its molecule.

In autumn 1946 we made a preparation of leghaemoglobin from the root nodules of soya bean by passing carbon monoxide into the solution and by taking care that after each precipitation and dissolution of the precipitate the solution was treated with carbon monoxide. In this way it was attempted to prevent the oxidation of the preparation to legmethaemoglobin. After the fifth precipitation with ammonium sulphate the preparation was dialyzed and no precaution was then taken to avoid its autoxidation. So, after dialysis, the preparation was for the most part legmethaemoglobin. Its Fe-content was now 0.34 % corresponding with fair accuracy to that of the crystalline haemoglobin of blood. Accordingly, the purity of the preparation would have been nearly 100 %.

Haematin and leghaemoglobin and/or legmethaemoglobin contents of the nodules

In order to determine the haematin content of the nodules formed by different strains and consequently varying in effectiveness we used the following technique. A weighed amount of the nodules of pea was crushed in a mortar with pyridine. The precipitate was separated by centrifuging and washed three times with a small volume of pyridine. The extinction was determined from the combined solution of pyridine which was deep red in colour. The results thus obtained gave a rough idea of the haematin content of the nodules in the early part of growth up till flowering. When the formation of the green pigment starts to a greater extent the method gives very erroneous results because the green pigment interferes with the results.

Table 3. Experiment in the sterile culture system with pea (Torsdag) in nutrient solution without combined nitrogen. 12 Woulff-bottles, 2 peas in each, inoculated with different strains of pea Rhizobium as follows:

5 bottles with strain H 7 (very effective)
5 » » » H 2 (less effective)
2 » » » H VIII (ineffective)

The seedlings were transferred to bottles 27—28. VIII. 1946. The first nodules appeared 5—7. IX. On appearance of the first buds (8. X) two plants, inoculated with different strains, were simultaneously taken for haematin determination.

	In 2 plants				Haematin in nodules		Leghaemoglobin in nodules	
Inoculation	Dry matter, mg	N, mg	Number of nodules	Dry weight of nodules, mg	γ per g dry matter	γ per plant	% of dry matter	γ per plant
Strain H 7	1 382	51.0	831	117	1 130	66	2.9	1 700
Strain H 2	1 023	36.5	678	77	910	35	2.3	900
Strain H VIII	664	17.3	420	25	0	0	0	0

These preliminary results, too, seem to suggest that the rate of nitrogen fixation depends on the haemoglobin content of the root nodules. In the future experiments this point will be taken into closer examination.

Since, however, the haematin component in the nodules seems, at any rate in the early days of growth, to belong mainly to the haemoglobin or methaemoglobin — denatured haemoprotein is then found only little — the approximate amount of leghaemoglobin + legmethaemoglobin in the nodules can be calculated on the basis of the haematin content provided that good growth conditions prevail until flowering. At a later stage of growth the amount of denatured haemoprotein increases and the calculation leads therefore to very erroneous results. By assuming the haem content of the pure leghaemoglobin to be 3.9 % we obtained the following values in a preliminary experiment for the haematin and leghaemoglobin + legmethaemoglobin contents of the nodules (table 3).

Iron content of the nodules

Some experiments have been made to determine the iron content of the nodules. The total iron was determined from the ashes. Some drops of conc. nitric acid were added to the ashes, and after evaporation 1 ml of 6 *N* hydrochloric acid was added and heated to boil. The solution was washed to a small measuring glass, made up to 2 ml, whereupon 1 ml of 4 % ammonium thiocyanate solution and 2 ml of ethylacetate were added and the mixture was shaken. The intensity of the red colour of the clear ethylacetate solution was determined by the Pulfrich photometer. By comparing the extinction values with the values obtained with known amounts of iron it is possible to determine the iron content. Table 4 gives the results.

Table 4. Two plants, inoculated with different strains were simultaneously taken for iron determinations from the sterile culture experiments described in table 3. The figures in parentheses indicate the haematin iron. Determinations were made on following dates:

I. *8. X on appearance of the first buds*
II. *15. X during flowering*
III. *22. X at the end of flowering*
IV. *25. X when pods had developed*

	Fe, γ per g dry nodules			Fe, γ per g dry roots (nodules separated)		
	H 7	H 2	H VIII	H 7	H 2	H VIII
I	850	685	—	—	—	—
II	825 (100)	580 (80)	440 (0)	315	270	340
III	680 (108)	580	—	315	310	—
IV	540	—	—	—	—	—

The data show that the iron content of the nodules is considerably higher than that of the roots and that the difference is not caused by leghaemoglobin alone. According to our preliminary experiments the other iron compounds of the nodules, excluding the leghaemoglobin iron, can for the most part be dissolved by boiling with 0.5 % hydrochloric acid. The iron in the roots is then likewise almost entirely dissolved.

Isolation and purification of the green pigment

The formation of the green pigment was started by removing the sterile cultures of peas, inoculated with effective bacterial strains, into a completely dark room for a few days. As a rule the colour of the nodules changes to green in 3—4 days. The nodules were then separated from the roots and crushed in a mortar with water. The purification of the green pigment was performed by the same method as that of the red one. In precipitating the green pigment the precipitate was collected which was formed by 50—75 % saturation with ammonium sulphate. The preparation thus obtained was dialyzed. The iron contents of three different preparations were 0.22 %, 0.27 %, and 0.29 %.

With the preparations of green pigment isolated from the root nodules of soya and precipitated with ammonium sulphate some experiments were made for liberation of the iron with hydrochloric acid. The preparation was boiled in two tubes for four hours in a 1 % solution of hydrochloric acid, a current of carbon monoxide was led to one tube during boiling, a current of air or oxygen to the other. Different amounts of preparation were used in both experiments. The iron was determined in the manner described above by using ethylacetate.

	Atmosphere of air	Atmosphere of CO
Experiment 1	Extinction 72	Extinction 78
	Atmosphere of O_2	Atmosphere of CO
Experiment 2	Extinction 33	Extinction 31

The experiment shows that CO has not prevented the liberation of iron during boiling. In view of the fact that approximately the same amounts of iron were found both in atmosphere of CO and in that of oxygen it is likely that the iron in the pigment is trivalent. Whether iron becomes oxidized on isolation and purification or whether it is trivalent already in the nodules, is unknown. According to a previous observation preliminarily communicated [3] carbon monoxide seemed to prevent at least to some extent the cleavage of iron. The possibility that the iron of green pigment would occur in the nodules both in di- and trivalent forms must therefore be considered.

With regard to the preparations of green pigment so far made it is highly probable that the question is about a mixture of several chromoproteins. The green pigment isolated from the nodules which have turned green after the cessation of growth resembles in its properties the pigment formed in young nodules in the dark. On the basis of the findings so far made it seems that the green pigment becomes more easily denatured than the leghaemoglobin.

Leg- and legmethaemoglobin turns greenish in a solution containing ascorbic acid and hydrogen peroxide. Absorption maxima between 500—600 mμ disappear at the same time. In a leghaemoglobin-containing solution (6.0 ml), the pH of which was adjusted to about 6.5 by means of phosphate buffer and which was 0.03 % in regard to l-ascorbic acid, a 0.4 ml addition of 3 % H_2O_2 affected at room temperature the change of the colour from red to greenish and the disappearance of absorption maxima in 1 minute. With an addition of 0.04 ml of 3 % H_2O_2 the change took place in about half an hour.

The Gmelin test was made with the green nodules of soya as follows: the nodules were crushed in glacial acetic acid, acetone was added and the solution evaporated to a small volume, whereby precipitate was formed. A small volume of the solution was carefully poured on nitric acid in a test tube. Various coloured rings, though faint, formed at the junction. The extract of green nodules also gave a similar reaction.

Nitrogen fixation with free-living legume bacteria

As the indispensability of leghaemoglobin to nitrogen fixation in the root nodules seemed very plausible some experiments were made in our laboratory in order to find out whether it is possible to bring about nitrogen fixation with free-living legume bacteria by supplementing the nutrient solution with a water extract of red nodules or with purified leghaemoglobin. In the first experiments we used a raw extract of nodules which was filtered through a bacterial filter and contained both leghaemoglobin and legmethaemoglobin (and naturally many other substances). The sterile nutrient solution without combined nitrogen, containing mannite 20 g, K_2HPO_4 1.2 g, $CaCO_3$ 1.0 g, $MgSO_4 \cdot 7\,H_2O$ 0.5 g, $MnSO_4 \cdot 7\,H_2O$ 0.5 g, Na_2MoO_4 0.005 g, $FeSO_4 \cdot 7\,H_2O$ 0.1 g, and tap water to 1 000 ml, was divided into Roux-flasks, 150 ml to each, and each flask was inoculated with a loopful of H 7 organism and stored for 3 days at 25°. After that nodule extract was added to 2 of the flasks, to 2 again nodule extract plus oxaloacetic acid, while 2 were kept as controls without any additions. The flasks were kept at 25° for 3 days, after which nitrogen was determined. Before Kjeldahl determination an equal amount of the root nodule extract, stored in the ice box, was added to the controls as had been added to the test flasks 3 days earlier. The added amount of combined nitrogen thus came to be the same in all flasks. In the first experiments[14] an increase of nitrogen was found in amounts far beyond experimental error, particularly in the flasks containing oxaloacetic acid and nodule extract, but also in some flasks with nodule extract alone. On the other hand, no increase of nitrogen was affected by the purified pigment.

In repeating these experiments in winter 1945—46 we failed to confirm the positive results of the first experiments[15]. In summer 1946 altogether seven further series of experiments were carried out by employing the same bacterial strain for inoculation as in the preceding summer. In the most experiments the nodule extract was prepared from the nodules of soya, in one again from those of pea. The oxaloacetic acid solution was neutralized, filtered through a bacterial filter and added to the flasks in four lots during a period of 3 days, 20 mg at a time. The bacteriological purity of the control cultures was tested at the end of the experiment microscopically and in sterile milk and on slanted agar. No contamination was noted in any experiments.

As can be seen from table 5 no nitrogen fixation was noted when to the culture of legume bacteria either a raw nodule extract or purified leghaemoglobin was added. Only in the culture 4 in experiment 5 a considerable increase of nitrogen seemed to occur, but this single exception is evidently due to an experimental error. The nitrogen analyses in parallel experiments have generally given concordant values. In the experiment made for comparison with *Azotobacter* (no. 10) a powerful nitrogen fixation was observed. — What accounts for the positive results obtained in summer 1945 has remained unrevealed.

Transfer of nitrogen from nodules to host

In order to follow the transfer of nitrogen from nodules to host at different stages of growth as well as the changes in the colour of the pigment we undertook several experiments on pea. A typical example is given below.

An experiment on inoculated Concordia pea was made 17. V.—30. VII. 1941. The plants were grown in quartz sand in unglazed clay pots 8 inches in diameter. The experiment comprised 15 pots, 3 plants in each. The watering solution contained: 3.6 g $MgSO_4$,

Table 5. Experiments in 1946 with free-living legume bacteria for ascertainment of nitrogen fixation. The quantity of nodule extract given in the table was equally divided between the individual culture flasks of each experiment. To the control the extract was added at the end of the experiment prior to determination of nitrogen. The volume of nutrient solution was 100 ml per flask, the additions appear from the table. The figures express ml of 0.1 N *sodium hydroxide used for back titration of 50 ml 0.1* N *sulphuric acid, except in experiment no. 7 where they express N in mg. Duration of experiment was 3 days.*

No. of expt.	Added	Extract and oxaloacetic acid		Extract		Control	
		Flask 1	Flask 2	Flask 3	Flask 4	Flask 5	Flask 6
1	55 ml water extract from 20 g of nodules of pea	—	47.1	47.2	47.1	47.2	47.2
2	20 ml water extract from 12 g of nodules of soya	47.9	48.4	47.4	48.2	48.2	49.6
3	26 ml water extract from 13.5 g of nodules of soya	47.8	—	47.8	47.8	47.8	47.8
4	16 ml water extract from 15 g of nodules of soya	46.7	47.1	46.8	47.1	46.9	46.9
5	26 ml water extract from 22 g of nodules of soya	45.7	—	—	38.7	46.1	46.4
6	26 ml water extract from 25 g of nodules of soya	44.6	44.6	44.9	45.0	—	45.6
7	50 ml extract[1] from 271 g of nodules of soya	16.9	—	17.4	—	17.7	—
8	10 ml purified leghaemoglobin[2] solution	47.6	47.3	—	—	47.6	47.5
9	4 ml sterile blood of sheep	29.2	29.3	—	—	28.3	29.8
	Azotobacter chroococcum (experiments inoculated, but no extract added; controls uninoculated)	41.9	40.4	—	—	49.1	48.9

[1] Nodules were crushed without addition of water and the juice was pressed through a cloth, centrifuged and filtered through a bacterial filter. Experiment comprised 15 flasks. Each figure represents the mean value of analyses from 5 flasks. (Experiment was carried out in August 1946 by J. Erkama to whom we express our thanks.)

[2] Leghaemoglobin was isolated from a 100 g sample of nodules of soya, and purified by precipitating twice with ammonium sulphate (66—75 % saturation). After dialysis the preparation was used to the experiment. (Experiment was carried out in autumn 1945 by T. Laine.)

4.5 g KCl, 4.2 g KH_2PO_4, 4.0 g $CaSO_4$ in 60 litres of tap water. Besides, at the time of seeding the sand was watered with the minor element solution of Hoagland and Broyer. The effective strain H 11 was used for inoculation. In different periods of growth plants from 3 pots were analyzed at a time, accordingly, the mean values of each period are based on analyses from 9 plants. The periods of growth are apparent from table 6.

Table 6. Experiment with Concordia pea in open pots. Substrate: quartz sand without combined nitrogen. Period of growth: 17. V—30. VII. 1941.

No. of expt.	No. of cultures	Period of growth	Duration of growth, days	Length of period, days	Stage of growth
I	1—3	17. V—30. VI	45	45	Before flowering
II	3—6	17. V—12. VII	57	12	Start of flowering
III	6—9	17. V—21. VII	66	9	End of flowering
IV	9—12	17. V—30. VII	75	9	Pods well developed

Table 7. Experiment belonging to that presented in table 6. Dry weights and N-amounts of plants and nodules during different periods of growth.

No. of expt.	No. of culture	Tops			Roots			Nodules		
		Dry weight, g	N, mg	N, % of dry weight	Dry weight, g	N, mg	N, % of dry weight	Dry weight, g	N, mg	N, % of dry weight
I	1	2.059	110.50	5.37	0.539	14.39	2.67	0.271	21.11	7.79
	2	2.013	80.40	3.99	0.679	16.97	2.50	0.274	22.84	8.34
	3	1.604	78.40	4.89	0.586	15.16	2.59	0.251	21.32	8.49
Mean value		1.892	89.77	4.75	0.601	15.51	2.59	0.265	21.76	8.21
II	4	4.670	211.92	4.54	0.647	18.04	2.79	0.424	28.94	6.83
	5	5.545	232.56	4.19	0.912	22.47	2.46	0.539	39.26	7.28
	6	7.452	273.34	3.67	1.091	34.99	3.21	0.865	61.87	7.15
Mean value		5.889	239.27	4.13	0.883	25.17	2.82	0.609	43.36	7.09
III	7	12.029	344.03	2.86	1.417	37.60	2.65	0.924	46.96	5.08
	8	8.853	339.96	3.84	1.269	44.59	3.51	0.788	53.30	6.76
	9	10.125	324.61	3.21	1.125	28.67	2.55	0.860	52.29	6.08
Mean value		10.336	336.20	3.30	1.270	36.95	2.90	0.857	50.85	5.97
IV	10	9.152	282.61	3.09	0.926	23.63	2.55	0.568	27.95	4.92
	11	12.520	388.62	3.10	1.317	30.25	2.30	0.709	37.63	5.31
	12	12.250	406.46	3.32	1.577	32.70	2.07	0.753	37.02	4.92
Mean value		11.307	359.23	3.17	1.273	28.86	2.31	0.677	34.20	5.05
Uninoculated		0.620	14.81	2.39	0.628	9.85	1.57	—	—	—

Table 8. Experiment belonging to that presented in tables 6 and 7. Nitrogen amounts and the ratio of N in the host plant to the N in the nodules at different stages of growth.

No. of expt.	No. of culture	Duration of growth days	N mg, control subtracted: Tops	Roots	Tops + roots	N in nodules, mg	Ratio of N in tops + roots to N in nodules	N in tops + roots, % of total N
I	1		95.7	4.5	100.2	21.1	4.75 : 1	82.6
	2	45	65.6	7.1	72.7	22.8	3.1. : 1	76.1
	3		63.6	5.3	68.9	21.3	3.24 : 1	76.4
Mean value			75.0	5.6	80.6	21.7	3.73 : 1	78.4
II	4		197.1	8.2	205.3	28.9	7.10 : 1	88.0
	5	57	217.8	12.6	230.4	39.3	5.89 : 1	85.4
	6		258.5	25.1	283.6	61.9	4.58 : 1	82.1
Mean value			224.5	15.3	239.8	43.4	5.86 : 1	85.2
		In 12 days	149.5	9.7	159.2	21.7		86.9
III	7		329.2	27.8	357.0	47.0	7.60 : 1	88.4
	8	66	325.2	34.7	359.9	53.3	6.72 : 1	87.1
	9		309.8	18.8	328.6	53.3	6.17 : 1	86.1
Mean value			321.4	27.1	348.5	51.1	6.83 : 1	87.2
		In 9 days	96.9	11.8	108.7	7.7		93.4
IV	10		267.8	13.8	281.6	28.0	10.10 : 1	91.0
	11	75	373.8	20.4	394.2	37.6	10.44 : 1	91.3
	12		391.7	22.9	414.6	37.0	11.23 : 1	91.8
Mean value			344.4	19.0	363.5	34.2	10.63 : 1	91.4
		In 9 days	23.0	—8.1	15.0	—16.9	—	—

The results are examined in detail in the discussion.

For analysis the plants were freed from sand. The tops and the roots were separated from each other. The roots were washed free from sand and the nodules were detached by means of forceps and knife. Tops, roots, and nodules were weighed fresh and dried at 105° to a constant weight. Nitrogen was determined separately from tops, roots, and nodules. The total quantity of nodules and roots was used for the analysis, of the tops a sample of 500 mg was taken. The results are given in tables 7 and 8.

DISCUSSION

The experimental results of this laboratory which have been recorded both in previous papers and in the present one show concordantly that the red pigment is always present in active N-fixing root nodules, whereas it is never

found in ineffective ones. It seems that factors of most various kind which inhibit nitrogen fixation effect harmfully on the formation or promotingly on the inactivation of the pigment in the root nodules. This is true, for instance, of the effect of nitrate. The question much discussed as to whether the nodules fix nitrogen when the plant is supplied with sufficient nitrate or ammonium nitrogens has got a positive solution in our experiments and it has been shown at the same time that the red nodules are then in action. In nitrate concentrations where the nodules remained white no nitrogen fixation took place. If the nitrate concentration of the nutrient solution is so high that it allows formation of a few small nodules only, it seems possible that a nitric oxide-leghaemoglobin compound may be formed preventing the action of leghaemoglobin. In one experiment on pea in which a part of the roots (inoculated) grew in nitrate-free nutrient solution, a part again in nutrient solution with very high nitrate concentration, active red nodules appeared on the roots in the nitrate-free solution and nitrogen was fixed considerably. It seems therefore likely that the concentration of nitrate in the roots is an inhibiting factor for the formation and action of the nodules. This is suggested by the fact that ammonium salts do not prevent formation of normal-sized leghaemoglobin-containing nodules nor ample N-fixation in such concentrations where the nitrate already completely prevents the N-fixation.

On the basis of these observations the hypothesis [12] that combined nitrogen reduces the level of carbohydrate in the host and thus prevents nitrogen fixation is insufficient to explain the specific effect of nitrate. The remarkable observations of Fred, Wilson *et al.*[16] of the inhibiting effect of too intensive continuous lighting upon the nitrogen fixation in soya are possibly also ascribable to the failure of the formation of leghaemoglobin.

Determinations of haematin and leghaemoglobin in the nodules imply that a positive correlation exists between the effectiveness of the nodules and their leghaemoglobin content. In the light of the ocular examination of the nodules in numerous parallel cultures (sterile system) it seems likely, that the more leghaemoglobin is found in nodules and the longer it remains undecomposed, the more nitrogen is fixed. The nodules formed by less effective strains often begin to turn green already at an early stage of growth. Under unfavourable conditions the same is also noted in nodules from effective strains.

The effective nodules contain more iron than the roots. The difference is due partly to the haematin iron, partly possibly to other iron compounds. The nodules of the ineffective strain H VIII contain only a little more iron than the roots. The iron of the roots and of the nodules, excluding

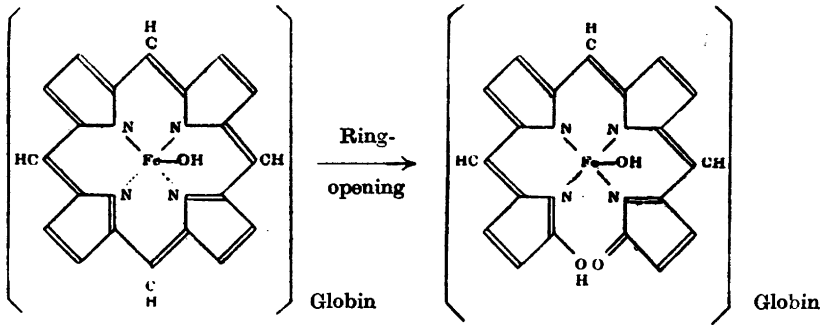
the leghaemoglobin iron, seems for the most part to dissolve on boiling with dilute hydrochloric acid. Since the iron content of the nodules seems to be the higher the more effective the nodules are, the extra iron has evidently accumulated in the interior of the nodule where the active bacterial mass is found.

The indispensability of leghaemoglobin to the function of root nodules may be entirely due to its ability to store and carry oxygen. This action of leghaemoglobin may promote the respiration of the nodule bacteria and it may also supply the oxygen for the oxidation of molecular nitrogen, if this is the first step in the nitrogen fixation. Since leghaemoglobin is autoxidized to legmethaemoglobin more easily than the haemoglobin of the blood of vertebrates it can also be assumed that the valency changes of iron in the leghaemoglobin may be involved in the nitrogen fixation.[3] This hypothesis is, however, fully speculative.

It is interesting to note, that Keilin and Wang [17] in their recent studies on the haemoglobin of *Gastrophilus*-larvae, which also is easily autoxidized, advanced the possibility »that the oxygen dissociating from its union with haem may possess a slightly higher level of reactivity than the ordinary molecular oxygen and that this level may vary with the haemoglobin». The easily autoxidizable haemoglobins, which seem to constitute the low-molecular haemoglobins (the molecular weight of leghaemoglobin is probably 34 000, of the haemoglobin of *Gastrophilus* 34 000, and of myoglobin 18 000), would thus occupy a position between the oxygen carriers and the oxidizing catalysts. A point of interest is that according to the determinations of Keilin and Wang the haemoglobin of *Gastrophilus* has a very low affinity for carbon monoxide ($K = [HbCO] \times pO_2 / [HbO_2] \times pCO = 0.67$), the corresponding value with phenoloxidase being 0.25—1, with cytochrome oxidase 0.1 and with the haemoglobin of vertebrates 120—550. With a very impure preparation of leghaemoglobin the said authors have obtained the value 37, which is of the same order as that with myoglobin.

Leghaemoglobin and legmethaemoglobin are easily changed to green pigment. A water-soluble green chromoprotein (probably a mixture of chromoproteins) which contains iron can be isolated from the nodules of plants kept some days in the dark as well as of plants which have normally ceased to grow. In addition, the nodules contain denatured green pigment. Ascorbic acid may have an important role in the formation of green pigment. The experiments *in vitro* have proved that leghaemoglobin and legmethaemoglobin are rapidly changed to green pigment at room temperature if the solution contains ascorbic acid and hydrogen peroxide. Thereby a considerable denaturation of protein also takes place. The ascorbic acid content of the nodules after several days' storage in the dark is still so high (0.01—0.02 % of the nodule juice after 4 days) that the formation of the green pigment may well

be linked to the ascorbic acid. As far as hydrogen peroxide is formed in the nodules in these circumstances all factors are provided for the formation of green pigment. The catalase activity of the nodules decreases if pea or soya is kept in the dark and consequently, the chances to formation of hydrogen peroxide in the nodules become greater. It is possible that the breaking of porphyrin ring may also occur without hydrogen peroxide at the presence of oxygen although the reaction is then very slow at 10—20°. Whether the opening of the ring is caused by the oxidation of leghaemoglobin and/or legmethaemoglobin is unknown. The latter alternative seems to be supported by the increase of legmethaemoglobin when the plants are kept in the dark. The reaction would then proceed in the following way.



Of course leghaemoglobin, too, may undergo the corresponding oxidation which leads to the opening of the porphyrin ring.

Lemberg [9] assumes that the precursor of bile pigments, choleglobin, is formed in animal tissues from oxyhaemoglobin in coupled oxidation with ascorbic acid (H_2A):

$$HbO_2 + H_2A \longrightarrow Hb \cdot H_2O_2 + A$$

$$Hb \cdot H_2O_2 \longrightarrow HbOH \text{ (methaemoglobin)}$$

$$Hb \cdot H_2O_2 \longrightarrow \text{choleglobin}$$

The mode of formation of the green pigment in the root nodules is still to a great extent obscure. It seems likely in any case that both in the dark and with cessation of growth either some substance disappears from the nodule juice which during the growth prevents the breaking of porphyrin ring in leghaemoglobin (or legmethaemoglobin) or a factor is formed which causes

the oxidation of the ring. According to our determinations oxaloacetic acid disappears in such conditions but so do probably some other active substances. The possible role of oxaloacetic acid in this connection has previously been pointed out.

Much attention was at first paid to Beijerinck's [19] observation of the occurrence of swollen and irregular »bacteroids» in the root nodules, since these were supposed to represent a type of nitrogen-fixing bacteria and the unchanged bacteria in the nodules were regarded as ineffective (for instance, Nobbe and Hiltner [20]). This conception was, however, later rejected. Especially the findings of Almon [21] according to which the bacteroids are not able to multiplication and do not cause nodulation indicated that the bacteroids are senile and biologically very inactive organisms. This does not, however, prove that bacteroids could not be active factors in the nitrogen fixation since this occurrence seems not to be associated with the propagation of bacterial cells (*cf.* below).

According to our observations the bacteria appear in the red nodules, formed by effective strains H 6 and H 7, chiefly as »bacteroids», in the ineffective white nodules, formed by H VIII strain, as rods which are surrounded by a slimy layer [2] (fig. 4). These observations thus support the old conception of the significance of »bacteroids» in the nitrogen fixation. The findings of some other workers (*e. g.* Pfeiffer [22]) of the absence of bacteroids from certain legume make, however, further investigation necessary and prevent generalization of our findings made with nodules of pea. The formation of red pigment and the development of bacteroids in the roots of pea seem to proceed, at any rate qualitatively, parallel.

An experiment recorded in the experimental part on the transfer of nitrogen from the nodules to the host over successive periods of growth after the appearance of nodules deserves still a closer examination. For this purpose we give below a graphical illustration of the results (fig. 7).

In the process of fixation and transfer of nitrogen several different phases can be distinguished. These have been marked in the graph by dotted vertical lines. Of course there is no sharp distinction between the different phases.

In the first few days the nodule-forming bacteria receive their nitrogen from the host plant (phase 1). The nodules are then white. However, after two-four days red pigment and the requisite machinery for nitrogen fixation begin to form in the nodules. Nitrogen fixation now starts and simultaneously begins the passage of nitrogen from the nodules to the host plant and a great number of new nodules appears. Lively multiplication of bacteria accompanied by protein synthesis still continues in the nodules for some time, and therefore appreciably nitrogen is retained in the nodules (phase 2). However, the

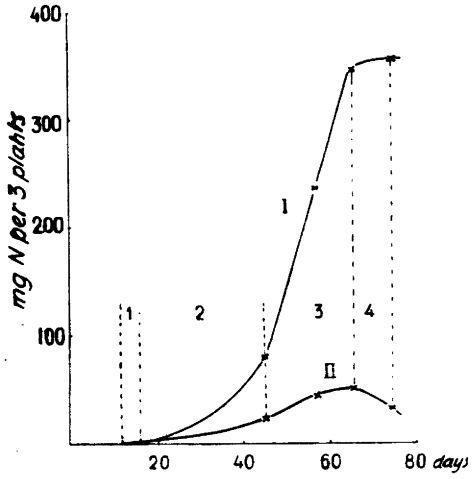


Fig. 7. Fixation of nitrogen in root nodules of pea and its transfer to host plant. Curve I: N in plants. » II: » » nodules.

percentage of nitrogen retained by the nodules decreases continuously during this phase. Nitrogen is fixed at a higher rate than new bacteria are formed within the nodules and as a consequence, the transfer of nitrogen to the host is proportionally increased. In the root nodules of pea the bacteria are now present chiefly as bacteroids. The transfer of nitrogen from nodules to host reaches gradually its maximal rate at phase 3. This phase during the middle and the later half of which about 90 % of the fixed nitrogen is regularly past to the host can be compared to the fermentation brought about in sugar solutions with »ready» bacterial or yeast masses (resting cells) when the enzyme machinery of the cells ferments sugar at an even rate. Nitrogen fixation is at phase 3 largely independent of the multiplication of bacteria and of the formation of bacterial proteins. The products of nitrogen fixation are probably excreted to the cytoplasm and used as nutrition by the host plants. The occurrence may be regarded as excretion especially as nitrogen compounds may also be excreted from the nodules to the culture medium. In connection with the latter phenomenon it was for the first time suggested [23] that the host plant obtains through excretion the amino acids (amino dicarboxylic acids) formed in the nitrogen fixation and that the earlier conception according to which proteins would first be formed in the nodules and only their decomposition products used by the host plant does not hold good.

Bond [24] has in his interesting experiments on soya arrived at the result »that a very high proportion probably in the region of 80 to 90 per cent is regularly liberated without appreciable delay into the host cytoplasm and that there is no retention or storage to any considerable extent of the fixed nitrogen within the bacteria or the nodules».

Wilson and Umbreit [25] have noted the same although they do not regard it as a proof of excretion.

The steady increase of nitrogen compounds in the host plant ceases during the last phase (phase 4) after the vegetative growth of the pea has come to an end. True, even after that the nitrogen content of the host plant is probably slightly increased (this is not easy to prove convincingly because of the variations in individual plants) and that of the nodules correspondingly decreased. The fixation of nitrogen has, however, completely ceased and soluble nitrogen compounds are passed from nodules to plant. This occurs possibly in certain measure already at the third phase especially towards the end of it as the nodules turn green. At the beginning of the fourth phase the red pigment has disappeared and the nodules have turned green. The association of leghaemoglobin with the nitrogen fixation is particularly apparent from experiments where the colour of the nodules and the increase of nitrogen are followed over the whole period of growth.

Nitrogen fixation could not be accomplished with free-living legume bacteria by adding to the nutrient solution an extract of crushed red nodules filtered through bacterial filter. The experiments made in winter and summer of 1946 failed to confirm the first positive results. In no experiment did the preparation of purified leg- and/or legmethaemoglobin cause nitrogen fixation in cultures of legume bacteria. The machinery which is required for nitrogen fixation has thus not been devised with free-living legume bacteria. It is possible that the occurrence is connected with a definite intranodular structure.

SUMMARY

The inability of the nodules formed by pea bacterial strain H VIII to fix nitrogen, at any rate in amounts detectable by ordinary analytical methods, has been shown. The H VIII nodules are devoid of leghaemoglobin.

Fixation of nitrogen by pea growing in nutrient solution with moderate concentration of nitrate has been demonstrated. Under such conditions the nodules are red. Nitrate prevents nitrogen fixation already in such concentrations which in regard to ammonium salts have no inhibiting effect on the formation of active red nodules of normal size. The causes of the harmful effect of nitrate nitrogen have been discussed.

Leghaemoglobin has been purified in so high degree that its iron content is the same as that of the haemoglobin of blood.

In parallel experiments on pea a positive correlation seems to exist between the leghaemoglobin content of the nodules and their N-fixing ability. More experiments are required in this respect.

The iron content of the effective nodules is considerably higher than that of the ineffective nodules and of roots.

The iron content of the purest preparation of the green pigment has been 0.29 %.

Nitrogen fixation has not been accomplished with free-living legume bacteria in nutrient solutions to which had been added either raw extract of nodules containing leghaemoglobin and/or legmethaemoglobin or purified preparation of these chromoproteids.

Transfer of nitrogen, fixed in the nodules, to the host has been examined over the successive stages of growth. During the main period when the major part of the nitrogen is fixed, on the average about 90 % is continuously past to the host. Nitrogen fixation is then largely independent of the multiplication of bacteria and of the formation of bacterial proteins. The bacteria appear then as swollen bacteroids at least in the nodules of pea. Towards the cessation of growth the red and brown pigments of the nodules begin to turn green. At the same time rod-shaped bacteria again occur in the nodules. At the end of nitrogen fixation the red pigment disappears and the nodules turn green. The plant then probably still receives some nitrogen nutrition from the nodules owing to the fact that soluble nitrogen compounds are past to the host and the nitrogen content of the nodules decreases.

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The Occurrence of a Diterpene-camphor in Scots Fir (*Pinus silvestris* L)

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The different role which each class of chemical compounds: hydrocarbons camphors, acids etc. plays among the monoterpenes, the sesqui-, di- and polyterpenes is a remarkable peculiarity in terpene chemistry.

The monoterpenes exhibit the most splendid variations in isoprene-hydrocarbons accompanied by a lot of alcohols, aldehydes and ketones, or camphors as we are accustomed to designate them. Apart from citronellic-, geranic- and teresantalic acid, carbonic acids are very scarce.

In the sesquiterpene group hydrocarbons and camphors again play the most important role, but in the plant family of the *Compositae* some interesting lactones (the alantolactones), keto-lactones (the santonins) and hydroxy-keto-lactones (artemisin) have been found.

The diterpenes on the contrary are overwhelmingly represented through unsubstituted carbonic acids: the resin acids make up the greater part of all wood resins. Diterpenehydrocarbons are scarce, in the camphor group the only pure representative is the aliphatic alcohol phytol, this compound being of quite outstanding importance. The investigation of Ruzicka [1] have proved sclareol of Salvia-oil to be a diterpenglycol. Three oxides *viz.* dicitronell-oxide,[2] manoyloxide [3] and the oxide $C_{20}H_{34}O$ from *Olearia paniculata* [4] are described in literature, besides manoyloxide keto-manoyloxide and a trihydroxy-diterpeneoxide were found.

The one of us (T. B.) has for some years studied the alterations in the terpenes of fir-stumps from Norwegian moors. To check the results we wished to convince ourselves that the supposed degradation products of the terpenes were all absent in living fir. For this purpose resinrich pine-material as the twig-roots and resinified trunks were destilled with superheated steam. The

resin acids were extracted from the ethereal solution of the destillate with very diluted sodium hydroxide. By fractionating the neutral material we got some fractions b. p. 180—220° at 7 mm (140—175° at 0.2 mm) having the composition of diterpenes with 1 and 2 O-atoms. The experiments were very much facilitated as it turned out that one of these compounds gave a semicarbazone sparingly soluble in methanol or benzene. After recrystallisation the semicarbazone melted at 223—224° and analysis gave $C_{21}H_{33}ON_3$ (+ H_2?) corresponding to a carbonyl compound $C_{20}H_{30}O$ (+ H_2?). The new diterpene-camphor ought to be a keto-diterpene as the very characteristic semicarbazone could be isolated even if the raw-fraction was treated with silver nitrate and alkali according to the excellent procedure of Curtius.[5] The free camphor was prepared by splitting the semicarbazone with oxalic acid according to Ruzicka.[6] The free diterpene-camphor distilled at 0.1 mm and bath temperature 160—165°, and crystallised in the recipient after short standing, m. p. 50—52°. Titration with perbenzoic acid: after 2 days 2.03 /⁼, after 10 days 2.53 /⁼. Only 1 /⁼ was readily hydrogenated with Pd or Pt-catalysts which do not reduce the carbonyl group of ordinary camphor. We therefore suppose that the new diterpene-camphor, for which we propose the name c r y p t o p i n o n e, is a tricyclic diterpene-ketone with one hindered ethylene bond. Work on its constitution is in progress.

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Catalase from Horse Kidney and Human Liver *

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Catalase was first crystallized from beef liver in 1937, [1] and since that time catalases have been prepared from horse blood and liver, beef blood, and human blood.[2, 3, 4]

It has long been known that the tissues richest in catalase are blood, liver, and kidney; [5, 6] and since the only difference between the catalases of horse blood and liver is the one verdohemochromogen group in liver catalase [4], it seemed of interest to prepare catalase from horse kidney. Human liver catalase was also prepared in order to compare it with the known blood catalase.

This paper describes the methods of preparation of these catalases in the crystalline state.

EXPERIMENTAL

Preparation of crystalline catalase from horse kidney

Six horse kidneys were trimmed of fat and connective tissue and ground. Catalase was extracted from the 3.7 kg of ground kidney with 7.4 liters of distilled water for 4 hours. It was estimated that this extract contained 2.5 g of catalase (Kat. f. 55000), and only 150 ml of blood which could account for only a negligible fraction of the catalase present.

While stirring rapidly, 3.2 liters of alcohol chloroform mixture (3 : 1) were added to the extract. The mixture was allowed to stand overnight in the cold room.

The solution was centrifuged and then filtered so that it was completely clear with a yellow-green color. There is very little loss of activity during the filtration which may take several hours at 20°. The filtrate was then evaporated *in vacuo* to a volume of 360 ml. It was dark brown in color, slightly turbid, and exhibited a distinct catalase absorption band at 625 mμ in a 1 cm layer.

* This project was supported by a grant from Statens Medicinska Forskningsråd.

Acetate buffer (4 *M*, pH 4.0) was added with stirring to bring the final acetate concentration to 0.1 *M*. After several hours at room temperature a brown denatured protein separated out and was removed by centrifugation. This solution was then fractionated with acetone as follows:

To 360 ml was added 188 ml of acetone and the grey-brown precipitate centrifuged off and discarded. Then a further 188 ml of acetone was added to the supernatant solution and the catalase separated as a dark brown precipitate. The yellow supernatant solution, which had no visible spectrum, was discarded.

The precipitate was dissolved in 110 ml of 0.1 *M* acetate buffer pH 4, centrifuged clear, and fractionated with alcohol. To 110 ml was added 84 ml of alcohol and the grey-white precipitate discarded. Upon further addition of 56 ml of alcohol to the supernatant solution a greenish precipitate of catalase was obtained.[8] This was redissolved in a small amount of 0.1 *M* acetate buffer (about 60 ml) clarified by centrifugation, and dialyzed overnight against distilled water.

A sediment appearing during dialysis was discarded, and then ammonium sulfate was added to the solution to make it 0.4 saturated. The brown precipitate was centrifuged off, and more ammonium sulfate slowly added to the supernatant solution to bring the degree of saturation to approximately 0.43. After a few hours the solution became thixotropic and the catalase began to crystallize.

The catalase crystals had the form of fine needles and dissolved readily in distilled water. The yield was 250 mg of crystalline catalase.

Preparation of human liver catalase

Preliminary to a study of catalase distribution in pathological tissues, a method was developed for accurately assaying human livers* for catalase. This method, which will be described in detail in a later publication involved a controlled extraction of the catalase from livers and titration with permanganate. The mean of analyses on 18 normal human livers was 0.61 g of catalase/kg liver. This compares with 0.5 g of catalase/1000 ml of human blood.

The catalase was isolated from human liver by the following procedure.

Two human livers were cleaned of connective tissues and of the larger blood vessels and ground. 3 kg of ground liver was extracted for 4 or 5 hours with 6 liters of distilled water. The amount of catalase in the extract was estimated as 1.8 g (Kat. f. 40000); the blood content 150 ml.

As the kidney preparation described in the foregoing section, the extract is treated with alcohol-chloroform (2.6 liters) and let stand overnight in the cold. Filtration yielded a yellow-green, clear solution with a reddish tint from the cytochrome c present in liver. Upon evaporating in vacuo to 400 ml the absorption bands of catalase (625 mμ) and cytochrome c (550 mμ) were visible.

The pH was adjusted to 4.0 with acetate buffer and the solution fractionated with acetone and alcohol as described above. In this case, 400 ml of solution were used and

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* Supplied by Rättsmedicinska Institutet, Stockholm.

the catalase fraction collected between 200 and 360 ml of acetone; the catalase dialyzed for a few hours; and then fractionated with alcohol.

Cytochrome c is usually insoluble after the alcohol fractionation. Any remaining trace, however, was removed by precipitating the catalase at 0.7 saturation of ammonium sulfate.

The catalase solution was dialyzed and crystallized with ammonium sulfate as described for the kidney preparation. The crystals were readily soluble in distilled water. Yield was 275 mg.

PROPERTIES

Measurement of Activity. The catalase activity was estimated as described by v. Euler and Josephson.[7] 0.4 to 0.5 g of catalase were used for each determination. Values of Kat. f. determined in this way are 55000 for kidney catalase and 40000 for human liver catalase. The figures are necessarily approximate, for the extrapolation to zero time is difficult. Preliminary work has indicated a rapid decrease in activity during the first few seconds so that the true Kat. f. may be considerably higher than indicated. This phenomenon may be due to adsorption on the walls of the reaction vessel and is being investigated further.

Hemin and Iron Analyses. Hemin was determined as pyridine hemochromogen. Iron was measured photometrically as the sulfosalicylic acid complex following combustion with H_2SO_4 and »Perhydrol».

	Hemin	Iron
	%	%
Horse kidney catalase	1.030	0.097
Human liver catalase	0.790	0.090

On splitting with conc. HCl and acetone [2] the kidney catalase yields a faint green color in the water phase. The human liver catalase does not give any blue color as the horse liver catalase does, but on standing for about 1 hour in the HCl and acetone solution a faint blue color developes. H_2SO_4 and acetone do not give any coloring of the water phase with human liver or horse kidney catalase, while the horse liver catalase in all cases gives a strong blue coloring of the water phase even with a very low HCl acid concentration (0.1 ml 1*N* HCl + 10 ml acetone).

Absorption Spectrum. The visible absorption spectra as measured with a Beckman spectrophotometer are shown in fig. 1. The Soret band for both has a maximum absorption at 405 mμ where β for kidney catalase is 84.5×10^7 cm^2 μM^{-1} and 67×10^7 for human liver catalase.

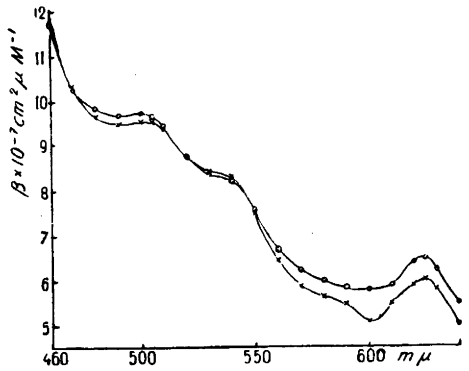

Fig. 1. Absorption spectra. —○— *Human liver catalase 0.0055 μM/ml and* —×— *horse kidney catalase 0.0042 μM/ml. 0.01* M *phosphate buffer pH 6.8, d = 1 cm.*

SUMMARY

Methods have been described for isolating crystalline catalase from horse kidney and human liver, and data presented on the distribution of human catalases in blood and liver. The visible absorption spectrum of the kidney and human liver catalases has been determined. Analyses for hemin and iron have been made.

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A New Method for the Synthesis of Arylstibonic Compounds

Preliminary Communication

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In a recent paper Hanby and Waters [1] have shown, that arylarsines are formed in following reaction (in acetone suspension):

$$3\ RN_2Cl + AsCl_3 + 3\ Zn = R_3As + 3\ N_2 + 3\ ZnCl_2 \qquad (1)$$

While working on the preparation of some antimony compounds, I have found a similar procedure advantageous.

By adding antimony trichloride to a diazonium salt solution, a double salt, of the composition $RN_2Cl,SbCl_3$ is formed in nearly quantitative yield. [2] The intention was to decompose this salt under such conditions that:

$$RN_2Cl,SbCl_3 = RSbCl_4 + N_2 \qquad (2)$$

would be the main reaction, but I found, that only compounds with reducing power were catalytically active, and therefore (2) was more or less accompanied by:

$$2\ RN_2Cl,SbCl_3 - 2\ Cl = R_2SbCl_3 + SbCl_3 + 2\ N_2 \qquad (3)$$

and:

$$3\ RN_2Cl,SbCl_3 - 4\ Cl = R_3SbCl_2 + 2\ SbCl_3 + 3\ N_2 \qquad (4)$$

Suspended in dry acetone the double salts are decomposed at 0° C by sodium iodide, iron, ferrous chloride, copper, cuprous chloride etc. Also many organic compounds, for instance formaldehyde, hydroquinone, etc., catalyze the decomposition, but require acetone with 5—10 % water.

With sodium iodide the yield of stibonic acid is as good as or better than by the Scheller reaction [3] and only small amounts of diarylstibonic chloride are formed. With iron (ferrum reductum) as a catalyst, (3) is the main reaction and the diarylstibonic chloride is formed in good yield. The experimental data and discussion of the reaction will be published later.

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Electron Diffraction Investigations of Molecular Structures

I. Application of Fourier Analysis to the Rotating Sector Method

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In the present paper we shall deal with the theory of electron diffraction in gases and of a method for the determination of molecular structures from diagrams obtained by the sector method. In a subsequent paper [1] the results will be given for determinations of about 40 molecular structures which have been carried through by this method. The apparatus used was completed and the method of interpretation worked out at the beginning of the war. The method has now been used in our laboratory for some years and a series of minor modifications have been introduced. Parts of the theory and the results have been published previously in Norwegian periodicals. As these papers, however, are not easily accessible in other countries we shall give a survey both of the method and the results.

THE BASIC THEORY

In this summary we intend to follow chiefly a paper by Finbak.[2] Parts of the theory will be discussed more thoroughly elsewhere.[3]

The diffraction of an electron beam by a point charge of q elementary units may, according to Gordon,[4] be written

$$I_q = \frac{I_0}{R^2}\left[\frac{8\pi^2 m\varepsilon}{h^2}\right]^2 \frac{\lambda^4 q^2}{(4\pi \sin \frac{\vartheta}{2})^4} = K \frac{q^2}{s^4} \qquad (1)$$

* The author wishes to express his gratitude to Dr Chr. Finbak and Professor O. Hassel for giving him the opportunity of working with them in this field, and to *Fridtjof Nansens fond* and *Statens Forskningsfond* for financial aid.

Where

I_0 = intensity of the primary beam

R = distance between the diffraction point and the point of observation

m, ε = mass and charge of an electron

h = Planck's constant

$\lambda = \frac{h}{mv_0}$ = wavelength of the electrons in the beam

v_0 = the velocity of the electrons

$s = \frac{4\pi}{\lambda} \sin \frac{\vartheta}{2}$ is used as variable instead of the scattering angle ϑ

The coherent scattering from a freely rotating system of point charges q_i, the mutual distances of which are given by r_{ij}, is then, according to Debye [5] and Ehrenfest.[6]

$$I(s) = \frac{K}{s^4} \Sigma\Sigma\, q_i q_j \frac{\sin sr_{ij}}{sr_{ij}} \tag{2}$$

If the two charges q_i and q_j are altering their mutual distance during the time of observation, the mean intensity is given by

$$I(s) = \frac{K}{s^4} \int_0^\infty \Sigma\Sigma\, q_i q_j\, p_{ij}(r) \frac{\sin sr}{sr}\, dr \tag{3}$$

The value of $p_{ij}(r)\, dr$ represents the probability of these two charges having a mutual distance in the interval from r to $r + dr$.

Eq. (3) may be written

$$I(s) = \frac{K}{s^4} \int_0^\infty \sigma(r) \frac{\sin sr}{sr}\, dr \tag{4}$$

where $\sigma(r)\, dr$ is twice the probability for *any* two charges in our system having a mutual distance between r and $r + dr$. For a neutral system we have

$$\int_0^\infty \sigma(r)\, dr = \Sigma\Sigma\, q_i q_j = (\Sigma q_i)^2 = 0 \tag{5}$$

The connection between the probability function $\sigma(r)$ and the density function $D(r)$ [7] is given by the equation:

$$\sigma(r) = 4\pi r^2 D(r) \tag{6}$$

Applying the function $\sigma(r)$ to actual systems we may assume that every volume element dv possesses a number of charges corresponding to $\varrho\, dv$ where ϱ is the charge density. We then have the following well known equation for the coherent scattering by a rigid system of spherical atoms

$$I_e(s) = \frac{K}{s^4}\left[\Sigma(Z_i - F_i)^2 + \Sigma\Sigma' \ (Z_i - F_i)\ (Z_j - F_j)\ \frac{\sin sR_{ij}}{sR_{ij}}\right] \tag{7}$$

Here the $F_i\ (s)$ is the atomic scattering functions for X-rays and R_{ij} is the distance between two nuclei. The sums must be taken over all the atoms in the system for which $i \neq j$.

For the coherent scattering by a single atom eq. (7) gives

$$I_i(s) = \frac{K}{s^4}\ (Z_i - F_i)^2 \tag{8}$$

Mott [8] has discussed the atomic scattering, employing wave mechanics, and finds that eq. (8) is valid when the energy of the incident electrons is greater than the binding energy of the K-electrons in the atom by which they are scattered. We then may assume that eqs. (4) and (7) also are valid under the same conditions.

Eq. (7) may now be written

$$I_c(s) = \frac{K}{s^4}\left[\Sigma(Z_i - F_i)^2 + \int_0^\infty \sigma_m(r)\ \frac{\sin sr}{sr}\ dr\right] \tag{9}$$

where $\sigma_m(r)\, dr$ is twice the probability of finding charges belonging to *different atoms* at a distance between $r + dr$ apart. Adding the incoherent scattering, the total intensity may be written

$$I_t(s) = \frac{K}{s^4}\left[\Sigma((Z_i - F_i)^2 + S_i) + \int_0^\infty \sigma_m(r)\ \frac{\sin sr}{sr}\ dr\right] = I_a(s) + I_m(s) \tag{10}$$

Using Fourier's integral theorem we have

$$\sigma_m(r) = \frac{2}{\pi} r \int_0^\infty \frac{I_m(s)}{K}\ s^5 \sin sr\ ds \tag{11}$$

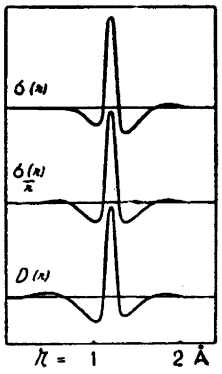

Fig. 1. Theoretical distribution curves without diffraction effects for a diatomic molecule.

The function $\sigma_m(r)$ may be written

$$\sigma_m(r) = \Sigma\Sigma' \sigma_{ij}(r)$$

where $\sigma_{ij}(r)$ depends solely on the charge distribution in two atoms i and j and the distance R_{ij} between their nuclei. Fig. 1 gives the function $\sigma_{ij}(r)$ for two oxygen atoms oscillating about the equilibrium distance 1.2 Å and the corresponding curves $D_{ij}(r) = \frac{\sigma_{ij}(r)}{4\pi r^2}$ and $\frac{\sigma_{ij}(r)}{r}$.

It may be proved that the function $\frac{\sigma_{ij}(r)}{r}$ is symmetrical with respect to the line $r = R_{ij}$, a property which is not shared by the function $\sigma_{ij}(r)$ or $D_{ij}(r)$. The function $\frac{\sigma_{ij}(r)}{r}$ should therefore be more suitable than the other two for the determinations of molecular structures (3). According to eq. (11), the function $\frac{\sigma_m(r)}{r}$ takes the form

$$\frac{\sigma_m(r)}{r} = \frac{2}{\pi}\int_0^\infty \frac{I_m(s)}{K} s^5 \sin sr \, ds \qquad (12)$$

The function $\frac{I_m(s)}{K}$ must be determined experimentally, and this may be done by the rotating sector method described below.

THE EXPERIMENTAL TECHNIQUE

It is a well-known fact that a photometric record of an ordinary electron diffraction diagram gives only meagre information regarding the molecular scattering $I_m(s)$. This is due to the form of the intensity curve $I_t(s)$

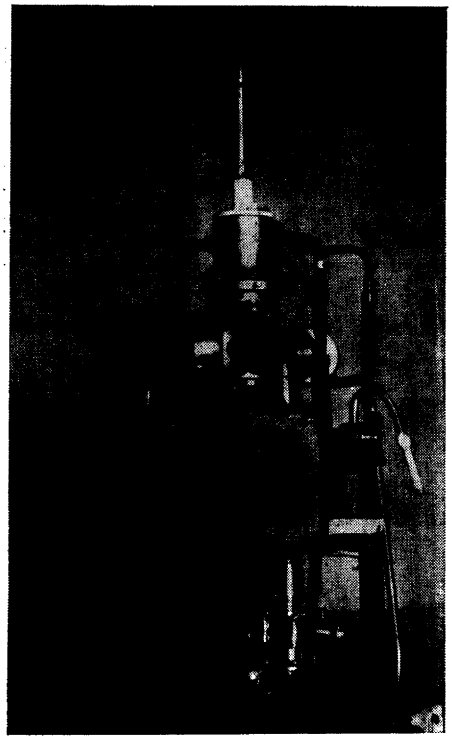

Fig. 2. Electron diffraction apparatus for gases.

which falls off very rapidly with increasing values of s. In order to obtain diagrams on which the blackening is more equally distributed over the whole range of the film, a sector of suitable form may be rotated in front of the photographic plate during the exposure. The sector must have a form such that its screening effect decreases with increasing values of s. The application of such a sector was first proposed by Finbak[9] and later by Debye.[10]

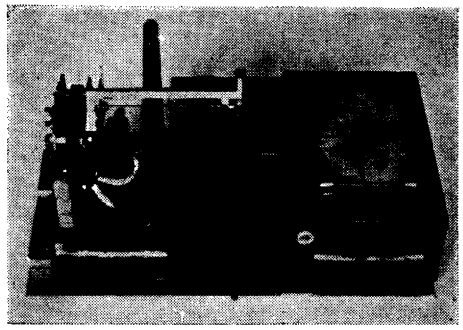

Fig. 3. The rotating sector in its place above the photographic plate.

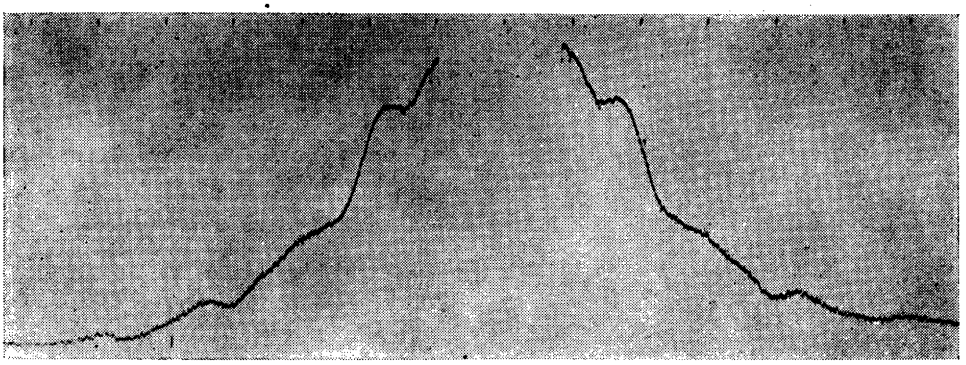

Fig. 4. Photometric record of a sector diagram (benzene)

The diffraction apparatus used in our laboratory, shown in fig. 2, has been described elsewhere.[11] A description of the apparatus will therefore not be given here, but some details concerning the rotating sector should be mentioned. This part of the apparatus is shown in fig. 3. The sector itself is rotated with the incident beam as an axis. Different forms of sectors have been tried, their screening effects being proportional to s^{-1}, s^{-2} and s^{-3}. Experience shows that the sector having the factor s^{-2} is most suitable and this sector has therefore been used almost exclusively in our laboratory. Fig. 4 gives photometric record of a sector diagram of benzene. The maxima and minima of the curve are very conspicuous and the curve is well suited as a basis for the determination of the structure.

The most strightforward way of determining the $I_m(s)$ function would appear to be the procedure used in the case of X-ray investigation of liquids,[12] but in the case of electron diffraction this method has not been successfull. We therefore adopted a different procedure originally proposed in a slightly modified form bv Finbak.[13]

The terms $I_t(s)$, $I_m(s)$ and $I_a(s)$, defined above, correspond to the intensities of the scattered beam before it strikes the rotating sector. Let $I_t'(s)$, $I_m'(s)$ and $I_a'(s)$ represent the same intensities after passing the sector and $P_t(s)$ the ordinate of the photometric record. In fig. 5 the curves $I_t'(s)$, $I_a'(s)$ and $P_t(s)$ are drawn with a common s-axis, the scale of which has been calibrated from gold-foil diagrams. In the same figure we have given the function $P_a(s)$, defined by the equation

$$\frac{I_t(s)}{I_a(s)} = \frac{I_t'(s)}{I_a'(s)} = \frac{P_t(s)}{P_a(s)} \qquad (13)$$

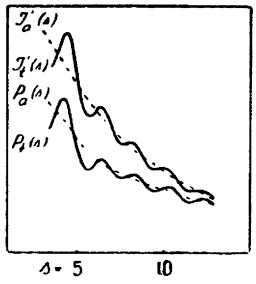

Fig. 5. The correction of the photometric record.

From eqs. (7), (10) and (13) follows that the function

$$\frac{I_m(s)}{I_a(s)} = \frac{P_t(s)}{P_a(s)} - 1 \tag{14}$$

consists of a sum of modified $\frac{\sin x}{x}$ functions. For a sufficiently great interval of s the mean value of this function is near to zero. Our experience indicates that it is possible to make a reliable determination of the curve $P_a(s)$ by applying these relations. A further control of the curve may be established by assuming that the curve $\frac{P_a(s)}{I'_a(s)}$ shall be of a smooth form. Lastly it may be mentioned that rather large modifications in the values of the function $P_a(s)$ have been found to produce very small alterations in the $\frac{\sigma_m(r)}{r}$ curve for $r > 1$ Å. For $r < 1$ Å, however, the form of the $\frac{\sigma_m(r)}{r}$ curve depends to some extent on the values of $P_a(s)$, but the part of the $\frac{\sigma_m(r)}{r}$ function between $r = 0$ and $r = 1$ Å. is of practically no consequense to the structure determination.

With the values of $\frac{P_t(s)}{P_a(s)}$ obtained in this way we may calculate the values of

$$\frac{I_m(s)}{K} s^5 = \left[\frac{P_t(s)}{P_a(s)} - 1\right]\left[\Sigma\left((Z_i - F_i)^2 + S_i\right)\right] s \tag{15}$$

if the functions F_i and S_i are known. These functions have been calculated by James and Brindly[14] and Bewilogua[15] for s values smaller than $s =$ ca 14.

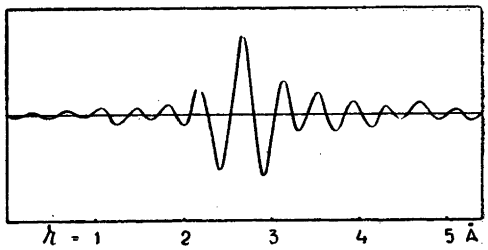

Fig. 6. Experimental σ_m (r) curve of a diatomic molecule.

For our purpose we have extended the calculations of the F_i and S_i functions to $s = 30$. These values will be published separately.

The experimental intensity curves may be obtained only for a limited interval of s between s_1 and s_2. This means that the function $\frac{\sigma_m(r)}{r}$ of eq. (12) has to be replaced by the function

$$\frac{\sigma_m(r)}{r} = \frac{2}{\pi}\int_{s_1}^{s_2} \frac{I_m(s)}{K}\, s^5 \sin sr\, ds \qquad (16)$$

The computation of this function is carried out in our laboratory with the help of sin sr tables, the intervals of which are $\Delta s = 0.25$ and $\Delta r = 0.0698$ Å. The limits s_1 and s_2 depend on experimental conditions and are generally $s_1 = 3$ and $s_2 = 20$ or 22 when the field accelerating the electron beam is about 33 K. V.

THE INTERPRETATION OF THE EXPERIMENTAL CURVES

Fig. 6 shows the $\sigma_m(r)$ curve of a diatomic molecule (I_2) determined experimentally from eq. (16). The difference between this curve and the theoretical curve $\sigma_m(r)$ in fig. 1 is rather remarkable. As pointed out by **Finbak**[2] this difference is undoubtedly caused by the fact that the experimental curve gives the integral from $s_1 = 6$ to $s_2 = 16.5$ while the curve of fig. 1 corresponds with the integral from $s_1 = 0$ to $s_2 = \infty$. The systematic errors thus introduced in the experimental $\sigma_m(r)$ curves may be called diffraction effects, as first proposed by **Bragg** and **West**[16] in the case of Fourier-analyses of X-ray diagrams from crystals. It should be pointed out, however, that in the case of electron diffraction this error is much more pronounced on account of the great influence of the nuclei on the scattering process.

The interpretation of the experimental $\sigma_m(r)$ curve may be based on the fact that every distance between two nuclei of the molecule gives a maximum

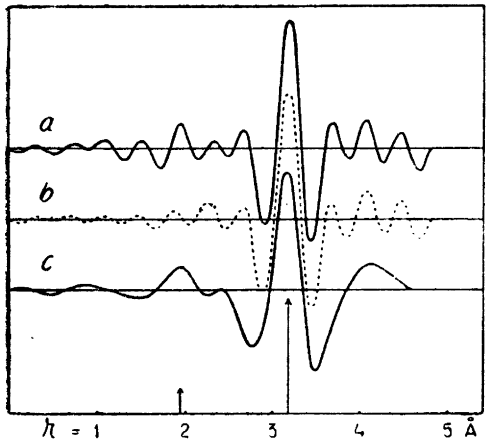

Fig. 7. (a) Experimental σ_m (r) curve of CBr_4. (b) Theoretical σ_m (r) curve with diffraction effects for the Br-Br distance. (c) Experimental σ (r) curve of CBr_4.

on the curve, the magnitude of which is approximately proportional to the product of the atomic number of the two nuclei. In addition to this maximum spurious undulations of period $\frac{2\pi}{s_2}$ are caused by the diffraction effects. Better results may be obtained by varying the limits s_1 and s_2 in the integral and thus altering the period of the diffraction error. In this way some molecular structures have been worked out on the basis of $\sigma_m(r)$ curves alone.

In the case of complicated molecules, however, the interpretation of the experimental $\sigma_m(r)$ curves very often seemed to be somewhat arbitrary because of the diffraction effects. Following a suggestion by Finbak [17], we may then introduce a new function $\sigma(r)$ defined by *

$$\sigma(r) = \frac{2}{\pi} r \int_{s_1}^{s_2} \frac{I_m(s)}{K} e^{-ks^2} s^5 \sin sr \, ds \qquad (17)$$

The exponential factor e^{-ks^2} has the same influence on our curves as an increasing thermal agitation and thus makes the diffraction effects less conspicuous. With suitable values of the constant k, we may compute $\sigma(r)$ curves the interpretation of which is more straightforward than that of the original $\sigma_m(r)$ curves. This may be seen from the fully drawn curves of fig. 7. Here the constant k was chosen equal to 0.018, and the integration carried out for the interval $s_1 = 4.5$ to $s_2 = 17$. The two r-values which correspond to inter-

* Originally this function was termed $\sigma_t(r)$ but for simplicity $\sigma(r)$ is now generally used.

atomic distances are indicated by arrows, the lengths of which are proportional to the theoretical heights of the maxima.

The influence of the diffraction effects on the $\sigma_m(r)$ curves may conveniently be studied by the use of so called »normal curves».[18] A combination of eqs. (7) and (16) gives

$$\frac{\sigma_m(r)}{r} = \Sigma\Sigma' \frac{2}{\pi R_{ij}} \int_{s_1}^{s_2} (Z_i - F_i)\,(Z_j - F_j)\,\sin sR_{ij}\,\sin sr\,ds \qquad (18)$$

which may be written

$$\frac{\sigma_m(r)}{r} = \Sigma\Sigma' \frac{1}{\pi R_{ij}} \left[\int_{s_1}^{s_2} (Z_i - F_i)\,(Z_j - F_j)\,\cos (R_{ij} - r)\,s\,ds - \int_{s_1}^{s_2} (Z_i - F_i)\,(Z_j - F_j)\,\cos (R_{ij} + r)\,s\,ds \right] \qquad (19)$$

The first integral in this equation has its greatest maximum at $r = R_{ij}$ and is symmetrical with respect to the line $r = R_{ij}$. For $r > 0$ the second integral has the same form as the first with $r < 0$. The form of the »normal-function»

$$\int_{s_1}^{s_2} \left(1 - \frac{F_i}{Z_i}\right)\left(1 - \frac{F_j}{Z_j}\right) \cos \varrho s\,ds \qquad (20)$$

for given limits s_1 and s_2 depends only slightly on the atomic numbers and will be discussed more thoroughly elsewhere [3]. If we use the function $\sigma(r)$ defined in eq. (17) instead of the $\sigma_m(r)$-function, the normal curves are given by

$$\int_{s_1}^{s_2} \left(1 - \frac{F_i}{Z_i}\right)\left(1 - \frac{F_j}{Z_j}\right) e^{-ks^2} \cos \varrho s\,ds \qquad (21)$$

With the help of the functions (20) or (21) theoretical $D(r)$, $\sigma_m(r)$, $\sigma(r)$ or $\frac{\sigma(r)}{r}$ curves including diffraction effects may be easily computed for any atomic distance and it is fairly simple to build up such curves for a whole molecule or parts of it. These curves should show the same diffraction errors as the corresponding experimental ones and may directly be compared with them. The dotted curve of fig. 7 gives an example of such a $\sigma_m(r)$ curve for the Br-Br distance. Besides the true maxima at $r = 3.1$ Å, the spurious ones are found in close agreement with the experimental curve.

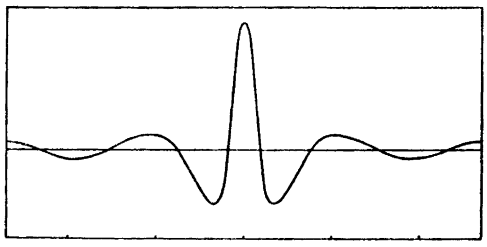

Fig. 8. Normal curve $\frac{\sigma(r)}{r}$ *with diffraction effects of a single interatomic distance.*

In our laboratory both the $\sigma_m(r)$ and the $\sigma(r)$-functions have been employed for the determination of molecular structures.[1] By the introduction of the exponential factor e^{-ks^2} the influence of the experimental errors has been reduced and in most cases a value of k equal to 0.01 was found to be suitable. Lately we have preferred to base our interpretation upon the $\frac{\sigma(r)}{r}$ curves alone, as this function has the most convenient form for the application of normal curves. Fig. 8 gives the normal curve of eq. (21) when the values of the constants are $k = 0.01$ $s_1 = 4$ and $s_2 = 20$. This curve corresponds closely with the $\frac{\sigma(r)}{r}$ curve of a single interatomic distance, and may be compared with the $\frac{\sigma(r)}{r}$ curves which have been worked out from the experimental data.

Some modifications of the interpretation technique which have been used in our laboratory should also be mentioned. When the $\sigma(r)$ function was introduced[18] it was pointed out that this function for a given system may be split into two or more different parts. Each of these parts gives information concerning smaller parts of the scattering system. According to eq. (19) the functions $\frac{\sigma_m(r)}{r}$ or $\frac{\sigma(r)}{r}$ may be written as a sum of functions each belonging to a single interatomic distance. Upon these facts various types of differential methods have been based.

If one or more interatomic distances are common to two molecules, the experimental $\sigma_m(r)$, $\frac{\sigma_m(r)}{r}$, $\sigma(r)$ or $\frac{\sigma(r)}{r}$ curves may be determined for both substances. The difference between the two sets of experimental curves may then give more information about other distances which are not common to these molecules. Sometimes the difference may be taken between the experimental curve and a theoretical curve including diffraction effects for a

certain number of distances in the molecule.[13] In both cases the difference curve will be less influenced by the diffraction effects and therefore yields more accurate information about the structure. In many cases the differential methods have made possible the determination of rather fine details in the molecular structures as will be seen from examples given in the following paper.

It must be emphasized that the methods described here demand intensity measurements of high accuracy. These may be obtained by the sector method described. Our experience is that these methods can give better results than any of those based on visually estimated intensities. The best justification of our methods seems to be satisfactory agreement between the experimental curves and the theoretical curves for which diffraction effects have been taken into consideration.

SUMMARY

The determination of molecular structures by use of the sector method is discussed and the different forms of distribution functions introduced by Fourier analysis are compared to each other. Because of the diffraction effects, the interpretation of the experimental distribution curves for complicated molecules seems to be somewhat arbitrary. By the use of normal curves, theoretical curves including the diffraction effects may be obtained for any atomic distance. These curves may then be compared directly with the experimental ones when the structures are to be determined. For the determination of fine details in the structure different types of differential method are introduced. The structures of about 40 molecules obtained by the sector method are given in a subsequent paper.[1]

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Über eine Methode zur Abspaltung von Carboxalkyl aus β-Keto- und β-Dicarbonsäureestern

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Die Ketonspaltung (1) von β-Ketocarbonsäureestern

$$R \cdot CO \cdot CH_2 \cdot COOR' \begin{cases} + H_2O \rightarrow R \cdot CO \cdot CH_3 + CO_2 + R'OH & (1) \\ + 2H_2O \rightarrow R \cdot COOH + CH_3 \cdot COOH + R'OH & (2) \end{cases}$$

wird, wie bekannt, gewöhnlich mittels Erhitzen derselben mit verdünnten Alkalien oder Säuren ausgeführt. Erhitzen mit konzentrierten Alkalien verursacht dagegen die Säurespaltung (2).

Schon seit längerer Zeit liegen aber Beobachtungen vor, dass die Ketonspaltung auch durch alleiniges Erhitzen mit Wasser erzielt wird. Nach Bonné[1] wird α-Benzoylacetessigester durch siedendes Wasser unter lebhafter Entwicklung von Kohlendioxyd zersetzt. Etwas später wurde gefunden[2], dass dabei auch Benzoylaceton gebildet wird. Ähnliche Beobachtungen haben auch andere Forscher gemacht, z. B. beim Kochen von Benzoylessigester mit Wasser Bildung von Acetophenon[3]; beim Erhitzen von α-Acetonylacetessigester mit Wasser im zugeschmolzenen Rohr bei 160° Bildung von Acetonylaceton[4]. Das Erhitzen mit Wasser im Rohr bei 140—150° haben Bouveault und Bongert[5] als eine allgemeine Methode zur Darstellung von Acylacetonen aus C-Acylacetessigestern benutzt; die Acylacetone werden dabei nicht, wie bei Anwendung von verdünnten Alkalien oder Säuren, weiter gespalten.

Meerwein[6] hat gezeigt, dass die durch Erhitzen mit Wasser erfolgende Ketonspaltung von β-Ketosäureestern einer noch allgemeineren Anwendung

fähig ist. Er hat verschiedene β-Ketosäureester mit dem halben bis gleichen Volumen Wasser im zugeschmolzenen Rohr kurze Zeit auf 200° bzw. 250° erhitzt und festgestellt, dass unter diesen Bedingungen nur die enolisierungsfähigen β-Ketosäureester der Ketonspaltung unterliegen, während die nicht enolisierbaren Verbindungen oder Gruppierungen vom Typus der Dialkylacetessigester unverändert bleiben. Die Ketonspaltung erfolgt umso leichter, je grösser die Enolisierungstendenz bzw. die Acidität des β-Ketosäureesters ist. So unterliegen der Ketonspaltung am leichtesten die cyclischen, schwerer die nicht alkylierten acyclischen, am schwersten die substituierten acyclischen β-Ketosäureester. Die Säurespaltung (2) wurde in den Meerweinschen Versuchen nur bei den acyclischen β-Ketosäureestern, und zwar in nur sehr geringem Grade (0,4—1,5 %), beobachtet. — Was den Verlauf der Ketonspaltungsreaktion betrifft, ist Meerwein der Ansicht, dass es sich hier nicht um eine einfache Verseifung und darauffolgende CO_2-Abspaltung handelt; nach ihm ist die Annahme am besten begründet, dass der Hydrolyse diejenige Enolform des β-Ketosäureesters anheimfällt, in der der Ester nach der Carboxalkylgruppe hin enolisiert worden ist:

$$R \cdot CO \cdot CH = C\begin{matrix} H_2 \quad O \diagup OH \\ \diagdown OR' \end{matrix} \longrightarrow R \cdot CO \cdot CH_3 + CO_2 + R'OH.$$

Als eine Ergänzung zu der obigen Untersuchung sei die Feststellung von Connor und Adkins [7] erwähnt, dass auch der Dimethylacetessigester, obwohl sehr langsam, der Ketonspaltung durch Wasser unterliegt. Nach 12-stündigem Erhitzen mit 10 Mol. Wasser in einem besonders konstruierten Apparat bei 250° waren aus diesem Ester 50 % Methylisopropylketon gebildet worden (keine Säurespaltung). Dagegen wurde der Diäthylacetessigester unter etwa denselben Bedingungen nicht gespalten, was nach diesen Autoren auf der grösseren sterischen Hinderung beruht.

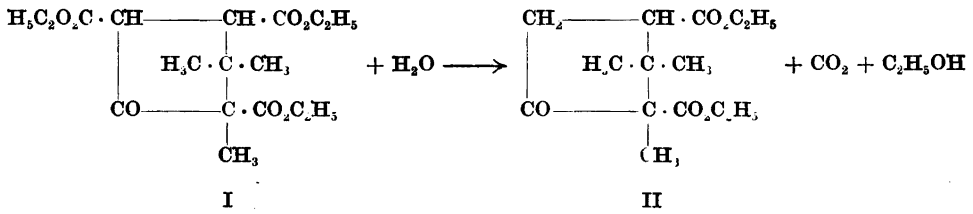
Das Erhitzen mit Wasser in geschlossenen Gefässen ist jedoch ein ziemlich unbequemes Verfahren zur Abspaltung des Carboxalkyls, besonders bei Behandlung grösserer Substanzmengen oder wenn man den Verlauf der Reaktion näher verfolgen will. Ich beabsichtigte deshalb, ein Verfahren zu entwickeln, mittels dessen man die Reaktion der β-Ketoester mit Wasser in offenen Gefässen ausführen könnte. Am zweckmässigsten schien es, das Erhitzen zusammen mit einer solchen Substanz auszuführen, die noch bei ziemlich hohen Temperaturen etwas Wasser zurückhält. Die Erwägung führte zu Glycerin, das bei den in Frage kommenden Temperaturen folgende Mengen Wasser zurückhält [8]:

Siedepunkt $_{760mm}$:	290°	244°	225°	196°	179°	168°	161°	156°	145,5°	137,5°	126,8°
Wassergehalt (%):	0	0,5	1	2	3	4	5	6	8	10	15

Es war auch anzunehmen, dass die wenigstens partielle Auflösung der zu behandelnden Ester in dem heissen Glycerin das Reagieren derselben mit Wasser seinerseits befördere. Die jedesmal erforderliche Quantität des Glycerins würde davon abhängig sein, welche Temperatur zur Zersetzung des Esters mit mässiger Geschwindigkeit erforderlich wäre und wieviel Wasser das bei dieser Temperatur siedende Glycerin enthält. Es musste auch möglich werden, den Verlauf der Reaktion durch Auffangen des Kohlendioxyds in einem Messrohr zu verfolgen.

Das oben skizzierte Verfahren wurde für präparative Zwecke im Zusammenhang mit meiner Camphersäuresynthese ausgebildet. Darüber wie auch über die Synthese im ganzen wurde seinerzeit nur kurz berichtet[9], weshalb Anlass vorliegt, das Verfahren etwas vollständiger zu beschreiben.

Um den 4-Carbäthoxy-5-Ketocamphersäurediäthylester (I) in 5-Ketocamphersäurediäthylester (II) überzuführen



I II

wurde folgendermassen verfahren.

In einem langhalsigen Rundkolben (500 ml), der mit einem Thermometer, mit einem etwa 20 cm hohen Steigrohr und mit einem mit dem letzteren verbundenen Kühler versehen war, wurde aus 300 g Glycerin (spez.Gew. 1,23; Siedep. 127°) so viel Wasser abdestilliert, dass der Siedepunkt (Thermometer in der Flüssigkeit) bis auf 160° stieg (Wassergehalt dann etwa 5 %). Jetzt wurden 171 g der Verbindung I zugefügt und mit dem Erhitzen fortgesetzt. Der Ester löste sich nicht wahrnehmbar in dem Glycerin, sondern bildete eine Schicht oberhalb desselben. Bei etwa 170° war eine deutliche Reaktion bemerkbar; es entwich Kohlendioxyd, und Alkohol samt etwas Wasser destillierten ab. Während einer Stunde wurde die Temperatur allmählich bis auf 200° gesteigert. — War die Ferrichloridreaktion des Esters noch nicht völlig verschwunden, liess man etwas erkalten, setzte einige ml Wasser hinzu und erhitzte von neuem wie oben. Gewöhnlich war dies nicht erforderlich, sondern die in dem Glycerin enthaltene Menge Wasser (13,5 g oder 1,5 Mol. auf 1 Mol. Ester) war genügend, um die Reaktion vollständig zu machen.

Als das Glycerin nach dem Erkalten mit Wasser verdünnt wurde, war die Lösung nur sehr schwach sauer. Das Reaktionsprodukt wurde in Äther aufgenommen, mit

verdünnter Sodalösung gewaschen und mit Calciumchlorid getrocknet. Durch Destillation des Ätherrückstandes im Vacuum wurde der Ketocamphersäureester (II, ein Gemisch von beiden Stereoisomeren) in guter Ausbeute erhalten. Eine kleine Vorfraktion bestand aus Ketomonocarbonsäureester, der durch Abspaltung auch der an dem tertiären Kohlenstoffatom haftenden Carbäthoxylgruppe entstanden war. Saure (sodalösliche) Reaktionsprodukte waren nur in sehr kleiner Menge gebildet worden. Die entstandenen Mengen Alkohol und Kohlendioxyd stimmten ziemlich genau mit den berechneten überein.

Das selbe Verfahren wurde später bei der Synthese der 1-Methylnor-Camphersäure [10] zur Abspaltung der Carbäthoxylgruppe aus dem 4-Carbäthoxy-5-keto-1-methylnorcamphersäurediäthylester benutzt und ist auch zu anderen präparativen Zwecken benutzt worden.

Später ist das Verfahren bezüglich der Apparatur so modifiziert worden, dass man es zur quantitativen Verfolgung der Ketonspaltungsreaktion benutzen kann. Zu diesem Zweck haben wir die folgende Apparatur (Fig. 1) benutzt.

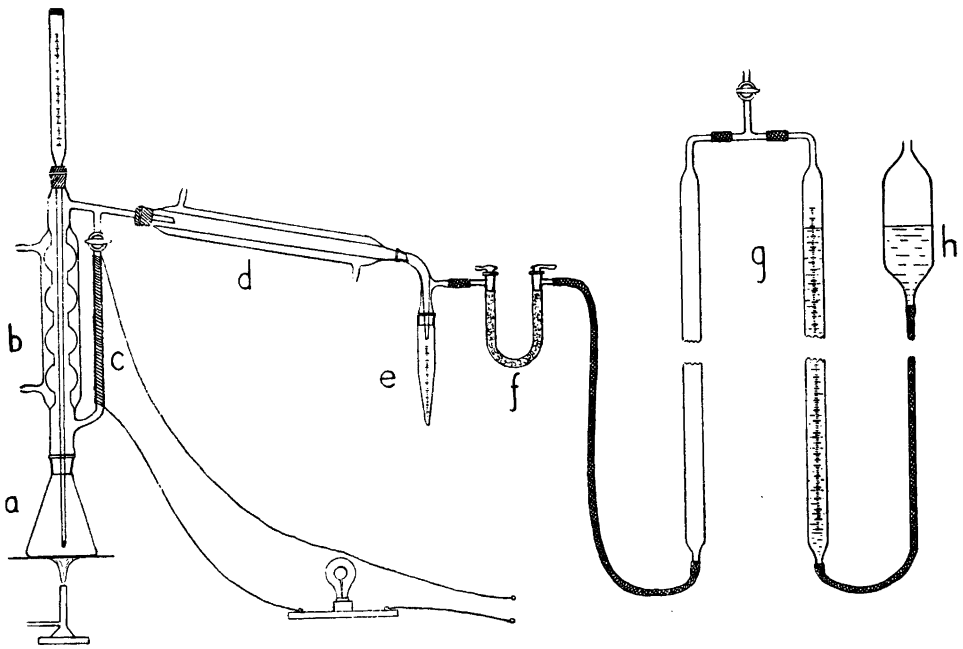


Fig. 1. Apparatur zur quantitativen Verfolgung der Ketonspaltungsreaktion.

Ein Gemisch von dem zu untersuchenden Ester und wasserhaltigem Glycerin wird in einem Kolben (a) erhitzt. Der Kolben, dessen Inhalt zweckmässig 50 oder 100 ml beträgt, ist mit einem Rückflusskühler (b) und einem Thermometer versehen. Mit dem

Rückflusskühler ist ein seitliches Hahnrohr (c) verschmolzen; durch umgewickelten Widerstandsdraht kann dessen Temperatur bei 70—80° gehalten werden. Durch dieses Rohr kann man den Alkohol und andere niedrig siedende Stoffe, die sonst den Siedepunkt des Glycerins herabsetzen würden, direkt zum Kühler (d) und Messrohr (e) austreten lassen. Auf diese Weise kann die Temperatur des Reaktionsgemisches leicht mit einer Genauigkeit von etwa 1° geregelt werden.

Das gebildete Kohlendioxyd wird durch vorher mit Kohlendioxyd gesättigtes Calciumchlorid (f) geleitet. Seine Menge wird in einem Messapparat (g) bestimmt. Das zweite, mit einer Messkala (0—500 ml) versehene Rohr dieses Apparats ist durch einen Gummischlauch mit einem beweglichen Flüssigkeitsbehälter (h) verbunden. Als Sperrflüssigkeit dient z. B. Diäthylphthalat, das nur wenig Kohlendioxyd absorbiert. Vor der Benutzung wird es mit Kohlendioxyd gesättigt und nach jeder Operation wird das Kohlendioxyd in dem Messapparat zurückgelassen, bis es kurz vor dem Beginn einer neuen Operation abgelassen wird.

Bei einem Versuch (s.Tabelle 1) wurden angewandt: 2,416 g (18,58 Millimole) Acetessigester und 67,605 g Glycerin, Siedep. 190°, Wassergehalt 2,25 %. Also Wasser 1,521 g (84,5 Millimole oder 4,55 Mole auf 1 Mol Acetessigester).

Bei Beginn der Operation liest man den Barometerstand und die Zimmertemperatur ab. Die Sperrflüssigkeit wird auf den Nullstrich eingestellt und der bewegliche Behälter in dem Masse gesenkt, wie das Volumen des Kohlendioxyds in dem Messrohr zunimmt. Nach dem Abstellen der Heizung lässt man die Apparatur erkalten und liest darauf den derzeitigen Stand der Sperrflüssigkeit ab. Die Differenz zwischen diesem und dem beim Abschluss des Erhitzens abgelesenen wird von allen während der Operation abgelesenen Werten abgezogen. Auch die Temperatur und der Barometerstand werden von neuem abgelesen.

Bisher haben wir mehrere genaue Bestimmungen nach dieser Methode in der Hauptsache nur mit Acetessigester als Untersuchungsobjekt ausgeführt. Er wurde in Glycerin-Wasser-Gemischen, deren Siedepunkte 190°, 200°, 210° und 220° betrugen, erhitzt. Diese wurden durch Zusatz von entsprechenden Mengen Wasser zu reinem, wasserfreiem Glycerin hergestellt. Die Menge des Wassers betrug etwa 3,5—4,5 Mol. auf 1 Mol. Ester. Die Erhitzungstemperatur wurde etwa 10° unterhalb des Siedepunktes des Glycerins gehalten.

Für die Reaktion wurde auch die Geschwindigkeitskonstante nach der üblichen Gleichung für bimolekulare Reaktionen berechnet (s. Tabelle). Die Konzentrationen sind dabei in Molen pro 1000 g wasserfreies Glycerin und die Zeit in Sekunden berechnet. Im folgenden seien diese Konstanten für einige bei verschiedenen Temperaturen ausgeführten Reaktionen wiedergegeben:

$k_{179°} \cdot 10^3 = 0{,}82$ $\quad$ $k_{197°} \cdot 10^3 = 3{,}12$

$k_{182°} \cdot 10^3 = 1{,}05$ $\quad$ $k_{205-206°} \cdot 10^3 = 4{,}79$

$k_{193°} \cdot 10^3 = 2{,}50$ $\quad$ $k_{208°} \cdot 10^3 = 5{,}49$

Tabelle 1. Quantitative Verfolgung der Ketonspaltung des Acetessigesters.

	Zeit Min.	Temp. °C	Volumen ml	Kohlendioxyd ml	Kohlendioxyd % d. Th.	$k \cdot 10^3$	
Der Ester löste	0	22	0	—	—		
sich bei 180°	3	105	30	—	—		
(5,35 Min.),	6	184	79	—	—		
Beginn der	8	182	137	55	12,1		
Reaktion.	10	181	191	109	24,1	0,98	
	12,5	182	251	169	37,2	1,03	
	15	182	299	217	47,8	1,04	$k_{182°} \cdot 10^3 = 1{,}05$ (im Mittel)
	17,5	182	339	257	56,6	1,04	
	20	182	371	289	63,6	1,05	
	25	182	419	337	74,3	1,05	
	30	182	454	372	82,0	1,06	
	35	183	478	396	87,3	1,07	
Nach dem Er-	40	184	493	411	90,5	1,05	
kalten		21	411				

Barometerstand 750,0 mm. Kohlendioxyd theor. 454 ml (unter den Versuchsbedingungen).

Auf die obenbeschriebene Weise haben wir das Verhalten einer Vielzahl verschiedener, sowohl acyclischer wie cyclischer β-Ketocarbonsäureester und auch β-Dicarbonsäureester beim Erhitzen in Glycerin-Wasser-Gemischen untersucht. Über die Ergebnisse dieser Untersuchungen sei hier nur erwähnt, dass sie in der Hauptsache mit den Versuchsergebnissen von Meerwein [6] sowie von Connor und Adkins [7] übereinstimmen. Die Unterschiede in der Zersetzungsgeschwindigkeit verschiedener Verbindungen sind aber mittels unserer Methode viel schärfer zum Vorschein zu bringen als nach den früheren Methoden. Unserer Erfahrung gemäss bietet diese Methode eine Möglichkeit für die Lösung mehrerer interessanter Fragen; so z. B. den Verlauf dieser Ketonspaltungsreaktion. Die obenerwähnte Annahme von Meerwein über den Verlauf lässt sich ja nicht mit der Spaltung der α-dialkylierten β-Ketosäureester vereinbaren.

Da jedoch u. a. die Reaktionsgeschwindigkeitskonstanten für mehrere Verbindungen noch nicht unter hinreichend übereinstimmenden Bedingungen (Temperatur, Wassergehalt) bestimmt worden sind, wollen wir die bisher

gewonnenen Resultate erst später näher beschreiben. Die kinetischen Untersuchungen mittels dieser Methode möchten wir uns für einige Zeit vorbehalten.

ZUSAMMENFASSUNG

Es wird eine Methode beschrieben, mit deren Hilfe man aus β-Ketocarbonsäureestern und β-Dicarbonsäureestern durch Erhitzen mit wasserhaltigem Glycerin in offenen Gefässen eine Carboxalkylgruppe hydrolytisch abspalten kann. Die Methode kann sowohl zu präparativen als auch, durch Anwendung einer Apparatur zur Messung des gebildeten Kohlendioxyds, zu kinetischen Zwecken benutzt werden.

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On the Chemical Nature of Plasteins

Preliminary Communication

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The chemical nature of the much discussed plasteins is illustrated by the following observations.

The egg albumen was hydrolyzed by crystalline pepsin at 37° C. The average molecular weight of the products of hydrolyzation was 233, determined by the diffusion method. The filtered clear hydrolysate was heated for half an hour in a water-bath. After cooling NaOH-solution was added to raise the pH of the hydrolysate to 4.1. After that crystalline pepsin was added to the solution which was placed at 37°. The amino groups were determined during the experiment according to Van Slyke and the carboxyl groups by the formol titration of Sörensen. The both groups decreased parallelly in about 2—4 h. Depending on the concentration of the hydrolysate and on the amount of pepsin the decrease in the amino groups was as follows (table 1).

Table 1. The decrease in the amino groups through the influence of crystalline pepsin on the hydrolysates of egg albumen at pH 4.1.

No. of expt.	Time of reaction	Decrease in amino groups	
	h	%	
1	4	about 25	(167 mg pepsin per 1620 mg hydrolysate-N in 100 ml)
2	4	» 45	(167 » » » 2940 » » » 100 »)
3	2	» 50	(167 » » » 4840 » » » 100 »)

The quantity of plastein precipitated corresponded approximately to the decrease in amino nitrogen. The molecular weights of three different preparations of separated plastein, determined cryoscopically in a phenol solution were 319, 300, and 267. Since the plastein which after determination was separated from the phenol solution was as difficultly soluble in water and equally hydrolysable in 0.1 *N* HCl-solution as it had been at the start it had evidently remained unchanged when dissolved in phenol. The

molecular weight sufficed to indicate that no high-molecular peptide, still less protein, could be concerned. — Svedberg observed with the plastein preparation of Folley[1] that no sedimentation took place in the ultracentrifuge and that accordingly, an upper limit 1 000 could be given for the molecular weight. Collier,[2] on the other hand, recently recorded a sedimentation in the ultracentrifuge when the plastein was dissolved in urea solution. The precipitate was inhomogeneous but contained particles of the size of protein molecules. He ascribes Svedberg's result to the decomposition of plasteins in alkaline phosphate solution.

Our plastein preparations contained about 13.6 % nitrogen. A 5 minutes' shaking after total hydrolysis yielded about 74 % amino-N of total N.

After hydrolysis with pepsin *plus* 0.1 *N* HCl at 30° the plastein hydrolysates were found to contain 20.9 % amino-N of total N (5 min. shaking). A 30 min. shaking yielded 38 % amino-N, copper method 37.5 %. The reason for the exceptionally high discrepancy between the results of 5 min. and 30 min. shakings is still unknown. A hydrolysis with HCl without pepsin gave 21.2 % amino-N of total N (5 min. shaking). Accordingly, plastein is decomposed at pH 1—2 as far in the absence of pepsin as in the presence of it.

The results obtained show that plasteins are substances of lower molecular weight than has been assumed. Since the substance is very difficultly soluble in water, at any rate within the region of pH 4—8, and since free amino groups are formed on dissolution at pH 1—2, it seems probable that peptides of ring structure are in question. Variations in the molecular weight of different preparations of plastein suggest a mixture of ring peptides, on the average of the size of di-tripeptides. As the formation of plastein occurs very rapidly it may be assumed that some plasteins are also formed in the digestive tract, the pH being higher when the contents of the stomach pass to the small intestine. — The investigations are continued.

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Zur Wirkungsweise der Hydrolasen

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Um die Wirkung der hydrolysierenden Enzyme auf ihre Substrate und speziell die Abhängigkeit der Reaktionsgeschwindigkeit von der Substratkonzentration zu erklären, haben Michaelis und Menten [1] angenommen, dass Enzym und Substrat eine Verbindung bilden, die mit den freien Komponenten im Gleichgewicht steht. Bezeichnen wir die Substratkonzentration mit [S], die totale Enzymkonzentration mit [E] und mit [ES] die Konzentration de Enzymsubstratverbindung, so ist

$$\frac{([E] - [ES]) \cdot [S]}{[ES]} = K_m \tag{1}$$

oder

$$\text{Relative Geschwindigkeit} = \frac{[ES]}{[E]} = \frac{1}{1 + \frac{K_m}{[S]}} \tag{2}$$

Die Gleichgewichtskonstante K_m wurde später oft die Michaelis-Konstante genannt.

Diese Betrachtungsweise hat sich in vielen Fällen als sehr nützlich erwiesen. Besonders hat dabei das rohrzuckerspaltende Enzym, die Saccharase, als Objekt gedient. Es hat sich gezeigt, dass die einfache Gleichung (1) oft durch kompliziertere Ausdrücke ersetzt werden muss. Besonders gilt dies für den Fall, dass die Untersuchungen über grössere pH-Gebiete ausgedehnt werden. In der Gleichung (1) rechnet man offenbar damit, dass [E] für alle Versuche mit einer gewissen Enzymmenge konstant ist. Es ist aber durchaus nicht sicher, dass dies zutrifft. Bekanntlich haben Michaelis und Davidsohn [2] die Annahme eingeführt, dass die pH-Kurven der Enzyme durch die

amphotere Natur derselben bestimmt werden. Speziell wurde angenommen, dass der alkalische Ast der pH-Kurve der Saccharase die Dissoziationsrestkurve einer schwachen Säure mit K_a = etwa $10^{-6,6}$ ist. Bezeichen wir das als Säure aufgefasste Enzym mit [HE], so sollte der alkalische Ast der pH-Kurve durch die Formel

$$\frac{[H^+] \cdot [E^-]}{[HE]} = 10^{-6,6} \tag{3}$$

dargestellt werden können. Nachdem dann die Annahme der Enzymsubstratverbindung durch Michaelis und Menten eingeführt wurde, haben Michaelis und Rothstein [3] die Theorie der pH-Kurve dahin abgeändert, dass die Enzymsubstratverbindung eine Säure mit der erwähnten Dissoziationskonstante sein soll. Kuhn [4] hat aber darauf hingewiesen, dass, wenn der nicht dissoziierte Teil des Enzyms (HE) das Substrat bindet und die Enzymsubstratverbindung eine Säure mit $K_a = 10^{-6,6}$ ist, eine Verschiebung des alkalischen Astes der pH-Kurve mit [S] erwartet werden muss. Von mehreren Seiten ist aber gezeigt worden, dass keine solche Verschiebung eintritt. Kuhn hat daraus den Schluss gezogen, dass die Theorie von Michaelis überhaupt falsch ist. Dies braucht indessen gar nicht der Fall zu sein. Wenn das Enzym ein Ampholyt mit gewissen K_a- bzw. K_b-Werten ist, das dank spezieller substratbindender Gruppen Rohrzucker binden kann, so ist nicht einzusehen, warum die Anlagerung des Rohrzuckers den K_a-Wert des Enzyms beeinflussen sollte. Vielmehr ist anzunehmen, dass sowohl Enzym wie Enzymsubstratverbindung Säuren mit derselben Dissoziationskonstante $K_a = 10^{-6,6}$ sind. [5] Statt der obigen Formeln (1—3) erhalten wir dann:

$$\frac{[HE] \cdot [S]}{[HES]} = \frac{[E^-] \cdot [S]}{[ES^-]} = K_m$$

und

$$\frac{[H^+] \cdot [ES^-]}{[HES]} = \frac{[H^+] \cdot [E^-]}{[HE]} = K_a$$

Wenn Σ die totale Enzymkonzentration bedeutet, so ist

$$\Sigma = [HES] + [ES^-] + [HE] + [E^-]$$

und man findet, unter der Annahme, dass nur die Molekülart HES zerfällt, für die relative Reaktionsgeschwindigkeit den Ausdruck

$$\text{Relative Aktivität} = \frac{[HES]}{\Sigma} = \frac{1}{(1 + K_m/[S])\,(1 + K_a/[H^+])} \qquad (4)$$

Aus dieser Formel geht hervor, dass, wenn K_m in dem untersuchten Aziditätsgebiet konstant ist, keine Verschiebung des alkalischen Astes der pH-Kurve eintritt.

Anders liegen aber die Verhältnisse im sauren Teil des Aziditätsgebietes; das pH-Optimum der Saccharase wird mit steigendem [S] breiter, indem sich der saure Ast der Kurve nach der sauren Seite verschiebt. Inaktivierungsversuche sprechen dafür, dass die Bindung des Rohrzuckers an das Enzym durch primäre Aminogruppen des Enzyms vermittelt wird, die auch für die basische Dissoziation des Enzyms verantwortlich sind.[6] Die Verschiebung der pH-Kurve mit [S] im sauren Gebiet (pH < 4) muss also gedeutet werden als die Folge einer Konkurrenz der H^+-Ionen und des Rohrzuckers um die Aminogruppen des Enzyms. Anders ausgedrückt: Rohrzucker wird an die NH_2-Gruppen, nicht aber an NH^+_3-Gruppen gebunden. (Als Analogon kann die Bildung der Schiffschen Basen herangezogen werden.) Bezeichnen wir das nicht dissoziierte Enzymmolekyl mit $HENH_2$, so erhalten wir die Gleichungen

$$\frac{([HENH_2] + [^-ENH_2]) \cdot [S]}{[HENH_2S] + [^-ENH_2S]} = K_m$$

$$\frac{([HENH^+_3] + [^-ENH^+_3]) \cdot [OH^-]}{[HENH_2] + [^-ENH_2]} = K_b$$

$$\frac{([^-ENH_2S] + [^-ENH_2] + [^-ENH^+_3]) \cdot [H^+]}{[HENH_2S] + [HENH_2] + [HENH^+_3]} = K_a$$

$$\Sigma = [HENH_2S] + [^-ENH_2S] + [HENH_2] + [^-ENH_2] + [HENH^+_3] + [^-ENH^+_3]$$

und daraus durch Elimination

$$\text{Relative Aktivität} = \frac{[HENH_2S]}{\Sigma} = \frac{1}{(1 + K_a/[H^+])\,[(1 + K_m/[S]\,(1 + K_b/[OH^-])} \qquad (5)$$

Für die Saccharase gibt diese Formel die Abhängigkeit der Aktivität des Enzyms von der Azidität und der Substratkonzentration im ganzen untersuchten Gebiet sehr gut wieder. Vergleicht man die Formel mit der einfachen Formel (2), so sieht man leicht ein, dass, wenn man von der Änderung des Enzyms

mit dem pH absieht und mit der Formel (2) rechnet, der K_m-Wert scheinbar mit der Azidität variiert.

In einer ganz anderen Weise betrachtet van Slyke [7] das Problem der Hydrolasenwirkung: Die für den Umsatz eines Substratmoleküls nötige Zeit t ist zusammengesetzt aus a) der für den Zerfall eines Enzymsubstratmoleküls in die Reaktionsprodukte und freies Enzym nötigen Zeit t_1 und b) der Zeit t_2 die verstreicht, bis ein neues Substratmolekül die substratbindenden Gruppen des Enzyms trifft und dort gebunden wird. Die Zeit t_2 muss der Substratkonzentration [S] umgekehrt proportional sein ($t_2 = 1/K_C \cdot [S]$), t_1 ist dagegen von der Substratkonzentration unabhängig ($t_1 = 1/K_D$). Für die Reaktionsgeschwindigkeit erhält man dann den Ausdruck

$$-\frac{dS}{dt} = \frac{1}{\frac{1}{K_C \cdot [S]} + \frac{1}{K_D}} \tag{6}$$

Die Geschwindigkeiten der beiden Reaktionen sollen nun nach van Slyke in verschiedener Weise vom pH beeinflusst werden: Die Geschwindigkeit der Bindung des Substrates an das Enzym soll in umgekehrter Proportion zur $[H^+]$ variieren, während die Zerfallsgeschwindigkeit der Enzymsubstratverbindung in neutraler Lösung am grössten sein soll.

Da die Michaelis-Konstante K_m, wie aus der Formel (2) hervorgeht, gleich dem Wert derjenigen Substratkonzentration ist, bei welcher das Enzym die halbe maximale Aktivität zeigt, so muss für diesen Fall in der Formel (6) gelten, dass $K_C \cdot [S] = K_D$ ist. van Slyke hebt hervor, dass bei Variationen von K_C, die nach seiner Auffassung z. B. durch Veränderung der Azidität verursacht sein können, der K_m-Wert umgekehrt mit K_C variieren muss. Die Michaelis-Konstante ist nach van Slyke wertvoll, kann aber nur für definiierte Bedingungen eine Konstante genannt werden. Van Slyke führt an, dass für Urease eine pH-Verschiebung um eine Einheit die Michaelis-Konstante 11-fach vergrössert. [8]

Es muss aber festgestellt werden, dass van Slyke bei diesen Überlegungen stillschweigend voraussetzt, dass sich die Zahl der substratbindenden Gruppen des Enzyms bei Variation der Azidität nicht ändert, eine Annahme, die kaum zulässig erscheint, da die Urease zweifelsohne ein amphoterer Eiweisstoff mit solchen Säure- und Basenkonstanten ist, dass sich ihr Dissoziationszustand in dem infragekommenden pH-Gebiet stark ändern muss. Nach Ansicht des Verfassers kann K_C (Formel (6)) an sich nicht mit der Azidität variieren; die Änderung des K_C-Wertes wird dadurch vorgetäuscht, dass sich die Anzahl der substratbindenden Gruppen ändert. Im Folgenden soll deshalb untersucht

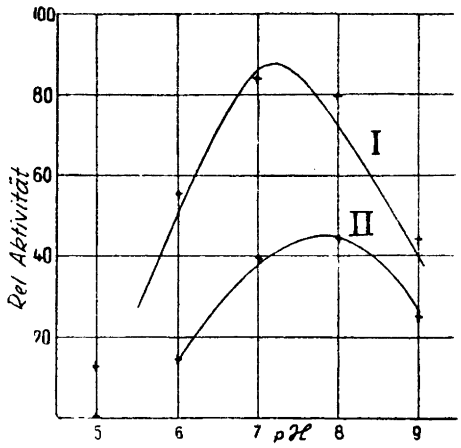

Fig. 1. Aktivitäts-pH-Kurven der Urease; Kurve I : [S] = 0,150 M: *Kurve II : [S] = 0,020* M *Harnstoff.*

werden, ob die von van Slyke gefundenen Verhältnisse von diesem Gesichtspunkt aus gedeutet werden können.

Als Beispiel sollen die von van Slyke gegebenen zwei Aktivitäts-pH-Kurven der Urease für [S] = 0,02 bzw. [S] = 0,150 *M* Harnstoff angeführt werden (ausgezogene Kurven in der Abbildung 1). Es fällt sofort auf, dass die durch die zwei Kurven veranschaulichten Verhältnisse denen bei der Saccharase ähnlich sind. Die alkalischen Äste der Kurven (pH > 8) geben den Eindruck, dass die Gruppe(n) des Enzyms, die seine Säurenatur bedingen, nicht für die Bindung des Substrats, wohl aber für die Zerfallsgeschwindigkeit der Enzymsubstratverbindung eine Rolle spielen. In Lösungen saurer als pH = 8 tritt dagegen eine Verschiebung der Kurven (und dadurch eine Verschiebung des pH-Optimums) mit steigender Substratkonzentration deutlich hervor. Zu diesem Resultat ist auch Lövgren[9] gelangt. Die Kurven erwecken also hier wie bei der Saccharase den Eindruck, dass die Gruppe(n), die die basischen Eigenschaften des Enzyms bestimmen, auch bei der Bindung des Substrats wirksam sind. Nimmt man an, dass die isoelektrische Urease aus Molekülen vom Typus $HENH_2$ besteht (wobei $-NH_2$ nur als Symbol einer basischen Gruppe aufzufassen ist), so deuten die Kurven darauf, dass einerseits die Salzbildung des Enzyms in saurer Lösung die Bindung des Substrats verhindert, dass aber andererseits die Dissoziation der sauren Gruppe(n) des Enzyms zwar mit der Anlagerung des Harnstoffs nichts zu tun hat, wohl aber den Zerfall der Enzymsubstratverbindung in Enzym und Reaktionsprodukte verhindert. Wir werden deshalb, am Beispiel der von van Slyke gegebenen Kurven, die Anwendung der für die Saccharase entwickelten Formel (5) auf die Urease versuchen.

Wenn wir also annehmen, dass der alkalische Ast der Kurven durch die saure Dissoziation des Enzyms bzw. der Enzymsubstratverbindung bedingt ist, so lässt sich der Abbildung entnehmen, dass K_a recht genau $= 10^{-9}$ sein muss. Da die Kurve für die hohe Substratkonzentration 0,150 M, die nicht sehr weit von der theoretischen Kurve für unendlich hohe Substratkonzentration liegen kann, um die Koordinate durch pH = 7,25 symmetrisch ist, so muss, wenn der saure Ast der Kurven durch die basische Dissoziation des Enzyms bestimmt wird, K_b = etwa $5 \cdot 10^{-8}$ sein. Merkwürdigerweise haben van Slyke und Zacharias[10] hervorgehoben, dass die pH-Kurve der Urease der Dissoziationsrestkurve eines Ampholyten mit fast gleichen Konstanten der sauren und basischen Dissoziation weitgehend ähnlich ist, ohne den naheliegenden Schluss zu ziehen, dass die Dissoziation die Anzahl der substratbindenden Stellen des Enzyms beeinflussen muss.

Aus älteren Untersuchungen, wie z. B. derjenigen von Lövgren ist ersichtlich, dass die Aktivität der Urease in 0,150 M Harnstoff nicht weit von der maximalen liegen kann. Schätzungsweise kann für die relative Aktivität beim pH-Optimum und [S] = 0,150 der Wert 0,88 angegeben werden. Man findet dann, dass K_m etwa 0,02 sein muss.

Wenn die Werte $K_a = 10^{-9}$, $K_b = 5 \cdot 10^{-8}$ und $K_m = 0{,}02$ in die Formel eingesetzt werden, findet man für die relative Aktivität die folgenden Werte (Tabelle 1):

Tabelle 1. Relative Aktivität der Urease.

pH	[S] = 0,02	[S] = 0,150
5	0,02	0,13
6	0,14	0,56
7	0,40	0,83
8	0,44	0,80
9	0,25	0,44

Die Werte der Tabelle sind in der Abbildung 1 durch Kreuze veranschaulicht. Die Übereinstimmung mit den experimentellen Kurven ist ganz gut und könnte zweifelsohne durch zweckmässige Wahl der Werte der Konstanten noch besser werden. Es ist also völlig klar, dass die Abhängigkeit der Ureasewirkung von Azidität und Substratkonzentration sehr gut unter der Annahme erklärt werden kann, dass Enzym und Substrat eine Verbindung mit einer wirklichen Gleichgewichtskonstante K_m bilden. K_m gilt für den ganzen pH-Bereich; die von van Slyke gefundene Variation von K_m mit der Azidität ist nur scheinbar und hängt damit zusammen, dass sich die Zahl der substratbindenden Gruppen mit der Azidität ändert.

Nachdem also gezeigt werden konnte, dass sich die Ureasewirkung unter gewissen Annahmen über die Affinität zum Substrat, über die sauren und ba-

sischen Eigenschaften des Enzyms und über die Art der substratbindenden Gruppen, formell beschrieben werden kann, so erhebt sich die Frage, ob die Bindung des Harnstoffes an das Enzym wirklich in der angenommenen Weise stattfinden kann. Angenommen wurde, dass die Bindung durch Aminogruppen oder andere basischen Gruppen des Enzyms vermittelt wird, und dass für den Zerfall der Enzymsubstratverbindung in Enzym und Reaktionsprodukte notwendig ist, dass die die sauren Eigenschaften des Enzyms bzw. der Enzymsubstratverbindung bedingenden Gruppe(n) nicht dissoziiert ist (sind). Wenn die substratbindende Gruppe des Enzyms eine basische Gruppe ist, so wäre wohl anzunehmen, dass der Harnstoff in einer polaren Form angelagert wird. Polare Formen des Harnstoffes sind ja von mehreren Seiten diskutiert worden, so dass eine diesbezügliche Annahme nicht befremdend erscheint.

Wenn man die enzymatische Hydrolyse der zusammengesetzten Kohlehydrate, der Peptide, der Ester etc. mit der Hydrolyse dieser Verbindungen durch Säuren verknüpfen will, liegt die Annahme nahe, dass die Wirkung der Enzyme, die ja in Aziditätsgebiete fällt, in welchen die H^+-Ionen an sich keine Rolle spielen können, damit zusammenhängt, dass die zu spaltende Bindung durch die Anlagerung des Substrats an das Enzym sehr nahe an Gruppen des Enzymmolekyls herankommt, die H^+ abgeben können und wo so zu sagen örtlich eine hohe H^+-Konzentration herrscht. Es erscheint dann wahrscheinlich, dass es eben diese Gruppen sind, die die saure Dissoziation des Enzyms bzw. der Enzymsubstratverbindung bedingen. So erscheint es begreiflich, dass das Substrat nur dann in die Reaktionsprodukte zerfällt, wenn die betreffenden sauren Gruppen undissoziiert sind. Werden sie durch Aziditätsverschiebung nach der alkalischen Seite hin in die entsprechenden negativen Ionen übergeführt, so ist die notwendige »H^+-Konzentration» nicht mehr vorhanden und die enzymatische Wirkung bleibt aus.

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Electron Diffraction Investigations of Molecular Structures

II. Results Obtained by the Rotating Sector Method

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An extensive work on the structure of gasous molecules was carried out in this laboratory during the period 1940—46, using the electron diffraction method designated as the »sector method». From photometer records of the diffraction diagrams scattering curves were obtained, and distribution functions were then computed using Fourier analysis. A survey of the methods developed for this purpose is given in a separate paper [1]. This paper is intended to give a corresponding survey of the more important results of structure determinations carried out during the period just mentioned. The difficult conditions, especially regarding facilities of publication, have so far prevented us from publishing our results in periodicals easily available outside Scandinavia. References to the original papers will be given throughout the following survey.

The paper is divided into three parts. The first part deals with the structure of simple compounds, chosen in order to get experience regarding the new methods before more complicated problems were tackled. In the second part results of the molecular structure determinations of some halogen derivatives of ethane are given. The third part of our survey has been reserved for structure determinations of molecules containing isocyclic or heterocyclic rings.

It should be emphasized that the structure of a number of the molecules in question is so complicated that a determination was possible only on the basis of a comprehensive study of simpler compounds and the use of differential methods described in some detail in the preceding survey.

* We wish to express our sincere gratitude towards *Fridtjof Nansens Fond* and *Statens Forskningsfond* for economical aid without which this work could not have been accomplished.

In order to save space, reproductions of intensity curves are not given here. An example of a photometric record of a sector diagram (benzene) is reproduced in fig. 4 of the preceding paper.

I. THE STRUCTURE OF SOME SIMPLE MOLECULES

Br_2 and I_2 [2]. The experimental distribution function $\sigma_m(r)$ for a diatomic molecule is given in fig. 6 of the preceding paper. From the distribution curves the internuclear distances are easily obtained. Iodine and bromine were investigated and the following distances obtained: Br-Br = 2.28 Å. I-I = 2.67 Å.

CO_2 and CS_2 [2]. The distribution curves indicate two internuclear distances with a ratio of 1 : 2 corresponding to a symmetrical, rectilinear molecule. The atomic distances obtained were: C-O = 1.15 Å. C-S = 1.56 Å.

AsI_3 [2]. The distribution curve reveals two internuclear distances only: As-I = 2.54 Å, I-I = 3.85 Å. The molecule is pyramidal and symmetrical with an angle I-As-I = 98.5°.

CCl_4 and CBr_4 [3]. The experimental distribution curves, $\sigma_m(r)$ and $\sigma(r)$ for CBr_4 are given in fig. 7 of the preceding paper [1]. Both for CCl_4 and CBr_4 the distribution curves indicate only the two internuclear distances to be expected for regular tetrahedrons. The atomic distances are found to be C-Cl = 1.76 Å and C-Br = 1.94 Å.

CH_3I and CH_2I_2 [4]. Because of the small scattering power of hydrogen, the $\frac{\sigma(r)}{r}$ curves, fig. 1, give only the two distances C-I and I-I. For both substances the C-I distance is found equal to 2.12 Å. The I-I distances are found to be 3.535 Å in CH_3I and 3.569 Å in CH_2I_2. This correspond to an angle I-C-I of 113.0° in iodoform and 114.7° in methylene iodide.

C_2Cl_2 [5]. The structure of dichloro-acetylene derived by de Laszlo [6] from electron diffraction diagrams could easily be shown to be incorrect. The $\sigma_m(r)$ curve is shown in fig. 2. The maxima of this curve correspond to the r values 1.20 Å, 1.64 Å, 2.83 Å and 4.425 Å. The molecule must therefore be linear and symmetrical and correspond to the formula Cl-C≡C-Cl, the atomic distances being C-C = 1.195 Å, C-Cl = 1.64 Å.

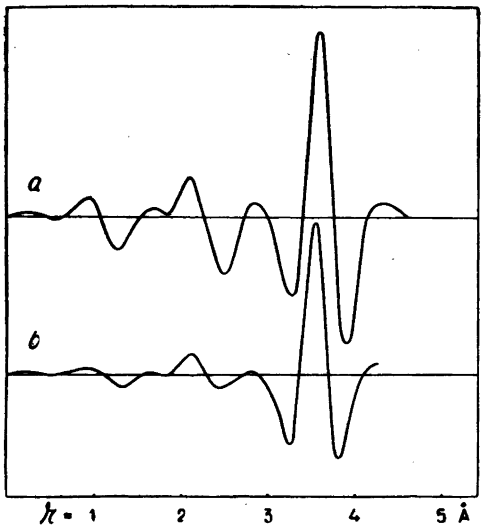


Fig. 1. Experimental $\frac{\sigma(r)}{r}$ *curves of iodoform (a) and methylen iodide (b).*

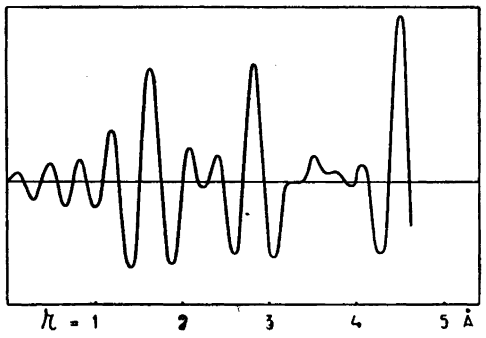


Fig. 2. Experimental $\sigma_m(r)$ *curve of* C_2Cl_2.

P_4S_3 [7]. The $\sigma_m(r)$ function of this substance — reproduced in fig. 3 exhibits two pronounced maxima at 2.15 Å and 3.48 Å, the origin of the other maxima may be explained on the assumption that they are the consequences of diffraction effects [1]. The most probable structure will be that of fig. 4 in which all the bond distances are about 2.15 Å and the distances between atoms not directly linked to each other about 3.48 Å. In order to demonstrate the relationship between the P_4S_3 and the P_4 structures, a model of the P_4 molecule is included in fig. 4. It is seen that the introduction of one S atom between

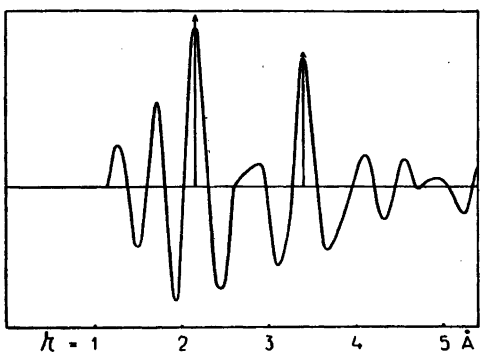


Fig. 3. Experimental $\sigma_m(r)$ *curve of* P_4S_3.

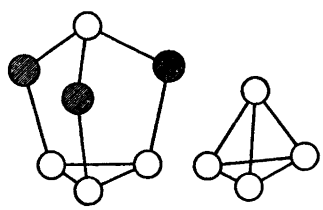


Fig. 4. Models of the P_4S_3 *and* P_4 *molecules.*

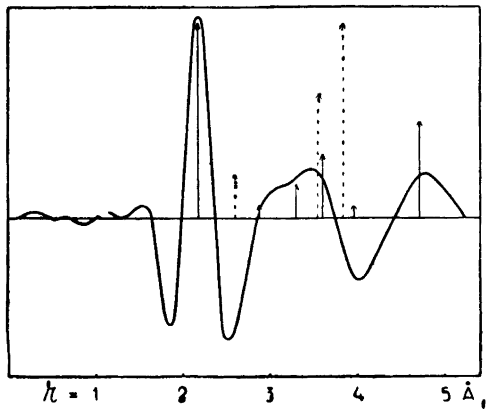
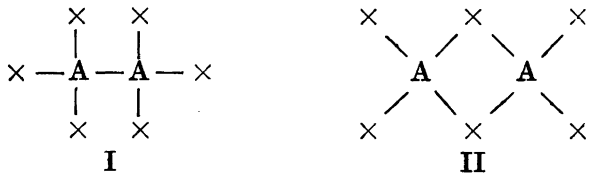


Fig. 5. Experimental σ(r) curve of Fe_2Cl_6.

one of the phosphorous atoms of P_4 and each of the three other P atoms transforms the P_4 molecule into a P_4S_3 molecule. The valency angles in P_4S_3 are all about 100° except those of the P_3 triangle. The near structural relationship existing between P_4S_3 and P_4 makes the conformity in chemical properties appear reasonable. A very striking example is the behaviour of the two substances towards gaseous oxygen.

Fe_2Cl_6 [8]. In the case of molecules A_2X_6 two possible structures have been proposed, the ethane-like structure I and the ethylene-like structure II:



I II

The $\sigma(r)$ curve of gasous Fe_2Cl_6 has one pronouced maximum only (fig. 5) and the r-value of this maximum — 2.17 Å — must no doubt correspond to the shortest Fe-Cl distance of the molecule. The dotted arrows of fig. 5 give the atomic distances of the ethane-like structure and the fully drawn arrows give the distances of the ethylene-like structure of the molecule. It is evident that only the ethylene-like structure is in agreement with the experimental $\sigma(r)$ curve.

The results of an investigation dealing with the aluminium halides Al_2Cl_6, Al_2Br_6 and Al_2I_6 has not been conclusive, chiefly because of the relatively poor scattering power of the Al atoms.

II. THE STRUCTURE OF SOME HALOGEN SUBSTITUTED ETHANES

The structure of ethane and its simple derivatives is of considerable interest because their elucidation will contribute to the knowledge about the forces between atoms not directly linked together and the nature of the rotation about C-C single bonds.

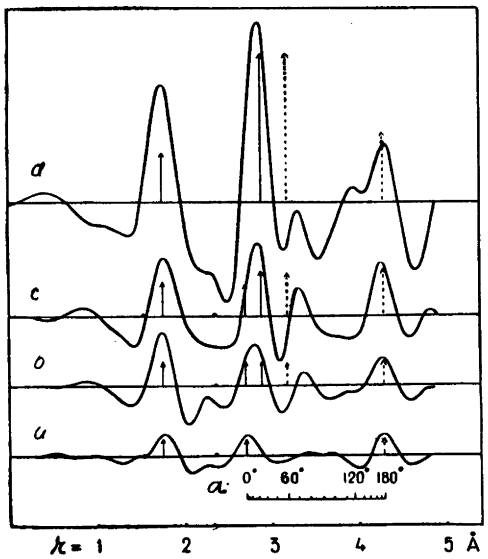


Fig. 6. Experimental $\sigma(r)$ curves of $CH_2Cl \cdot CH_2Cl$ (a); $CHCl_2$-CH_2Cl (b); $CHCl_2$-CH_2Cl (c) and CCl_3-CCl_3 (d).

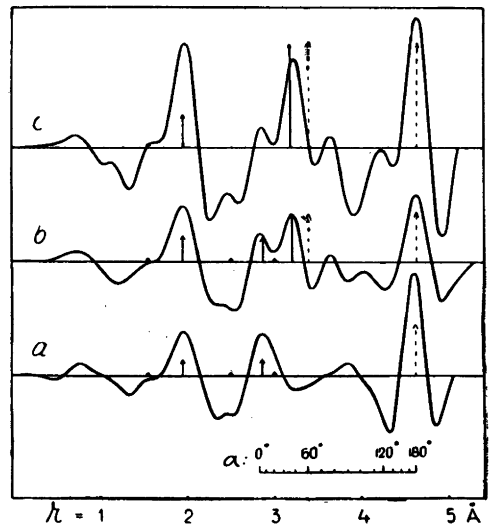


Fig. 7. Experimental $\sigma(r)$ curves of CH_2Br-CH_2Br (a); $CHBr_2$-CH_2Br (b) and $CHBr_2$-$CHBr_2$ (c).

The results published by different authors and based on dipole moment measurements, spectroscopical studies and electron diffraction analysis using the »visual» method have led to diverging interpretations. In tackling the question by the new methods of electron diffraction we chose the chlorine and bromine derivatives because of the relatively great scattering power of the halogens. The $\sigma(r)$ curves of the following seven compounds were worked out and discussed in detail [9]:

1,2 dichloro-ethane; 1,2 dibromo-ethane;
1,1,2 trichloro-ethane; 1,2,3 tribromo-ethane;
1,1,2,2 tetrachloro-ethane; 1,1,2,2 tetrabromo-ethane;
hexachloro-ethane.

The $\sigma(r)$ curves are reproduced in figs. 6 and 7. The resemblance between curves of corresponding chlorine and bromine compounds is striking. The existing differences are those to be expected because of the greater scattering power of the bromine atom. The C-C distance is 1.54 Å, the smallest C-Cl distance in all the chlorine compounds (fig. 6) is 1.76 Å and the corresponding C-Br distance (fig. 7) is 1.94 Å. In both figures the r values of atomic distances which should be constant and independent of an intramolecular rotation about

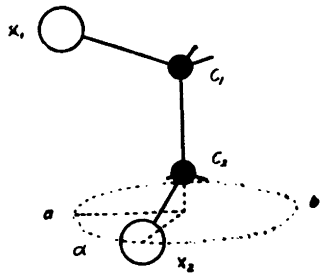


Fig. 8. Relative position of two halogen atoms bonded to different carbon atoms. At (a) $a = 0°$ (cis position) and at (b) $a = 180°$ (trans position).

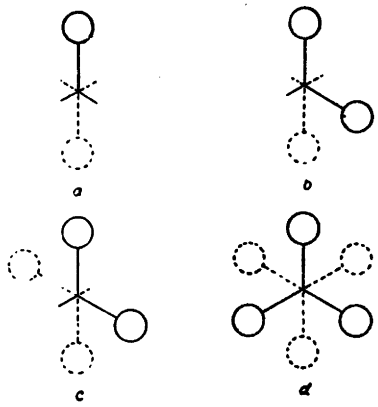


Fig. 9. Trans models of halogen substituted ethanes. (a) CH_2X-CH_2X; (b) CHX_2-CH_2X; (c) CHX_2-CHX_2; (d) CX_3-CX_3.

the carbon-carbon bond are indicated by fully drawn arrows, the length of the arrows being proportional to the theoretical $\sigma(r)$ values. There is no evidence for a distortion of the tetrahedral valency angles.

The distance between two halogen atoms X_1 and X_2 of which X_1 is directly linked to the carbon atom C_1 and X_2 to C_2 will be influenced by a rotation about the C_1-C_2 bond. The relative position of the halogen atoms is most conveniently described by giving the angle a between the two planes $X_1C_1C_2$ and $X_2C_2C_1$ (fig. 8). The possible values of this angle range from $a = 0°$ to $a = 180°$. In the »cis» position ($a = 0°$) the X_1-X_2 distance has its minimum value, and attains it greatest possible value in the »trans» position ($a = 180°$). The scales at the bottom of figs. 6 and 7 are intended to illustrate in which way the X_1-X_2 distances depend on the angle a. As a matter of fact all the experimental curves exhibit sharp maxima corresponding to trans positions of two halogen atoms ($a = 180°$). The mutual position of the two tetrahedral groups in the trans models of the different molecules is given by fully drawn and dotted lines in fig. 9. The dotted arrows in figs. 6 and 7 indicate the atomic distances in these models which depend on the relative position of the two groups. The r values corresponding to $a = 180°$ are in good agreement with the experimental curves. No maxima appear, however, for distances corresponding to $a = 60°$. These discrepancies are removed if we interpret the models of fig. 9 as representing the position of equilibrium of the different molecules and assume an oscillation of the two tetrahedral groups to take place about this position. Such an oscillation will alter the distance

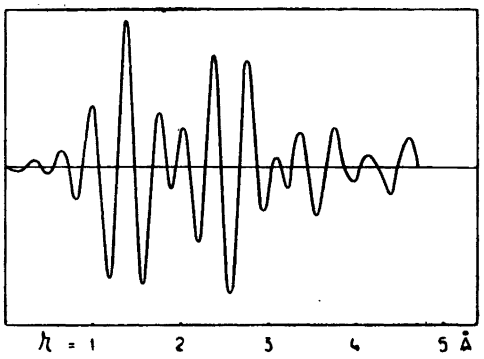


Fig. 10. Experimental $\sigma_m(r)$ curve of benzene.

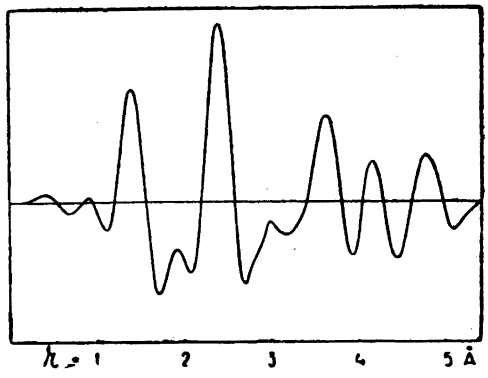


Fig. 11. Experimental $\sigma(r)$ curve of phloroglucinol.

between atoms in trans position very little. In the chlorine and bromine compounds which we are considering, a variation of α from 180° to 170° will cause a decrease of the halogen-halogen distance smaller than 0.01 Å. Distances corresponding to $\alpha = 60°$, however, will be much more sensitive to a change in α, a decrease or increase of 10° producing a decrease or increase of the distance of about 0.15 Å. In accordance with this, the maxima corresponding to $\alpha = 180°$ are always very pronounced and the r values only a little smaller than those calculated for the true trans position. Maxima corresponding to $\alpha = 60°$, however, are flattened out and cannot be observed because of the influence of other maxima and their diffraction effects. Although no accurate information concerning the amplitude of oscillation is given, the small displacement of the trans maxima indicates a mean value of about 15—20°.

The distances between hydrogen and halogen atoms, one of which is linked to C_1, the other to C_2, is 2.88 Å (Cl) and 3.10 Å (Br). As pointed out [10] these distances agree very well with the sums of van der Waals radii of a hydrogen atom and a halogen atom. This relation seems to be confirmed by the investigations of cyclohexane derivatives which will be discussed below.

III. THE STRUCTURE OF SOME CYCLIC COMPOUNDS

Benzene [1]. The experimental $\sigma_m(r)$ curve of benzene vapour is given in fig. 10. The curve is in complete agreement with the planar, hexagonal structure. The shortest C-C distance is equal to 1.40 Å, the C-H bond distance to 1.04 Å.

Fluorobenzene [2] was also investigated using the sector method. The results concerning the benzene ring were confirmed. The observation indicate that

the fluorine atom is situated on the line drawn between two para C atoms, the C-F distance being 1.31 Å.

Phloroglucinol[12]. The $\sigma(r)$ curve, reproduced in fig. 11, give conclusive evidence for the fact that the C- and O-atoms are coplanar and the internuclear distances correspond to those which would be expected for 1,3,5 trioxybenzene. This distances given by the curve are C-C = 1.40 Å, C-O = 1.36 Å. The corresponding distances to be expected for 1,3,5 cyclohexanetrion are C-C = 1.54 Å; C-O = 1.16 Å. A calculation of the energy shows that the enol structure must be considerably more stable than the keto structure, so that only a very small fraction of the latter should be present in the equilibrium mixture. The results of Kohlrausch[13] concerning the C-X bond energies in aliphatic and aromatic compounds are in good agreement with this result.

Cyclopropane[14]. The $\frac{\sigma(r)}{r}$ curve of this compound is very simple, as may be seen from fig. 12. It is obvious that the second of the three maxima observed at $r = 1.08$ Å, $r = 1.54$ Å and $r = 2.24$ Å gives the C-C distance in the three-membered ring and the other maxima the two C-H distances occurring in a symmetrical molecule. From these distances the valency angles C-C-H and H-C-H are found to be 116.4° and 118.2° respectively.

Cyclopentane[15]. The experimental $\frac{\sigma(r)}{r}$ curve is given in the upper part of fig. 13 and is seen to have a maximum for $r = 1.54$ Å, the C-C bond distance. If the ring is planar and symmetrical the second C-C-distance will have the value 2.49 Å. It is easily seen, however, that a C-H distance must occur which will tend to displace the maximum due to this C-C distance. In order to obtain the value of this C-H distance a special sort of subtraction process was performed. A theoretical $\frac{\sigma(r)}{r}$ curve including the diffraction effects was computed for the C-C distances of the regular pentagon. At the bottom of the figure the curve representing the difference between the experimental curve and this theoretical curve is given. This curve would be expected to exhibit maxima for r values equal to the three C-H distances of the symmetrical molecule. The first maximum of the curve occurs for $r = 1.09$ Å. If the C-H distance has this value and the H-C-H angle of the methylene groups has its normal tetrahedral value, the second and third C-H distances should be 2.17 Å and 3.22 Å. The r values of the corresponding maxima of the differential curve are 2.18 Å and 3.13 Å. The last maximum is broad and little

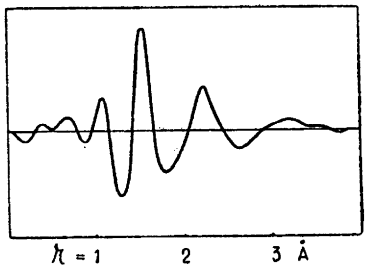


Fig. 12. Experimental $\frac{\sigma(r)}{r}$ *curve of cyclopropane.*

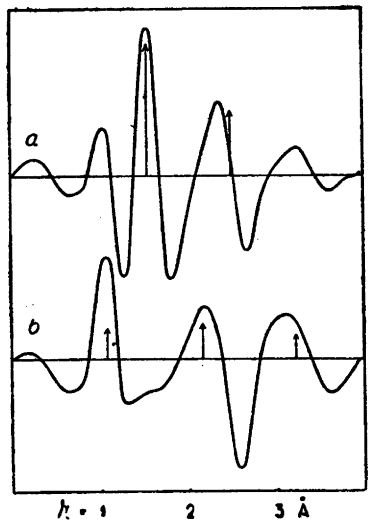


Fig. 13. (a) Experimental $\frac{\sigma(r)}{r}$ *curve of cyclopentane. (b) Difference between the curve (a) and the theoretical curve for the carbon skeleton.*

pronounced. The conclusion seems justified that a deviation from a symmetrical pentagonal structure with H-C-H angles of about 109.5° if it exists, must be very limited.

Cyclohexane. Earlier investigations based on the visual method [16] agreed with the staggered form of the molecule (fig. 14) in which all valency angles are assumed to be 109°28′. Results from our laboratory, some of which have been published [17], confirm this conclusion.

It is found that the model, when based upon a C-C bond distance of 1.54 Å and a C-H distance of 1.10 Å, gives atomic distances which are in satisfactory agreement with the experimental $\sigma(r)$ curve (fig. 15). In no case has an examination of the structure of cyclohexane or its derivatives so far given reliable indications of the presence of a less symmetrical form of the carbon skeleton. The reason seems to be that other possible forms would make distances between atoms linked to different carbon atoms of the ring smaller than the sum of their van der Waals radii [9].

The bonds between the carbon atoms of the symmetrical cyclohexane ring and other atoms are of two geometrically different kinds. Bonds of one kind are parallel to the trigonal axis of the ring. These »upright» bonds

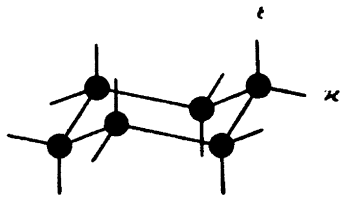


Fig. 14. The staggered form of the cyclohexane molecule. κ- and ε-bonds are indicated.

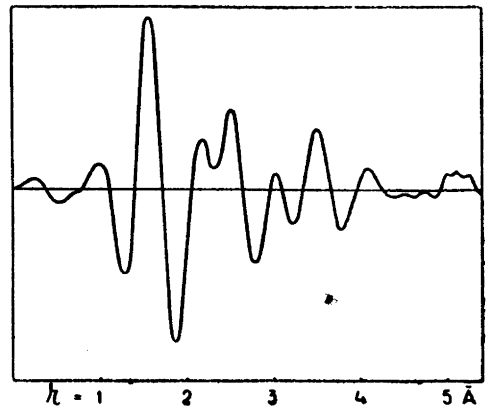


Fig. 15. The experimental σ(r) curve of cyclohexane.

(ἐσιηκώζ) have been designated as ε-bonds. Bonds of the second kind are roughly orthogonal to the principal axis (the angle beeing 109°28′ instead of 90°) and have been called κ-bonds (κειμενοζ, prostrate). Now, without breaking the carbon-carbon bonds, a conversion of the ring may take place into a new ring of the same shape in which, however, each ε-bond is a κ-bond of the old ring and vice versa. The significance of the distinction between κ- and ε-bonds will, we hope, be brought out clearly in the following part of our paper where the configurations of molecules containing six-membered rings are discussed.

Chloro-cyclohexane[18]. In a single mono-derivative of cyclohexane, like chloro-cyclohexane, there are two possible configurations, the ε-form and the κ-form. The only atomic distances which are different in the two forms and contribute some weight to the electron scattering of the molecule are the distances from the chlorine atoms to the carbon atoms. The fully drawn curve in the upper part of fig. 16 is the experimental $\sigma(r)$ curve of chloro-cyclohexane. It is difficult indeed to draw any conclusion regarding the structure of the chlorine compound directly from this curve. An examination of the curve, reproduced in the lower part of fig. 16 and representing the difference between the experimental $\sigma(r)$ curves of the chloroderivative and that of cyclohexane (fig. 15) is able, however, to give better informations concerning the position of the chlorine atom. The maxima of this curve should correspond to r values giving the C-Cl distances of the molecule. Arrows at the bottom of fig. 16 indicate the calculated r values in the ε- and κ-form respectively. The

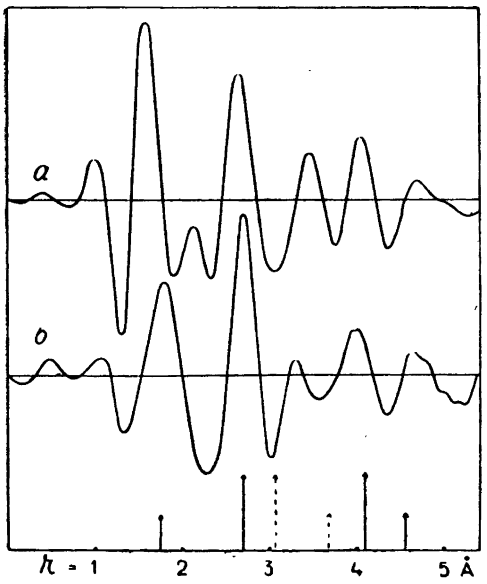


Fig. 16. (a) The experimental $\sigma(r)$ curve of chloro-cyclohexane. (b) The difference between the experimental $\sigma(r)$ curves of chloro-cyclohexane and cyclohexane.

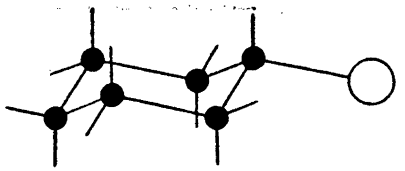


Fig. 17. Model of the chloro-cyclohexane (and cyclohexylmercaptane) molecule (κ-form).

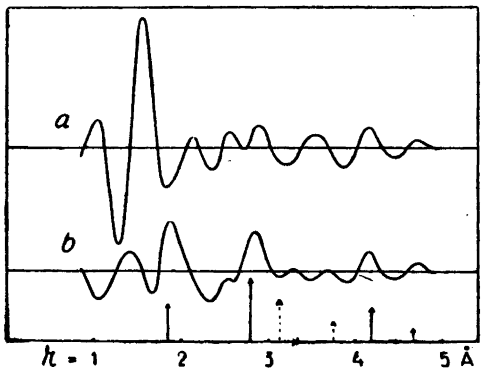


Fig. 18. (a) The experimental $\frac{\sigma(r)}{r}$ curve of cyclohexylmercaptane. (b) The difference between the experimental $\frac{\sigma(r)}{r}$ curves of cyclohexylmercaptan and cyclohexane.

two first arrows to the left indicate C-Cl distances common to both models whereas the two remaining fully drawn arrows correspond to distances characteristic of the κ-form given in fig. 17. The two dotted arrows indicate C-Cl distances present in the ε-form only. The calculations are based on the bond distances C-Cl = 1.76 Å and C-C = 1.54 Å. The lengths of the arrows are proportional to the weights of the distances in question. It is seen that the curve does not give any indication of the presence of the ε-form, but the presence of the κ-form seems obvious. The conclusion must be drawn that the concentration of the κ-form is at least considerably greater than that of the ε-form.

The energy of the ε-form must therefore be greater than that of the κ-form, a fact which is not surprising. In a model of the ε-form the distance between the chlorine atom and the two nearest hydrogen atoms (both of which are ε-atoms) would be nearly equal to the distance between a chlorine and a hydrogen atom linked to different carbon atoms in »cis» forms of chlorinated

ethanes (p. 153). This distance of about 2.5 Å is no doubt smaller than the sum of the van der Waals radii of the two atoms. On the other hand, the distance between a $\varkappa$-chlorine atom and the hydrogen atoms linked to neighbouring C atoms is nearly equal to the distance characteristic of the stable forms of chlorinated ethanes.

Cyclohexylmercaptan[19]. The discussion of the structure of this compound was based on the experimental $\frac{\sigma(r)}{r}$ curve reproduced in the upper part of fig. 18. The curve obtained by subtracting the experimental $\frac{\sigma(r)}{r}$ curve of cyclohexane from that of the mercaptan is given in the lower part of the figure. The arrows below this curve indicate the r values of C-S distances to be expected in the ε- and $\varkappa$-forms of cyclohexylmercaptan where the C-S bond distance is assumed to be 1.87 Å. In complete analogy with the chlorine compound the two smallest distances would be the same in both models. Here also the two remaining, fully-drawn arrows indicate distances present only in the $\varkappa$-form and the two dotted arrows the distances to be expected in the ε-form. The experiment thus gives clear indications of the presence of the $\varkappa$-form but not the ε-form.

1,4 dioxan. A series of compounds containing six-membered rings of oxygen and sulphur atoms is of interest when stereochemical problems related to the structure of cyclohexane are discussed. The structures of 1,4 dioxan and 1,4 dithian were therefore worked out in our laboratory by Ore.

The most pronounced maxima of the experimental $\frac{\sigma(r)}{r}$ curve (fig. 19 *a*) of 1,4 dioxan correspond to the r values 1.44 and 2.33 Å. A determination of the structure is not possible, however, on the basis of this fact alone. A comparison of the experimental curve with theoretical curves including diffraction effects was therefore carried out for different molecular models. The procedure of calculation has been described in a previous paper[1]. Neither a planar model or any model of cradle type was able to deliver a theoretical curve in satisfactory agreement with the experimental one. The only model fullfilling this requirement was the staggered form shown in fig. 20. So far the result obtained by Sutton and Brockway[20] using the visual method was thus confirmed. According to these authors the bond distances and valency angles have the following values: C-O = 1.46 Å, C-C = 1.51 Å, $\angle$ C-C-O = 109.5°, $\angle$ C-O-C = 110°.

The theoretical $\frac{\sigma(r)}{r}$ curve based on this model is given in fig. 19 (dotted

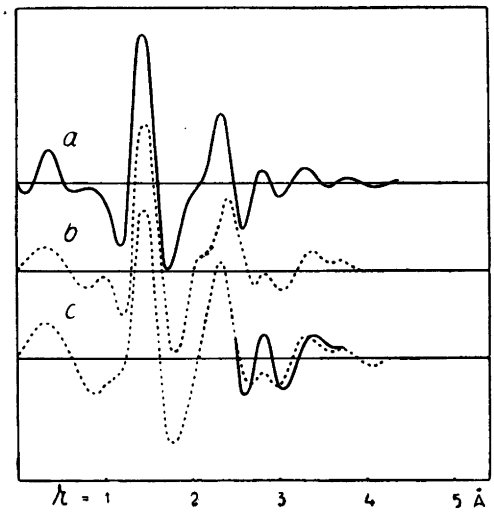


Fig. 19. (a) The experimental $\frac{\sigma(r)}{r}$ *curve of dioxane. (b) Theoretical* $\frac{\sigma(r)}{r}$ *curve of Sutton and Brockways model. (c) Theoretical* $\frac{\sigma(r)}{r}$ *curve of the model given in this paper.*

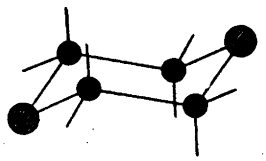


Fig. 20. Staggered form of the dioxane molecule.

curve *b*). A somewhat better agreement with our experimental curve was obtained when introducing slight modifications in the distances given by Sutton and Brockway. We found the best agreement to be reached when chosing C-C = 1.54 Å, C-O = 1.42 Å, ∠ C-C-O = 106° and ∠ C-O-C = 108°.

In calculating the theoretical curve corresponding to this model, the hydrogen atoms have also been taken into consideration. The C-H distance was assumed to be 1.10 Å and the H-C-H angle = 112°. The resulting curve is the dotted curve *c* of fig. 19. In this model there are four ε-hydrogen atoms, and the distance between two and two of them is only 2.16 Å. This distance is smaller than the double van der Waals radius of hydrogen — 2.5 Å — which nearly coincides with the distance in cyclohexane or the stable form of ethane[10]. We should expect, therefore, that the C-H bonds are not rigorously parallel but bent a certain angle out from the middle of the ring. In fact, the part of the theoretical $\frac{\sigma(r)}{r}$ curve lying in the region about $r = 2.8$ Å is in better agreement with the experimental curve if a bending of 10—12° is supposed to take place (*cf.* the fully drawn part of curve *c* in fig. 19). It is difficult to decide to which extent minor details like these can be relied on. They have

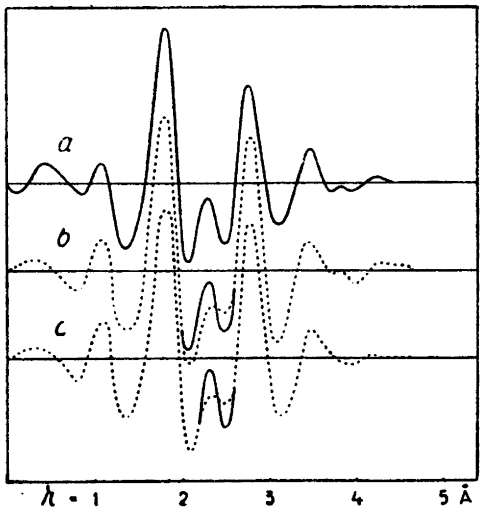


Fig. 21. (a) The experimental $\frac{\sigma(r)}{r}$ *curve of dithian. (b) Theoretical* $\frac{\sigma(r)}{r}$ *curve of the staggered form of the molecule. (c) Theoretical* $\frac{\sigma(r)}{r}$ *curve of the model given in fig. 22.*

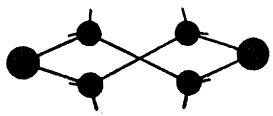


Fig. 22. Possible model of the dithian molecule.

been mentioned, however, because similar results were obtained also in the case of dithian.

1,4 dithian. The experimental $\frac{\sigma(r)}{r}$ curve is the fully drawn curve *a* of fig. 21. A comparison between this curve and a series of theoretical curves based on different molecular models led to the result that the staggered form of fig. 20 is the most probable in this case also. The atomic distances and the angles leading to the most satisfactory agreement with the experimental curve are: S-C = 1.81 Å, C-C = 1.54 Å, ∠ S-C-C = 111° and ∠ C-S-C = 100°.

The theoretical $\frac{\sigma(r)}{r}$ curve for this model is the dotted curve *b* of fig. 21. In addition to the distances and angles given above, the distance C-H was assumed to be 1.10 Å, and the angle S-C-H 109.5°. Here again, however, as in the case of dioxane, the theoretical $\sigma(r)$ curve is in better agreement with the experimental curve if the S-C-H angle is assumed to be smaller than 109.5°. The fully drawn part of curve *b*, fig. 21 which gives a better agreement in the region near 2.3 Å, is based on the angle S-C-H = 105°.

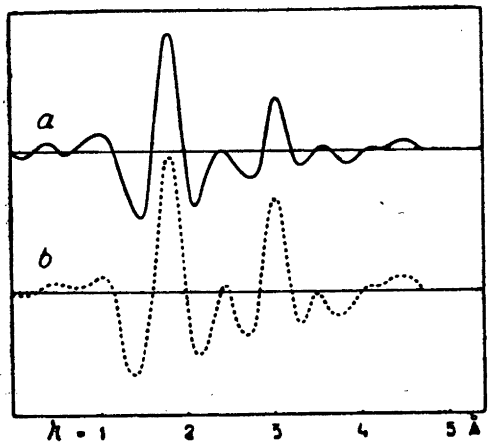


Fig. 23. (a) Experimental $\frac{\sigma(r)}{r}$ *curve of trithian. (b) Theoretical* $\frac{\sigma(r)}{r}$ *curve of the staggered form fig. 24.*

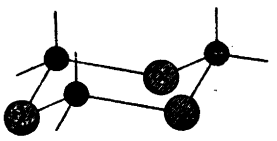


Fig. 24. Model of the trithian molecule.

Besides the staggered form of dithian, another model of the molecule has to be taken into consideration because it leads to a theoretical curve (dotted curve *c* of fig. 21) which agrees surprisingly well with the experimental curve. This model, given in fig. 22, is strainless and it may in fact be transformed into the »cradle» form without deformation of the valency angles. The model posesses two digonal axis of symmetry, the atomic distances and valency angles are C-S = 1.81 Å, C-C = 1.54 Å, ∠ S-C-C = 109.5° and ∠ C-S-C = 104.5°.

The remarkable conformity between the $\frac{\sigma(r)}{r}$ curve of dithian and the theoretical curve based on this model suggests that molecules of this form may be present in equilibrium with molecules of the staggered form. This seems probable because the energy differance between the two forms cannot be expected to be considerable.

Sym-trithian. This substance contains a symmetrical ring of alternate sulphur and carbon atoms and is of special interest because we are going to discuss the structures of *a*- and *β*-trithioacetaldehyde belov. The experimental $\frac{\sigma(r)}{r}$ curve of trithian exhibits two pronounced maxima at $r = 1.81$ Å and $r = 3.05$ Å, as may be seen from the fully drawn curve *a* of fig. 23. The atomic distance S-C being 1.81 Å, the second maximum should correspond to the

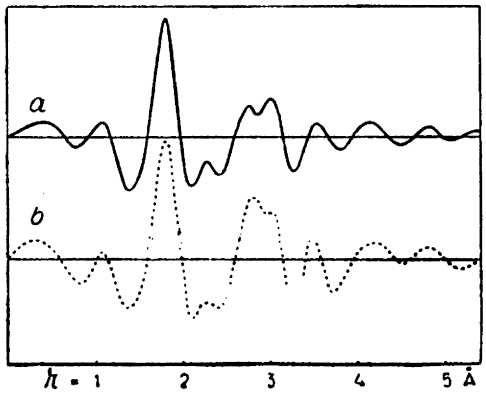


Fig. 25. (a) Experimental $\frac{\sigma(r)}{r}$ *curve of α-trithioacetalehyde. (b) Theoretical* $\frac{\sigma(r)}{r}$ *curve of the κ,κ,ε-model (fig. 27).*

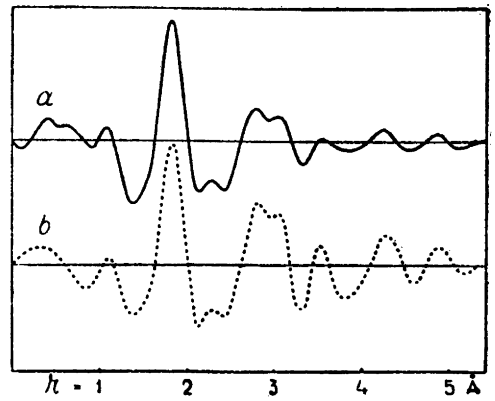


Fig. 26. (a) Experimental $\frac{\sigma(r)}{r}$ *curve of β-trithioacetaldehyde. (b) Theoretical* $\frac{\sigma(r)}{r}$ *curve of the κ,κ,κ-model (fig. 28).*

S-S distance. This leads to a C-S-C angle of 114.5°. The influence of other atomic distances on the $\frac{\sigma(r)}{r}$ curve is not great and consequently the determination of these distances is difficult. It seems reasonable to assume, however, that the ring will have very nearly the same form in trithian, α- and β-trithioacetaldehyde and, if that is so, the trithian molecule has the staggered form of fig. 24 with an angle S-C-S = 106.5°. Assuming the C-H distance to be 1.10 Å the theoretical $\frac{\sigma(r)}{r}$ curve including diffraction effects (dotted curve *b* of fig. 23) is seen to be in very good agreement with the experimental curve.

α- and β-trithioacetaldehyde[21]. Baumann and Fromm[22] and later Chattaway and Kellett[23] based their discussion of the configurations of these molecules on the assumption of a planar ring. The first-mentioned authors arrived at the conclusion that the α-compound (m. p 101° C) is the »cis» form and the β-compound (m. p 125—126° C) the »trans» form. Chattaway and Kellett, on the other hand, discovered that the α-compound is able to give two different monosulphones but the β-compound only one, and consequently came to the opposite conclusion.

The results to which we were led in the case of trithioformaldehyde makes it seem improbable that the ring is planar in either of these substances. Electron diffraction investigations confirmed this view. The fully drawn curve

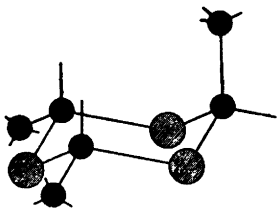


Fig. 27. Model of the a-trithioacetaldehyde molecule (κ,κ,ε-form).

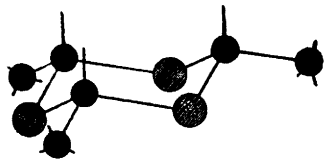


Fig. 28. Model of the β-thioacetaldehyde molecule (κ,κ,κ-form).

a of fig. 25 gives the experimental $\frac{\sigma(r)}{r}$ curve of the *a*-compound, the corresponding curve of fig. 26 that of the β-compound. The conformity of these experimental curves is very striking. The most pronounced maxima of both curves correspond to r values equal to 1.81 Å and 3.05 Å, the values observed also in the case of trithioformaldehyde. These indicate that the C_3S_3 ring must be very similar for all three substances. A comparison between the experimental $\frac{\sigma(r)}{r}$ curves and theoretical curves shows that a ring based on the distances C-S = 1.81 Å, the angle C-S-C = 106.5° and the angle S-C-S = 114.5° leads to a very satisfactory agreement in all cases.

The circumstance that the S-C-S angle is greater than 109.5° makes it very probable that the angle between the two other carbon valencies is somewhat smaller than the tetrahedral angle. The ε-valencies are not far from perpendicular to the C_3 and S_3 triangles, the angle they form with the normal of these plans being 2—3° only. It is evident that the properties of the two sorts of bonds (ε- and κ-bonds) already discussed in the case of cyclohexane derivatives will be of importance for trithioacetaldehyde as well. Thus steric hindrance will tend to prevent molecules having more than one ε-methylgroup from being formed, but the two configurations κ,κ,ε and κ,κ,κ should both be possible. The first of these forms is shown in fig. 27 the second in fig. 28.

Theoretical $\frac{\sigma(r)}{r}$ curves were computed for both forms chosing a C-C distance of 1.54 Å and a S-C-C angle of 106°. The curve corresponding to the configuration κ,κ,ε is the dotted curve of fig. 25. The dotted curve of fig. 26 corresponds to the κ,κ,κ form. The agreement between experimental and theoretical curves is so marked that very little doubt can remain as to the correctnes of our models. It is obvious that the changes produced in the $\frac{\sigma(r)}{r}$ curve when one ε-methyl group in the κ,κ,κ form is replaced by an ε-group is relatively unimportant. So in order to decide which of the two

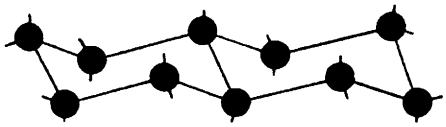


Fig. 29. Model of the »trans» decalin molecule.

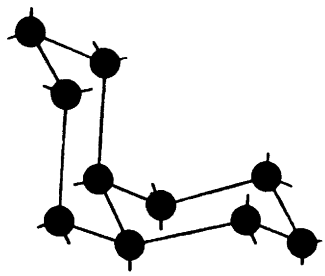


Fig. 30. Model of the »cis» decalin molecule.

compounds has the $\varkappa,\varkappa,\varkappa$ configuration and which the $\varkappa,\varkappa,\varepsilon$ configuration the fact mentioned above, that the α-compound forms two monosulphones, the β-compound only one, has to be taken into consideration. The result is then unambigous: the α-compound corresponds to the configuration $\varkappa,\varkappa,\varepsilon$ (fig. 27) the β-compound to $\varkappa,\varkappa,\varkappa$ (fig. 28).

The decalins [24]. It was pointed out some years ago [10] that the symmetrical model of decalin (fig. 29) containing two staggered cyclohexane rings will probably be the correct model of the »trans» isomer, but that the »cis» model proposed by Mohr [25] and containing two »cradle»-shaped rings has to be rejected and replaced by a model (fig. 30) based on two staggered cyclohexane rings. Of these models the former results if two cyclohexane ring are linked together in 1,2 position using two $\varkappa$ bonds, the latter if one $\varkappa$ bond and one ε bond is used. The proposed structure of the so-called »cis» decalin should be energetically more stable than the Mohr structure because of its more favourable hydrogen-hydrogen distances.

The upper curve *a* of fig. 31 is the experimental $\frac{\sigma(r)}{r}$ curve of »trans» decalin, the lower curve *b* that of »cis» decalin. The conformity of the two curves is very striking. Only in the region between 2 and 4 Å differences are observed. In order to test the correctnes of the proposed structure of the »cis» compound the experimental $\frac{\sigma(r)}{r}$ curve of the »trans» compound was subtracted from the corresponding curve of the »cis» compound.

The resulting curve (fig. 32 *b*) was compared with theoretical curves calculated under the assumption that the »trans» decalin has the structure of fig. 29. Fig. 32 *a* gives this theoretical differential curve based on the »cis» model of fig. 30, and fig. 32 *c* gives the corresponding curve based on the Mohr »cis» model. A comparison between these curves seems to settle beyond doubt that »cis» decalin has the structure given in fig. 30.

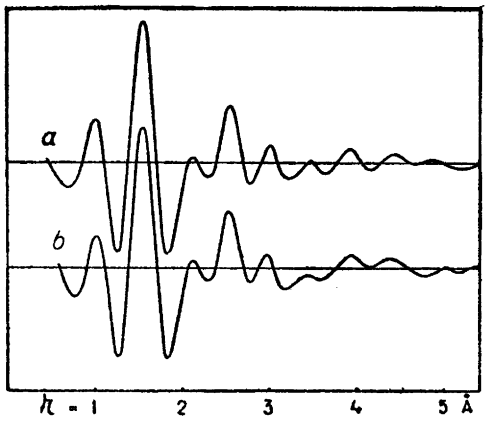


Fig. 31. (a) Experimental $\frac{\sigma(r)}{r}$ *curve of »trans» decalin. (b) Experimental* $\frac{\sigma(r)}{r}$ *curve of »cis» decalin.*

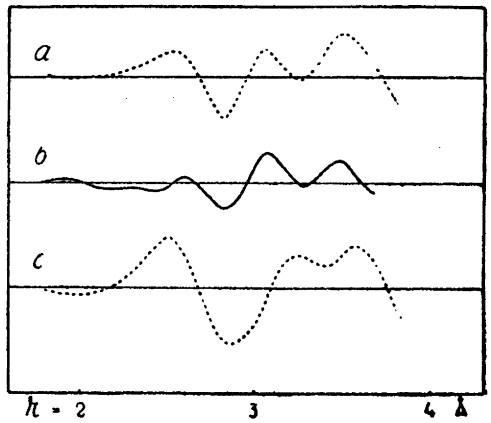


Fig. 32. (a) The difference between the theoretical $\frac{\sigma(r)}{r}$ *curves of »trans» and »cis» decalin (»cis» model fig. 30). (b) The difference between the experimental* $\frac{\sigma(r)}{r}$ *curves of »trans» and »cis» decalin. (c) The difference between the theoretical* $\frac{\sigma(r)}{r}$ *curves of »trans» and »cis» decalin (»cis» model proposed by Mohr).*

SUMMARY

Results of molecular structure determinations carried out in Oslo in the years 1940—46 by the rotating sector method of electron diffraction in vapours with subsequent Fourier analysis are given. The substances investigated include some simple organic compounds, halogen derivatives of ethane and finally a series of cyclic compounds. Among the latter substances containing six-membered rings identical with, or closely related to that of cyclohexane have been most extensively studied.

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Some Experiments in the Preparation of the Isotopes U^{239} and Np^{239}

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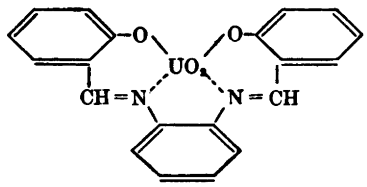
Some years ago, Starke [1] succeeded in an attempt to obtain a Szilard-Chalmers concentration of the uranium isotope U^{239} by means of the uranyl benzoylacetonate. He had a good concentration factor and his product appears to have possessed a high degree of purity. However, it was not possible to irradiate the compound in solution owing to the rapid exchange between the free, activated uranium ions and the uranium in the complex. In this case the compound, dissolved in the treatment after irradiation, had to be reprecipitated and dried before it could be used again. For the same reason, it was necessary for the activated uranium to be carried down very rapidly by adsorption when the irradiated compound was dissolved.

Duffield and Calvin [2] have made Szilard-Chalmers experiments with metal salts of salicylaldehyde-*o*-phenylenediimine. They achieved good results with the copper complex and mention that they also obtained good results with uranyl salicylaldehyde-*o*-phenylenediimine:



In the present paper some experiments are described, which have been performed with this uranium compound in order to obtain a sample of U^{239} and Np^{239} with high specific activity and as free from other activities as possible.

SZILARD-CHALMERS CONCENTRATION

In their copper experiments, Duffield and Calvin [2] obtained the best result when the complex was irradiated in pyridine solution and this solution was diluted with aqueous acetic acid after the addition of a small amount of copper salt as carrier. The precipitated complex was filtered off and the copper ions in the filtrate were precipitated as CuS.

In the beginning an attempt was made to use a similar method in the case of uranium. After the irradiation with slow neutrons a small amount of an aqueous solution containing a mixture of $U(SO_4)_2$ and UO_2SO_4 was added, and the pyridine solution diluted with ten times its volume of 15 % acetic acid. According to γ-activity measurements about 80 % of the activity accompanied the filtrate. Thus there is only little, if any, exchange between the complex and the liberated uranium.

The difficulty in this method is to find a method for the precipitation of the small quantity of uranium in the large filtrate corresponding to the precipitation of CuS. Several precipitation methods were tried under the appropriate conditions: with Na_2HPO_4, oxine, ammonia and various organic bases (iron was added in order to facilitate the precipitation) and cupferron. Since it was not improbable that the liberated U^{239} was in the quadrivalent state, in the first two cases oxidation was performed with $KBrO_3$. In no instance were both of the following two requirements fulfilled: the uranium (and if possible the neptunium also) should be precipitated with a good yield, and it should be precipitated free from the complex (or its decomposition products formed after the irradiation) still remaining in the aqueous filtrate. The instability of the complex and the buffering action of the solution were serious difficulties.

When minute amounts of a solution containing a mixture of $U(SO_4)_2$, UO_2SO_4, Na_2SO_4, and some H_2SO_4 are added to the pyridine solution, a gelatinous uranium-containing precipitate is formed. In some experiments the pyridine solution was shaken with small amounts of such a precipitate during the irradiation in a shaking device surrounded by paraffin and placed near the D-tank of the cyclotron. After the irradiation the precipitate was filtered off and washed with pyridine. In this manner it was possible to capture more than 50 % of the activity, but the use of uranium carrier reduced the specific activity obtainable.

In order to avoid uranium as a carrier it was attempted to carry down the activity by means of other precipitates, for instance by the addition of Li_2SO_4 solution, but the yeild was very poor.

Adsorption on charcoal after the irradiation was found to be the best method. Though somewhat irregular, yields as high as 80 % have been obtained.

SEPARATION FROM NATURAL DECAY AND FISSION PRODUCTS

Various experiments were performed in order to purify the product resulting from the adsorption on the precipitate containing uranium, but since the results were not very good and the method was abandoned, they are not discussed here.

The most important natural β-emitting decay product in a uranium preparation, the age of which is some months, is UX_1. Its activity is weak, but after some minutes it is already in equilibrium with its decay product UX_2, the β-radiation of which is rather penetrating. In the present case it is possible to handle both products as if the radiation were due to the Th isotope UX_1.

The main part of the UX_1 was removed by adsorption on charcoal immediately before the irradiation. However, a quantitative separation was not conveniently achieved in this way, and the remainder had to be removed in the treatment after the irradiation.

In the purification of the product advantage was taken of the (not very common) property possessed by uranium and neptunium [3, 4] of forming soluble complex carbonates with an excess of $(NH_4)_2CO_3$, irrespective of whether their valency is IV or VI. The charcoal was shaken with saturated $(NH_4)_2CO_3$ solution, and in order to provide a carrier for the insoluble carbonates some $Ba(NO_3)_2$ was added.

The Th isotope UX_1 will probably follow U and Np since Th forms soluble complex carbonates. Among the more frequent fission products that might be feared to go the same way are Zr and Ce. These three, however, form iodates, insoluble in nitric acid solution (Ce as Ce^{IV}). (As to Y, see below; Cb seems to give no trouble.) In order to avoid simultaneous precipitation of Np, it is necessary to ensure that it has the valency VI before the addition of iodate.

According to McMillan and Abelson [4], and to Strassmann and Hahn [3], $KBrO_3$ in strong nitric acid solution has the correct oxidizing power to convert Np^{IV} to Np^{VI}. Hence the following procedure was followed.

The carbonate filtrate was acidified with strong nitric acid and the carbon dioxide boiled off. Some $Ce(SO_4)_2$ was added and the mixture boiled with $KBrO_3$. After cooling KIO_3 was added; generally a drop of $Ce(SO_4)_2$ solution was necessary to start the precipitation. The iodate precipitate was filtered off.

In preliminary experiments there was some evidence of a 6—10 hour activity, which later appeared to have a somewhat longer period, 11—12 hours (see below). However, it was first thought to belong to the fission product I^{135} [5], though it seemed strange that the iodine should appear in the product and not be expelled at an early stage of the treatment. It is a well-known fact that there is generally no exchange of iodine between I^- and IO_3^-.

It was therefore thought that if some KI was added to the filtrate, the iodine immediately liberated would ensure a carrier for the activity. The iodine could simply be boiled off. This course was adopted in the experiment, the decay curve of which is shown below. As was to be anticipated, this treatment was without effect.

Finally the solution was made ammoniacal. The residue of Ce, not precipitated by KIO_3, was sufficient as carrier for U and Np. The precipitate was washed thoroughly and ignited.

Alternatively, the precipitate may be dissolved in an acid if it is desired to make radiochemical investigations on neptunium, which may easily be separated from the inactive Ce.

EXPERIMENTAL *

Preparation of uranyl compound. The uranyl salicylaldehyde-*o*-phenylenediimine was prepared according to Pfeiffer *et al.*[6] When the complex was prepared from pure salicylaldehyde-*o*-phenylenediimine and pure uranyl acetate, it had a high degree of purity and no recrystallization was necessary. When prepared in this manner it contains one molecule of ethanol of crystallization.

Treatment before irradiation. 2 g of the uranyl compound is dissolved in 20 ml pyridine and shaken for 10 min. with 500 mg adsorption charcoal. The charcoal is filtered off by suction without washing. This treatment is repeated once.

Irradiation. The solution, which has been newly shaken with charcoal, is placed in the cyclotron behind about 3 cm paraffin near the internal beryllium target, which is bombarded with 6.5 MeV deuterons. The irradiation has usually been continued for 2—3 half-life periods of U^{239}.

Concentration of the activity. 100 mg adsorption charcoal is added to the irradiated solution, and the mixture is shaken for 5 min. The charcoal is filtered off by suction (the filtrate is saved for future experiments) and washed well with pyridine, which is finally sucked off.

Purification of the activity. The charcoal is transferred to a flask containing 5 ml saturated $(NH_4)_2CO_3$ solution. The flask is shaken for about one minute, a few drops of $Ba(NO_3)_2$ solution, corresponding to 5 mg $Ba(NO_3)_2$, are added, and the shaking is continued for some minutes. The charcoal is filtered off and washed with 2 ml saturated $(NH_4)_2CO_3$ solution. 3 ml HNO_3 is carefully added, and the CO_2 is boiled off. A few drops of a nitric acid solution of $Ce(SO_4)_2$, corresponding to some mg $Ce(SO_4)_2$, and subsequently about 10 drops of N-$KBrO_3$ are added to the boiling solution. The solution is cooled in ice water, and $Ce(IO_3)_4$ is precipitated by means of KIO_3 solution. Generally one or two drops of the $Ce(SO_4)_2$ solution are added at this point in order to facilitate the rapid precipitation of $Ce(IO_3)_4$. After some minutes' shaking the precipitate is filtered off without washing. Finally, the filtrate is made ammoniacal and boiled. The faint colourless precipitate is filtered off and washed with dilute ammonia.

* Much of the experimental work was performed by Mr. K. Halvarson, whom I wish to thank.

If the preparation is to be used for radiochemical purposes, the precipitate on the filter may be dissolved in a small volume of acid. The only inactive material in this solution will be a minute amount of Ce, which may be removed by an appropriate method.

In the present case, where only the activity was to be measured, the filter was ignited. The weight of the residue was about 1 mg. The uranium content in the ash was not determined, but to judge from the faint colour of the ammonia precipitate and the ash it cannot have exceeded 10 %.

The process was completed in 40—45 min., counted from the end of the irradiation.

For the purposes of β-spectrography the tenfold amount of pyridine solution was irradiated. Ten times the amounts of charcoal given above were used, but the amounts of the other reactants were not more than doubled, except $Ba(NO_3)_2$, the amount of which was not increased. The residue was 2—3 mg.

Measurement of the activity *. In order to establish the yield in one of the large-scale experiments a sample of the original irradiated pyridine solution was saved and its γ-activity compared with that of the product. The result, 12 % yield, represents a lower limit since all fission products still remained in the pyridine together with U and Np.

The decay was followed by means of a Geiger-Müller tube with a 12 μ mica window. The sample was covered with a thin film of nitrocellulose. The Al absorption equivalent of the mica window, the air, the nitrocellulose and the self-absorption in the sample was calculated as 32 μ. The decay was followed by this absorption and by the addition of 27 and 59 μ Al, that is by the total absorption equivalents of 32, 59, and 91 μ Al respectively. The decay curves from a small-scale experiment (irradiation 46 min.) are shown in fig. 1 and fig. 2.

DISCUSSION OF RESULTS

In the present case, where the decay constants are far apart, it is possible to treat the consecutive activities as quite independent. Apart from U^{239} and Np^{239} the curves indicate the presence of a 11.8 hour activity, the radiation of which is rather penetrating (no influence of the difference in absorption could be detected). For physical and chemical reasons it seems most probable that this radiation originates from the fission product Y with a period of 10 hours (probable mass number 93)[5]. Only a detailed study will confirm this. However, the physical investigations (β-spectrographic studies by Slätis [7]) were not disturbed by this impurity, and radiochemical investigations on Np will also be possible without separation since after only 2 days the foreign

* The activity measurements have been performed in collaboration with Prof. H. Slätis, to whom my gratitude is due.

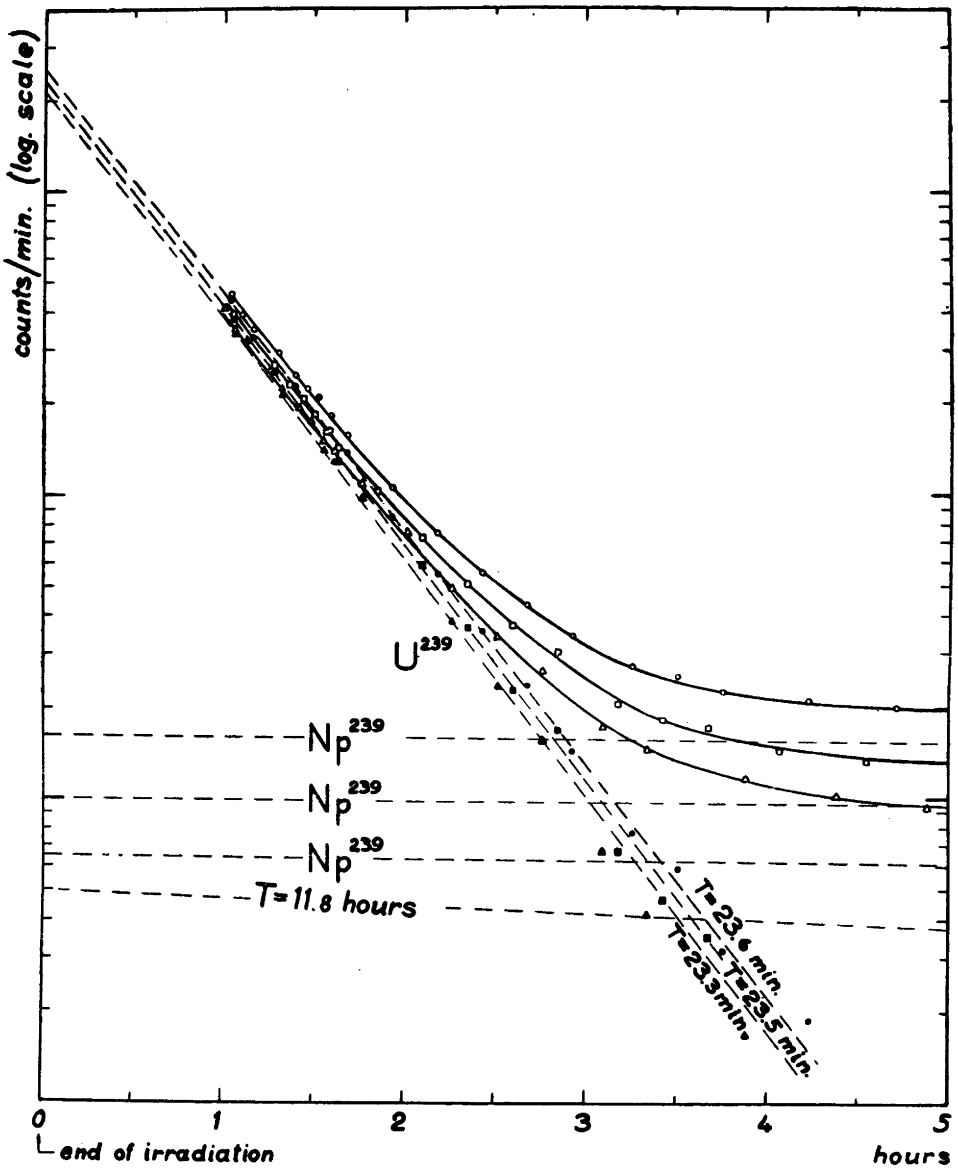


Fig. 1. Decay curves of U^{239} + Np^{239} with 32, 59, and 91μ Al absorption equivalents (first 5 hours).

component of the activity is only about 3 % if measured with 30 μ Al absorption and still less with smaller absorption.

At the ends of the curves there are certain indications of a longer period.

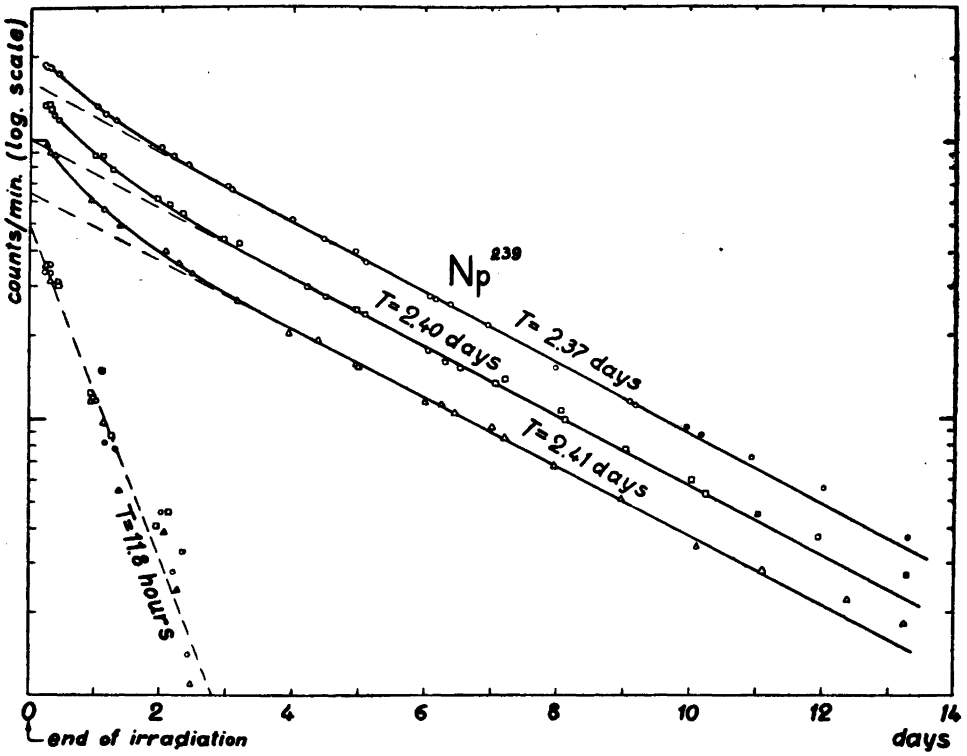


Fig. 2. Decay curves of U^{239} + Np^{239} with 32, 59, and 91μ Al absorption equivalents (the values for the first 5 hours not represented).

The influence of the absorption on this radiation cannot easily be settled, and it may be due to traces of UX_2 or U^{237} (formed by fast neutrons) as in the case reported by Starke [1].

The mean values of the half-life periods are found to be 23.5 min. for U^{239} and 2.39 days for Np^{239}.

The yield in the entire process, determined as above, was 12 % or more. In a large-scale concentration 7.6 g U (calculated as the element) is used. The U content of the product must be less than 0.7 mg. This together with the yield gives a concentration factor of 10^3 or more.

In order to gain information about the behaviour during the chemical operations of the Np already formed, the (extrapolated) activities at the end of the irradiation were extrapolated to the absorption 0 as shown in fig. 3. For purposes of comparison the figure includes the absorption curve of Np^{239} obtained by Segrè [8], to a certain extent confirmed by Starke [1]. The curve obtained in the present investigation (almost unchanged throughout three

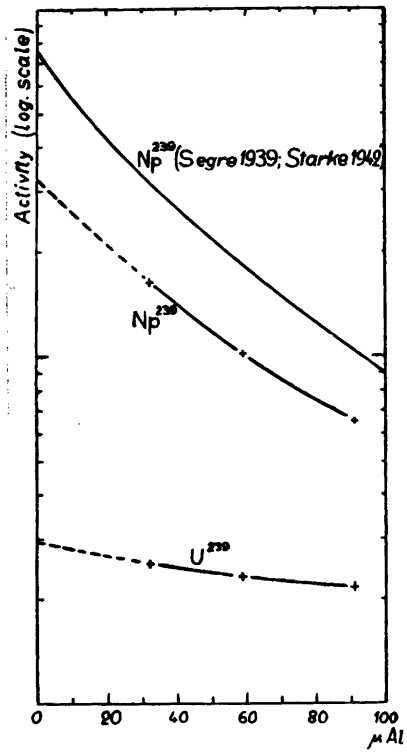


Fig. 3. Extrapolation of the (extrapolated) activities at the end of the irradiation to the absorption 0, compared with the absorption curve obtained by Segrè (confirmed by Starke).

half-life periods) corresponds to a somewhat smaller absorption coefficient than that found by Segrè.

If the extrapolation is performed as indicated in fig. 3, a ratio between the initial activities of Np and U of 1 : 89.8 is obtained, corresponding to an atomic ratio of about 1.60 : 1 if the period values 2.3 days and 23 min. are used. The theoretical atomic ratio (U + Np) : U after 46 min. uniform irradiation is 1.85. The extrapolation of the Np absorption curve is very uncertain, and if the strong curvature in the beginning of Segrè's curve is correct, a higher experimental value would have been obtained. The lower limit of the self-absorption in the sample has been used; an increase here will act in the same direction.

It therefore seems as if U and Np are concentrated in about the same proportions.

SUMMARY

The technique, proposed by Duffield and Calvin [2], of employing uranyl salicylaldehyde-*o*-phenylenediimine for the Szilard-Chalmers process on uranium, has been developed, and the method applied to the preparation of U^{239} and Np^{239}. A method for the purification of the product is described. The overall yield in the process is at least 12 %, about the same for U and Np, and the concentration factor at least 10^3. The product is somewhat contaminated by a 12 hour activity, which, however, does not influence physical and chemical applications.

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On the Crystal Structure of the Antimony Oxychloride $Sb_4O_5Cl_2$ and Isomorphous Oxybromide

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X-ray crystallography and electron diffraction have shown that trivalent antimony forms in the oxides and the halides typical covalent bonds with definite valence angles.

The antimony halides SbX_3 form pyramidical molecules, with Sb at the top of the triangular pyramid and the three X in the base plane. This was shown by Gregg, Hampson, Jenkins, Jones and Sutton [1] and by Hassel and Sandbo [2] by electron diffraction investigations on SbX_3 in the gaseous state. Gregg *et al.* found the following distances Sb — X and valence angles X-Sb-X: $SbCl_3$: 2.37 $\pm$ 0.02 kX and 104° $\pm$ 2°, $SbBr_3$: 2.52 $\pm$ 0.02 kX and 96° $\pm$ 2°, SbI_3: 2.75 $\pm$ 0.02 kX and 98° $\pm$ 2°. Hassel and Sandbo found 2.47 kX and 98° for $SbBr_3$ and 2.70 kX and 99° for SbI_3. Byström and Westgren [3] have carried out an X-ray analysis on SbF_3 in the solid state. Their structure gives the angles of antimony as 81.9° and 104.3°.

Trivalent antimony forms two types of oxides: the cubic, or low temperature modification, and the orthorhombic, or high temperature modification. They have been investigated by X-ray methods. Cubic Sb_2O_3 was first investigated by Bozorth [4], and was found to be built up from Sb_4O_6 molecules. The structure of orthorhombic Sb_2O_3 has been determined by Buerger and Hendricks [5]. It is composed of indefinitely long molecular chains of $(Sb_2O_3)_\infty$. The smallest Sb-O distances within molecules are all 2.00 kX. The valence angles O-Sb-O are 81°, 93°, and 99°, and the angles Sb-O-Sb are 116° and 132°. Later on more exact determinations of the parameters of the cubic Sb_2O_3 were made by Almin and Westgren [6]. They found an Sb-O distance of 2.0 $\pm$ 0.1 kX, which agrees well with the distance found by Buerger and Hendricks for the orthorhombic form. From Almin and Westgren's values O-Sb-O angles may be calculated as 97.2° and the Sb-O-Sb angles as 130.5°.

In the mineral nadorite, $PbSbO_2Cl$, however, antimony enters into MeO layers, where one antimony atom has four oxygen atoms as its nearest neighbours, which indicates an ionic binding [7]. Nadorite is closely related to the Me_2O_2X compounds which contain trivalent Bi, and which were investigated by Sillén [8]. These compounds form typical layer structures, which contain the same MeO layers as BiOX [9]. This suggests that antimony might form oxyhalides of the same types as does bismuth.

It therefore seemed of interest to investigate the oxyhalides of trivalent antimony, in order to obtaine some idea of the bond character of antimony in these compounds.

PREPARATION

Antimony oxyhalides have been prepared by numerous workers. Among the earlier investigators are Sabanejew (1871) [10] and Cooke (1877) [11]. A detailed study of the system Sb_2O_3-HCl-H_2O was made by Lea and Wood [12]. They examined both the action of hydrochloric acid on hydrated antimonous oxide and the action of water on antimonious chloride. They found SbOCl, $Sb_4O_5Cl_2$ and Sb_2O_3 only to be stable solid compounds. Two metastable compounds were observed. One was assigned the formula $Sb_4O_3(OH)_5Cl$, and $Sb_4O_3(OH)_3Cl_3$ was suggested for the other.

In the present investigation solid SbX_3 (X = Cl, Br, or I) was hydrolysed with various amounts of water either at room temperature or at about 50° C. In some cases Sb_2O_3 was dissolved in HX and then various amounts of water added. A precipitate was obtained, which crystallised after some time when X was Cl or Br. The precipitates were filtered off with suction and dried in an exsiccator with concentrated H_2SO_4 or P_2O_5 and then washed free from adhering SbX_3. $SbCl_3$ was removed with ether and $SbBr_3$ and SbI_3 with carbon disulfide.

It was found that $Sb_4O_5Cl_2$ gave quite good crystals suitable for X-ray investigation. The best crystals were obtained when about 10 g $SbCl_3$ were dissolved in 20 ml concentrated HCl and then diluted with 110 to 150 ml water. When this mixture was kept in a conical flask with a stopper at 50° C, crystals of a size $\leq$ 1 mm were obtained within 5 to 10 days. The extension of the crystals was of about the same magnitude in the three dimensions. When the same mixtures were kept at room temperature, crystals appeared only after a very long time. These crystals formed fairly thin plates of a distorted hexagonal shape. After fourteen months plates of a length of more than 5 mm were obtained. These different types of crystals proved to give identical powder photographs.

It was more difficult to obtain crystals of $Sb_4O_5Br_2$ suitable for X-ray investigation. As a rule they were too small to give good rotation photographs. Sufficiently large crystals were obtained when Sb_2O_3 and HBr were sealed into a glass tube and kept first at about 180° C for 10 days and then at room temperature for 18 days.

It seemed of interest to investigate $Sb_4O_5I_2$ at the same time as $Sb_4O_5Cl_2$ and $Sb_4O_5Br_2$, as they might be isomorphous. It would then be possible to obtain more exact information on the positions of the X atoms from $Sb_4O_5I_2$. I have, however, not yet been able to obtain crystals of $Sb_4O_5I_2$, though such crystals are reported by Cooke [11]. I have tried methods similar to Cooke's and in addition several other methods. On one occasion

crystals in the form of hexagonal plates appeared and showed hexagonal symmetry from Laue photographs. The Weissenberg photographs were not very good, and a closer investigation and chemical analysis have not yet been carried out.

ANALYSES

Antimony was determined by titration with 0.1 n $KBrO_3$*. The oxyhalide was dissolved in 25 % HCl and titrated at about 60° C using methyl red as indicator. At the equivalence point the solution was colourless.

Halogen was determined by boiling the oxyhalide with Na_2CO_3 solution in a platinum dish. The filtrate was acidified with HNO_3 and precipitated by $AgNO_3$. The silver halide was then filtered off, dried, and weighed.

	Antimony %	Halogen %
Calc. for $Sb_4O_5Cl_2$	76.34	11.12
Obs.	76.0, 75.7	11.23, 11.15
Calc. for $Sb_4O_5Br_2$	67.01	21.99
Obs.	66.3, 66.5	22.34, 22.87

UNIT CELL AND SPACE GROUP

Single crystals of the oxychloride and the oxybromide were picked out and set, and rotation photographs and the following Weissenberg photographs were taken: the photographs *h*0*l*, *h*1*l*, 0*kl*, 1*kl*, *hk*0, and *hk*1 for $Sb_4O_5Cl_2$ and the photographs *h*0*l*, *h*1*l*, *hk*0, and *hk*1 for $Sb_4O_5Br_2$. The compounds proved to be monoclinic.

The cell dimensions were determined more accurately from the powder photographs. Thus

$a = 6.229$ $b = 5.107$ $c = 13.50$ kX $\beta = 97.27°$ for $Sb_4O_5Cl_2$
$a = 6.593$ $b = 5.133$ $c = 13.43$ kX $\beta = 97.89°$ for $Sb_4O_5Br_2$

with an accuracy of about $\pm$ 0.05 % for the axes, and $\pm$ 0.05° for the angles.

If two formula units are assumed per unit cell the density would be:

	$Sb_4O_5Cl_2$	$Sb_4O_5Br_2$
$d_{calc.}$	4.94	5.33
$d_{obs.}$	4.98, 4.94, 4.95	5.19, 5.15, 5.25

The agreement is quite good.

* J. M. Kolthoff, Die Massanalyse, Berlin (1928) p. 443.

(Density determination: A sample was weighed in a small glass bulb, first in air and then in benzene. Air bubbles were driven out by evaporation. The temperature of the benzene was measured at each determination, and the corresponding density of benzene was used.)

In the Weissenberg photographs all reflections $h0l$ with l odd, and all reflections $0k0$ with k odd are absent. This is characteristic of the space group C_{2h}^5. It was observed that all reflections $h0l$ with $l = 2 + 4n$ are very weak.

STRUCTURE ANALYSIS

Patterson analysis

In order to obtain the positions of the eight antimony atoms of the cell, it seemed appropriate to apply Patterson analysis to $Sb_4O_5Cl_2$, where antimony is much heavier than the other atoms of the cell. The Patterson xz-projection and yz-projection were calculated. The values of $I(h0l)$ and $I(0kl)$ were estimated visually from the Weissenberg photographs in question. These I values were used instead of the F^2 values. Spots with large Θ were not taken into account (*cf.* Sillén)[13].

Because of the symmetry of space group C_{2h}^5, the parameters need only to be varied within the limits: $0 \leq x \leq 1$, $0 \leq y \leq \frac{1}{2}$, and $0 \leq z \leq \frac{1}{2}$. If positions 4(e) only are considered, it would be possible to make the limits even narrower for one set of parameters, thus for instance: $0 \leq x_1 \leq \frac{1}{2}$, $0 \leq y_1 \leq \frac{1}{4}$, $0 \leq z_1 \leq \frac{1}{4}$ and $0 \leq x_2 \leq 1$, $0 \leq y_2 \leq \frac{1}{2}$, $0 \leq z_2 \leq \frac{1}{2}$.

The xz-projection

In the xz-projection of space group C_{2h}^5 the following point positions are possible: 2(a) or 2(c) : 00, $0\,\frac{1}{2}$; 2(b) or 2(d) : $\frac{1}{2}\,0$, $\frac{1}{2}\,\frac{1}{2}$; 4(e) : $\pm\,(x,z)$, $\pm\,(x, \frac{1}{2} + z)$.

The Patterson xz-projection actually found is given in fig. 1, which is referred to during the following discussion.

On account of the symmetry it is necessary to calculate only the area $0 \leq x \leq \frac{1}{2}$, $0 \leq z \leq \frac{1}{2}$.

For the eight antimony atoms in the unit cell, all combinations involving twofold positions of different kinds can be excluded, as there is no peak in the projection corresponding to an interatomic vector at $(\frac{1}{2}\,\frac{1}{2})$. On the other hand, two twofold positions of the same kind may be regarded as a special case of 4(e). Therefore, only a combination of two fourfold positions 4(e) need be considered. Thus

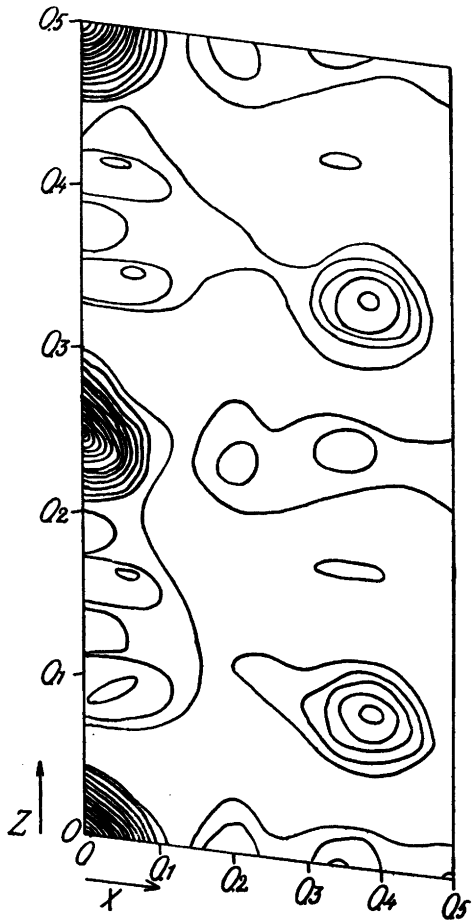

Fig. 1. Patterson projection P(xpz) for $Sb_4O_5Cl_2$

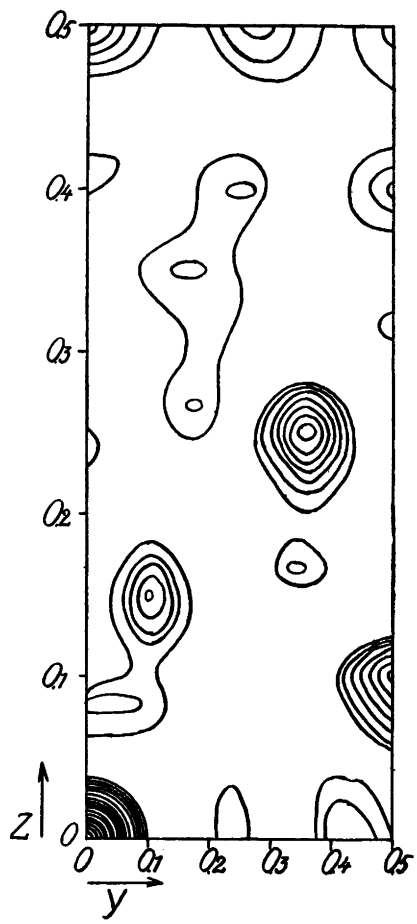

Fig. 2. Patterson projection P(pyz) for $Sb_4O_5Cl_2$.

$4Sb_1$ in $x_1,z_1\ \bar{x}_1,z_1\ \bar{x}_1,\frac{1}{2}-z_1\ x_1,\frac{1}{2}+z_1$
$4Sb_2$ in $x_2,z_2\ \bar{x}_2,\bar{z}_2\ \bar{x}_2,\frac{1}{2}-z_2\ x_2,\frac{1}{2}+z_2$

Peaks in the Patterson projection corresponding to interatomic vectors can be expected at the following points:
Distances $Sb_1 - Sb_1$ and $Sb_2 - Sb_2$:

I of weight 2 $\pm(2x_1,\ 2z_1)$
$\pm(2x_2,\ 2z_2)$
II of weight 2 $\pm(2x_1,\ 2z_1-\frac{1}{2})$
$\pm(2x_2,\ 2z_2-\frac{1}{2})$
III together of weight 8 $\pm(0\ \frac{1}{2})$

Distances $Sb_1 - Sb_2$

IV of weight 4 $\pm(x_1 - x_2, z_1 - z_2)$ $\pm(x_1 - x_2, z_1 - z_2 - \frac{1}{2})$
$\pm(x_1 + x_2, z_1 + z_2)$ $\pm(x_1 + x_2, z_1 + z_2 - \frac{1}{2})$

On inspecting more closely the limits of the parameters necessary to explain a Patterson xz-projection of space group C_{2h}^5, it was found from the symmetry that only x-parameters between 0 and $\frac{1}{2}$ need be considered, and that one z-parameter can be limited to $0 \leq z_2 \leq \frac{1}{4}$ and the other to $0 \leq z_1 \leq \frac{1}{2}$.

In the region $0 \leq x \leq \frac{1}{2}$, $0 \leq z \leq \frac{1}{2}$ two representatives of group I or II, two of group IV and the only vector of III will be found.

With the exception of the two high peaks at (00) and $(0\,\frac{1}{2})$, the Patterson projection reveals only three high peaks instead of the expected four. One is situated at $(0,\frac{1}{4})$ and is almost as high as the peaks at (0,0) and $(0,\frac{1}{2})$, which indicates that its weight must be eight. The other two which are lower, are found at $x \approx 0.4$, $z \approx 0.1$ and $x \approx 0.4$, $z \approx 0.35$. They have the same height and are therefore assumed to have the weight four. As the z-values 0.1 and 0.35 cannot be transformed into each other by $\pm\frac{1}{2}$, both peaks cannot belong to group IV. This shows that $2x_1$ must almost coincide with $+2x_2$ or $-2x_2$. The x values to be considered in the Patterson projection are 0, 0.4, 0.6, and 1.0.

The peak at $(0,\frac{1}{4})$ probably belongs to group IV, which will give it the weight 8, if $x_1 - x_2 = 0$ and $2x_1 \approx 2x_2 \approx 0.4$ or 0.6, which gives $x_1 \approx x_2 \approx 0.2$ or 0.3.

If it is assumed that $x_1 - x_2$, $z_1 - z_2$ is (0, 0,25) which gives $z_1 \approx z_2 + 0.25$, solutions of the following two types are possible:

A. Assuming $x_1 + x_2$, $z_1 + z_2$ is (0.4, 0.6) we have $x_1 \approx x_2 \approx 0.2$, $z_1 \approx 0.425$, $z_2 \approx 0.175$

B. Assuming $x_1 + x_2$, $z_1 + z_2$ is (0.4, 0.35) we have $x_1 \approx x_2 \approx 0.2$, $z_1 \approx 0.3$, $z_2 \approx 0.05$

From the yz-projection it will later on be evident that only solutions of type B are possible.

The yz-projection

From the xz-projection it seems probable that the antimony atoms occupy two fourfold positions 4(e). This gives the following point positions in the yz-projection:

4 Sb_1 y_1, z_1 $\bar{y}_1, \bar{z}_1$ $\frac{1}{2} + y_1, \frac{1}{2} - z_1$ $\frac{1}{2} - y_1, \frac{1}{2} + z_1$
4 Sb_2 y_2, z_2 $\bar{y}_2, \bar{z}_2$ $\frac{1}{2} + y_2, \frac{1}{2} - z_2$ $\frac{1}{2} - y_2, \frac{1}{2} + z_2$

Using the same group notations as in the preceeding section the corresponding maxima in the vector space are:

	Weight 1	
I	$\pm (2y_1, \pm 2z_1)$	$\pm (2y_2, \pm 2z_2)$
	Weight 2	
II	$\pm (\frac{1}{2}, 2z_1 + \frac{1}{2})$	$\pm (\frac{1}{2}, 2z_2 + \frac{1}{2})$
III	$\pm (2y_1 + \frac{1}{2}, \frac{1}{2})$	$\pm (2y_2 + \frac{1}{2}, \frac{1}{2})$
IV	$\pm (y_1 - y_2, \pm (z_1 - z_2))$	$\pm (\frac{1}{2} + y_1 - y_2, \frac{1}{2} \pm (z_1 + z_2))$
	$\pm (y_1 + y_2, \pm (z_1 + z_2))$	$\pm (\frac{1}{2} + y_1 + y_2, \frac{1}{2} \pm (z_1 - z_2))$

Just as for the xz-projection, it was found that the limits of the parameters for the Patterson yz-projection can be made narrower than for the spatial structure. Thus $0 \leq y_1 \leq \frac{1}{2}$, $0 \leq y_2 \leq \frac{1}{4}$, $0 \leq z_1 \leq \frac{1}{2}$ and $0 \leq z_2 \leq \frac{1}{4}$.

The Patterson projection was calculated for $0 \leq y \leq \frac{1}{2}$, $0 \leq z \leq \frac{1}{2}$. This area should contain eight peaks of weight 2 and two of weight 1. Now, excluding the high peak at (0,0) the projection contains four peaks, which are much higher than the others, namely at $(0, \frac{1}{2})$, $(\frac{1}{8}, 0.15)$, $(\frac{3}{8}, \frac{1}{4})$, $(\frac{1}{2}, 0.1)$, cf. fig. 2. The height of the last two seems to indicate a weight of 4, which was taken as a starting point.

At $y = \frac{1}{2}$ there are peaks at $z \approx 0.1$ (high), 0.4, 0.5, 0.6 and 0.9 (high), which should correspond to vectors of group II. Accordingly, possible values of z_1 and z_2 would be about 0, 0.05, 0.2, 0.3, 0.45 or 0.5. The xz-projection could be explained only by assuming $z_1 - z_2 \approx 0.25$ or $z_1 + z_2 \approx 0.25$ or 0.75*. This excludes the z-values 0 and 0.5. The possible z-combinations are therefore, $z_1 \approx 0.05$ or 0.45 $z_2 \approx 0.2$ or 0.3.

At $z = \frac{1}{2}$ there are peaks at $y \approx 0$, $\frac{1}{4}$, $\frac{1}{2}$, $\frac{3}{4}$ and 1.0, which should correspond to vectors of group III. This gives y_1 and $y_2 \approx 0$, $\frac{1}{8}$, $\frac{1}{4}$, $\frac{3}{8}$, or $\frac{1}{2}$.

The high peak at about $(\frac{3}{8}, \frac{1}{4})$, which, according to the possible z-values, must be of group IV and thus contain the sum or the difference between y_1 and y_2, indicates a combination of $y \approx \frac{1}{8}$ or $\frac{3}{8}$ with $y \approx 0$, $\frac{1}{4}$ or $\frac{1}{2}$. As the peaks at $y = \frac{1}{2}$ are not of the same height, it seems probable that one peak of group I coincides with (0.5, 0.1) or (0.5, 0.9). This would be possible if $y_1 \approx \frac{1}{4}$. From the previous discussion on the limits of the parameters, it is clear that we need only consider $y_2 \approx \frac{1}{8}$. The possible combinations are:

$$y_1 \approx \tfrac{1}{4} \quad z_1 \approx 0.05 \text{ or } 0.45$$
$$y_2 \approx \tfrac{1}{8} \quad z_2 \approx 0.2$$

* In the xz-projection the possibility of $z_1 + z_2 \approx 0.25$ or 0.75 was not discussed, as this would give x-parameters outside the given limits.

As $y_1 + y_2 \approx \frac{3}{8}$, the z-values 0.25 or 0.75 of the Patterson projection must be equal to $\pm (z_1 + z_2)$. This is only possible if $z_1 \approx 0.05$ and $z_2 \approx 0.2$. Thus we obtain: $y_1 \approx \frac{1}{4}$ $z_1 \approx 0.05$
$y_2 \approx \frac{1}{8}$ $z_2 \approx 0.2$

Now the parameter values from the two projections have to be combined into a structure in space. As the two Patterson projections can be explained independently the indices of the parameters were chosen arbitrarily. In combining the two projections the numerically equal z-values must be taken and not those with the same index. It should also be observed that the limits of the parameters are larger in space than in the Patterson projections. The parameter limits were discussed in the introductory part of the Patterson analysis. The narrowest space limits need not, of course, all belong to the same fourfold position.

From the xz-projection we obtain only two alternatives

$x_1 \approx 0.2$ $z_1 \approx 0.3$ and $x_1 \approx 0.8$ $z_1 \approx 0.2$
$x_2 \approx 0.2$ $z_2 \approx 0.05$ $x_2 \approx 0.2$ $z_2 \approx 0.05$

and from the yz-projection only one

$y_1 \approx \frac{1}{4}$ $z_1 \approx 0.05$
$y_2 \approx \frac{1}{8}$ $z_2 \approx 0.2$

(Other combinations give both z_1 and z_2 or both y_1 and y_2 larger than $\frac{1}{4}$). Thus we obtain as the only possible combination

$x_1 \approx 0.2$ $y_1 \approx \frac{1}{4}$ $z_1 \approx 0.05$
$x_2 \approx 0.8$ $y_2 \approx \frac{1}{8}$ $z_2 \approx 0.2$

where the indices are the same as in the yz-projection.

DISCUSSION OF INTENSITIES

In order to get more exact values for the parameters, these were varied around the approximate values obtained from the Patterson analysis, and the intensities were calculated. At first the influence of the halogen and the oxygen atoms was neglected.

For $h00$ the structure factor is

$$\frac{F_{Sb}}{4f_{Sb}} = \cos\ 2\ \pi\ hx_1 + \cos\ 2\ \pi\ hx_2.$$

As $|x_1| \approx |x_2| \approx 0.2$, it is simpler to write $\xi_1 = \frac{|x_1| + |x_2|}{2}$ and $\xi_2 = \frac{|x_1| - |x_2|}{2}$, thus giving $\frac{F_{Sb}}{4f_{Sb}} = 2 \cos 2\pi h \xi_1 \cos 2\pi h \xi_2$.

It is possible to fix ξ_1 to within fairly narrow limits as 0.197 for the oxychloride, whereas ξ_2 is more difficult to determine. Presumably $0 \leq \xi_2 \leq 0.015$. At first ξ_2 was assumed to be 0.

It is obvious that the antimony atoms are situated in two layers at $x \approx 0.2$ and $x \approx 0.8$. I thought it then probable that the ten oxygen and the four halogen atoms are also situated in layers, as, for instance, in the Me_2O_2X-compounds investigated by Sillén. Considerations of space as well as a rough intensity calculation of $h00$ for $Sb_4O_5Br_2$ make it probable that the four halogens are situated at $x \approx \frac{1}{2}$. However, they must occupy a fourfold position, as 2(b) and 2(d) with $x = \frac{1}{2}$ would give a distance halogen — halogen equal to $\frac{1}{2}b$ or about 2.5 kX, which seems very improbable. Then there is no room for oxygen atoms at $0.2 < x < 0.8$. It seems most probable that $x_0 \approx 0$. The ten oxygen atoms must then occupy one twofold position with $x = 0$, and two fourfold positions 4(e).

Assuming $\xi_2 \approx 0$, all Br at $x \approx \frac{1}{2}$, and all oxygen at $x \approx 0$, ξ_1 was determined to be about 0.181 for the oxybromide. Then the Br- and the Sb-parameters were varied alternately to obtain the best intensity agreement. When the intensities were calculated from $I \sim \frac{1 + \cos^2 2\Theta}{\sin 2\Theta} F^2$ it proved very difficult to obtain a satisfactory agreement for $I(100)$ and $I(200)$, which are fairly weak and seem to have almost the same intensities. On assuming all the ten oxygens at $x = 0$, $I(200)_{calc.}$ was much weaker than $I(100)_{calc.}$. The influence of varying the oxygen x-parameters was then examined. It was assumed that the two x_0 are nearly equal, as the two x_{Sb} seemed to be equal, and it would be difficult to make separate variations of the former, considering their low contribution to the intensities. The best intensity agreement was obtained with $\xi_1 = 0.185$, $x_{Br} = 0.515$ (0.485), 2 O at $x = 0$ and 8 O at $x = 0.11$, ξ_2 was assumed to be $= 0$.

The x-parameters of the oxychloride were then inspected more closely, which led to the conclusion that $\xi_1 = 0.197$, $0 < \xi_2 < 0.01$, $x_{Cl} = 0.515$ (0.485) and 2 O at $x = 0$, 8 O at $x = 0.13$.

For $00l$ the structure factor is

$$\frac{F_{Sb}}{4f_{Sb}} = \cos 2\pi l z_1 + \cos 2\pi l z_2.$$

As the y- and the z-axes of $Sb_4O_5Br_2$ are almost equal to those of $Sb_4O_5Cl_2$, it was assumed that the y- and z-parameters of the two compounds are nearly equal. Considering antimony only, the best agreement was preliminarily obtained with $z_1 = 0.049$ and $z_2 = 0.201$ for the oxychloride. As the reflections $h0l$ with $l = 2 + 4n$ are very weak for both the oxychloride and the oxybromide, it was assumed that the halogens cannot contribute much to these reflections. This would indicate $z_{hal.} \approx \frac{1}{8}$.

To determine the y-parameters of Sb more accurately, the reflections $0kl$ from a Weissenberg photograph of $Sb_4O_5Cl_2$ were used, as the few reflections $0k0$ were not sufficient. Assuming $y_1 = \frac{1}{4}$ and $y_2 = \frac{1}{8}$ we have $I(043)_{calc} \sim 0$. As the observed intensity is between medium and strong, this reflection cannot depend only on Cl and O. The best agreement was obtained with $y_1 = 0.225$ and $y_2 = 0.113$.

The $hk0$ reflection of $Sb_4O_5Br_2$ were then scrutinized. Here the contribution of Br to the intensities cannot be entirely neglected. A variation in the y-parameters of Sb indicates $y_{1Sb} = 0.220$ and $y_{2Sb} = 0.12$, when considering $0k0$ reflections, if it is assumed at the same time (1) that $F_{Br} \approx -F_{Sb}$ for 040, which is absent, and $F_{Br} \approx 0$ for 060, and (2) that F_{Br} and F_{Sb} have the same sign (negative) for 020. It seemed desirable to obtain as high a calculated intensity of 020 as possible. Values of y_{Br} fulfilling the conditions (1) are approximately 0.045, 0.205, 0.295, 0.455, 0.545, 0.705, 0.795 and 0.955. Of these values only 0.205, 0.295, 0.705 and 0.795 fulfil condition (2). If $y_{hal.} \approx 0.205$ and 0.295 are used for the oxychloride, and at the same time we assume $z_{Cl} \approx \frac{1}{8}$, $x_{Cl} \approx \frac{1}{2}$, a distance 2.1 kX (with more preliminary parameters 1.9 — 2.0 kX) is obtained for Sb — Cl, which is too small, as Sb — Cl is 2.37 $\pm$ 0.02 kX in $SbCl_3$[1]. These y-values were therefore rejected.

It is not possible to distinguish between $y_{Br} = 0.705$ and 0.795 by means of $hk0$. It is, however, possible to do so by means of hkl with l odd. When the other parameters were more definitely fixed, the influence of oxygen is, however, of little importance, a comparison was made between $I(012)$ and $I(013)$. $I(012)_{obs}$ was stronger than $I(013)_{obs}$. Taking the polarizing factor into account, the calculated values were:

1. $y_{Br} = 0.705 : I(012) \sim 17.1,\ I(013) \sim 8.6.$
2. $y_{Br} = 0.795 : I(012) \sim 17.1,\ I(013) \sim 25.6.$

This favours $y = 0.705$. When fixing the oxygen atoms, space considerations also seemed to favour $y = 0.705$.

THE OXYGEN POSITIONS

The rough parameter values of antimony and halogen were now used to determine the oxygen positions, which would render possible a more exact calculation of the parameters.

The Sb-O distance was assumed to be the same as in Sb_2O_3, where it is 2.0 kX, (*cf.* p. 178), and the sum of the ionic radii of oxygen and halogen are:

O—Cl	3.13 kX	(Goldschmidt),		3.21 kX	(Pauling),
O—Br	3.28 »	»		3.35 »	»
O—O	2.64 »	»		2.80 »	»

(Values of ionic radii are taken from *Internationale Tabellen* [14].)

Spheres of appropriate radii, inside which no oxygen ions could occur, were then drawn around the Sb and Cl (Br) centers, and cuts were made at $x = 0$ and $x = 0.13$ (chloride) $x = 0.11$ (bromide), since these x-parameters seemed probable for oxygen from the intensities of $h00$.

Cut $x = 0$. There are two twofold positions with $x = 0$, 2(a): (000), $(0\,\frac{1}{2}\,\frac{1}{2})$ and 2(c): $(00\,\frac{1}{2})$, $(0\,\frac{1}{2}\,0)$. This cut shows that only 2(c) is possible. It also seems as if some O — O distances would be rather short, if all oxygen atoms were placed at $x = 0$.

Cut $x = 0.11$ (0.72 kX) (bromide). Fig. 3 (The coordinates are referred to an orthogonal coordinate system, where the directions of the y- and the z-axis of the monoclinic cell and the orthogonal system coincide.) Possible oxygen

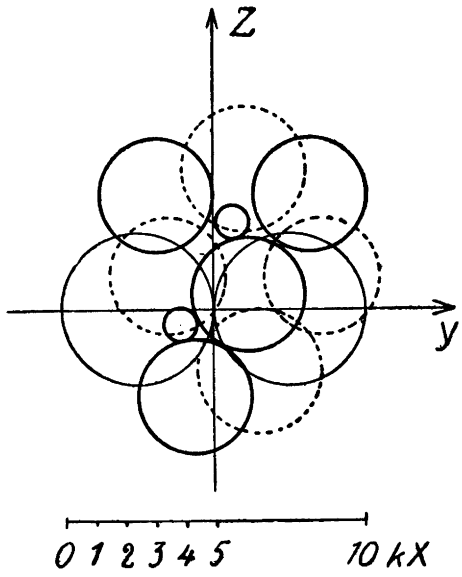

Fig. 3. $Sb_4O_5Br_2$. Spheres of appropriate radii are drawn around the Sb, Br, and O centers. Cut at $x = 0.11$. Thick circle = the Sb-O distance, dotted circle = the Br-O distance and thin circle = the O-O distance. Possible oxygen positions are approximatly: I y — 0.3 kx $z \approx$ — 0.9 kx, II $y \approx 0$, $z \approx 3$ kx and III $y \approx 1.5$ kx $z \approx 2.5$ kx.

positions are approximately I. $y \approx -0.3$ kX, $z \approx -0.9$ kX, II. $y \approx 0$, $z \approx 3$ kX and III. $y \approx 1.5$ kX, $z \approx 2.5$ kX. It does not seem possible to combine II and III. With the preliminary coordinates there would be a distance O — O about 1.6 kX. The oxygen can, however, be moved, so that this distance will become larger or about 2.25 kX. It might be possible to make it even larger, but this does not seem to be of interest, as these oxygen positions appear to give a rather improbable coordination around Sb_1. It is seen from fig. 4 that between two adjacent Sb_1 (distance between them 3.4 kX) there are no other negative charges than Br at distances 3.3 and 3.5 kX.

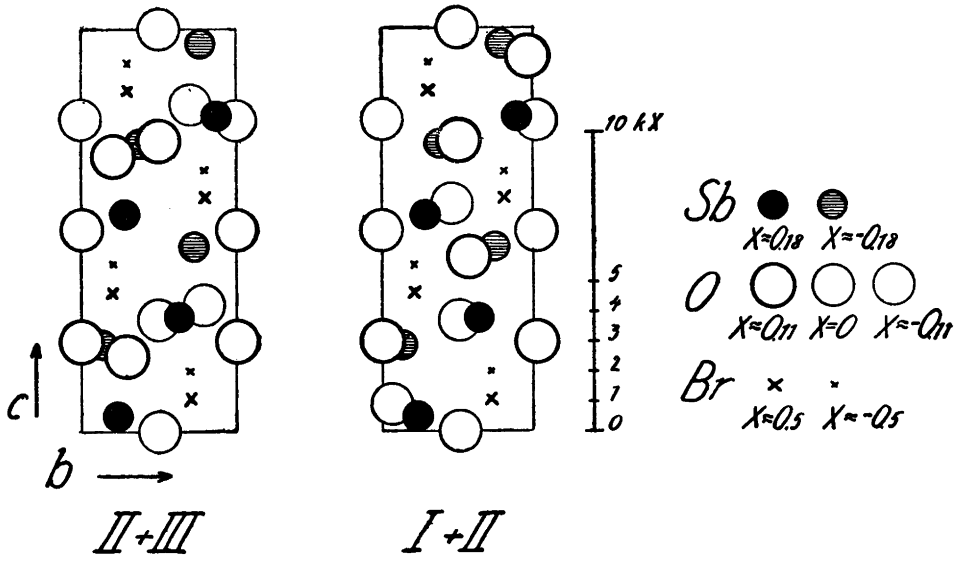

Fig. 4. Orthogonal projections of the unit cell of $Sb_4O_5Br_2$, giving the possible oxygen positions which could later on be excluded.

This would mean that only a combination of I and II or I and III is possible. Now the distance between two oxygen atoms of O_I would be about 2.4 kX, which seems rather short. If O_I is moved to $x \approx 0.6$, $y \approx 0.2$ and $z \approx -1.2$ kX, this distance will be better (about 2.7 kX), though the distance O_I-Br will be a little short. This was however thought to be less objectionable, as the halogens are more easily deformed. If I is combined with II the O — O distance of two adjacent oxygens of I and II would be about 2.6 kX, if the coordinates $x \approx 0.72$, y ≈ -0.3, $z \approx -0.9$ kX are used for O_I, and about 2.2 kX with the coordinates $x \approx 0.6$, $y \approx 0.2$, $z \approx -1.2$ kX. There seems to be some difficulty in obtaining good O — O distances with a combination of I and II. A projection of this configuration is shown in fig. 4.

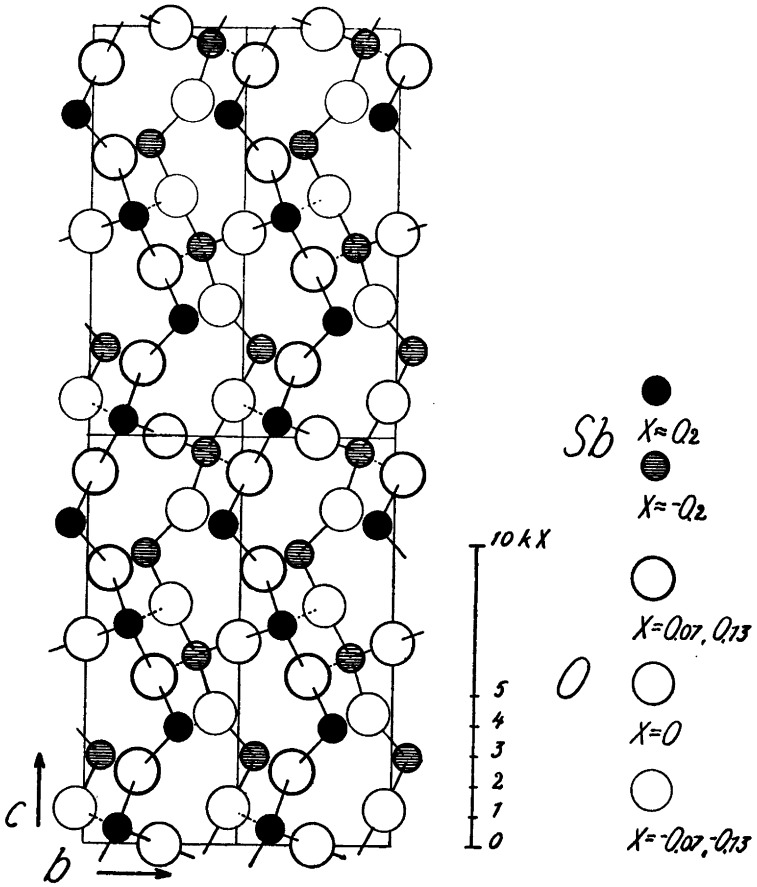

Fig. 5. Orthogonal projection of four adjecent unit cells of $Sb_4O_5Cl_2$ on $x = 0$, showing the Sb-O layer. The Cl-atoms have been omitted, to make the Sb-O chains more obvious.

A combination of I and III gives quite reasonable O — O distances. The shortest distance $O_I - O_{III}$ is about 2.5 kX for both positions of O_I. A projection of this configuration is shown in fig. s 5 and 6. (This is for $Sb_4O_5Cl_2$ with the final parameters).

In I + II there will be no oxygen or halogen atoms immediately between Sb_1 and Sb_2. In I + III, however, there is one oxygen between them. This seems to favour I + III, especially as Sb — O chains will be formed in I + III, which seems fairly plausible, as the antimony atoms do in fact appear to form chains.

To fix the x-parameters better, cuts were later on made at $z \approx 2.4$ kX and $z \approx -1.2$ kX, when the other atomic positions had been determined more accurately. This gave the following oxygen positions:

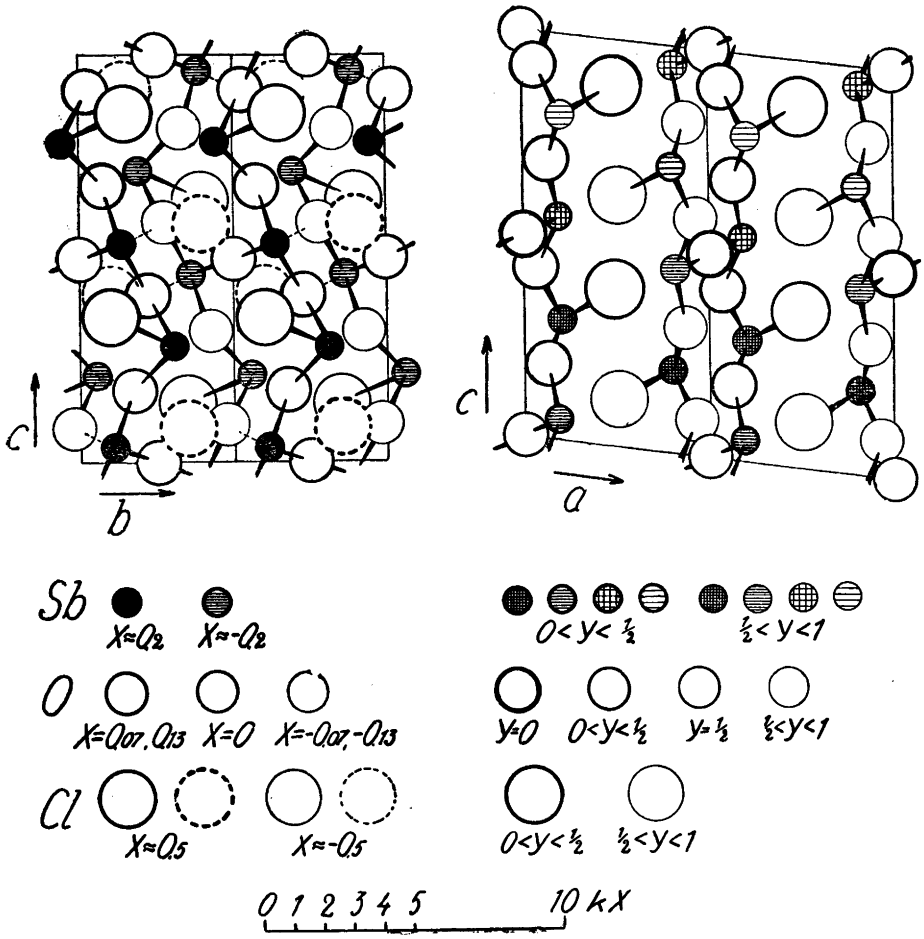

Fig. 6. Orthogonal projections of the structure of $Sb_4O_5Cl_2$ on $x = 0$ ($-\frac{1}{2} \lessapprox x \lessapprox \frac{1}{2}$) and on the xz-plane.

$Sb_4O_5Br_2$: O_1 2 (c) (00 ½), (0 ½ 0) O_2 4 (e) $x = 0.13$, $y = 0.37$, $z = 0.187$ O_3 4 (e) $x = 0.08$, $y = 0.04$, $z = -0.084$.

and similarly

$Sb_4O_5Cl_2$: O_1 2 (c) (00 ½), (0 ½ 0) O_2 4 (e) $x = 0.13$, $y = 0.35$, $z = 0.185$ O_3 4 (e) $x = 0.07$, $y = 0.05$, $z = -0.085$.

FURTHER DISCUSSION OF INTENSITIES

When a more exact intensity calculation was carried out for *hk*0 of $Sb_4O_5Br_2$, it was found impossible to obtain good agreement for the weak

reflections $h10$ as long as it was assumed that $|x_{1Sb}| = |x_{2Sb}|$. Assuming them to be unequal, the best agreement was obtained with:

Sb_1 at $x = 0.174$, $y = 0.225$
Sb_2 at $x = -0.194$, $y = 0.125$
Br at $x = 0.51$, $y = 0.705$

Table 1 shows that the agreement between observed and calculated intensities for the Weissenberg photograph $hk0$ is quite good.

Table 1. Calculated and observed intensities of hk0 from Weissenberg photograph of $Sb_4O_5Br_2$. CuKa radiation.

k	$0k0$	$1k0$	$2k0$	$3k0$	$4k0$	$5k0$	$6k0$	$7k0$	$8k0$
0		1.9 m.+	2.8 m.+	64 v.st.	1.2 w.+	9.2 st.—	41 st.	9.4 m.	13 st.
1	— —	1.2 w.—	1.6 w.	1.8 w.—	1.4 w.	1.8 w.—	3.9 w.	4.1 m.—	3.1 m.
2	18 st.	0.7 v.w.	0.2 —	24 st.	0.4 —	0.3 —	21 st.	0.7 —	
3	— —	20 st.	10 st.—	2.5 w.+	29 st.—	4.2 m.—	5.5 m.	27 st.	
4	0.1 —	0.5 —	4.2 w.+	0 —	0 —	4.1 w.+	2.5 w.+		
5	— —	17 m.	11 m.	1.6 w.	17 st.—	2.9 m.+			
6	2.0 w.	0.9 —	2.1 w.+	2.8 m.+					

Similarly for $Sb_4O_5Cl_2$ (table 4):

Sb_1 at $x = 0.186$, $y = 0.225$
Sb_2 at $x = -0.204$, $y = 0.113$
Cl at $x = 0.51$, $y = 0.703$

Table 2. Calculated and observed intensities of h0l from Weissenberg photograph of $Sb_4O_5Br_2$. CuKa radiation.

l	$00l$	$10l$	$20l$	$30l$	$40l$	$50l$	$60l$	$70l$	$80l$
16	9.2 v.w.	23 m.—							
14	0 —	3.6 —	0.3 —	2.2 v.w.					
12	44 w.+	8.1 v.w.	18 w.+	35 w.+	22 m.—	17 m.			
10	1.9 —	0 —	0.7 —	0.3 —	0.7 —	0 —	0.8 w.		
8	11 w.	63 m.—	4.9 v.w.	16 w.+	44 m.—	17 w.+	15 m.—		
6	0.8 v.v.w.	0 —	0.7 —	0.4 —	0 —	3.1 v.v.w.	3.6 v.v.w.	0.8 v.w.	
4	0 —	4.1 w.—	51 m.	7.4 w.+	17 m.	45 m.+	14 w.+	15 m.—	
2	0 —	0.1 —	0.1 —	0 —	0.7 v.v.w.	1.3 v.v.w.	2.9 v.w.	4.8 v.w.	5.5 w.
0		1.9 w.	2.9 w.	64 st.	1.3 v.w.	9.2 m.—	41 m.	9.4 w.+	13 m.
—2		0 —	3.0 v.v.w.	0 —	0.4 —	8.5 w.	0.6 v.v.w.	2.2 v.w.	12 w.+
4		61 st.—	0.1 v.v.w.	5.2 v.w.	59 m.	1.5 —	13 w.+	46 m.	4.6 v.w.
6		0.1 —	0 —	7.1 v.w.	0 —	0.3 —	13 w.	2.5 —	5.0 w.
8		0.1 —	69 m.	4.9 —	0.8 —	45 w.+	0.2 —	8.1 w.+	
10		2.7 —	0 —	0.1 —	14 v.w.	0 —	0.1 —	21 m.—	
12		7.8 w.	0 —	58 w.+	4.1 —	3.0 —	49 m.—	0.5 —	
14		1.3 —	10 —	0 —	0.4 —	22 w.	0 —		
16		40 w. +	16 w.	0.8 —	40 m.				

Table 3. Calculated and observed intensities of h1l from Weissenberg photograph of $Sb_4O_5Br_2$. CuKa radiation.

l	01*l*	11*l*	21*l*	31*l*	41*l*	51*l*	61*l*	71*l*	81*l*
16	0.3 —	0.4 —							
15	3.5 w.	2.8 w.	1.2 v.w.						
14	13 w.+	33 m.—	11 m.	13 st.					
13	0.7 v.w.	4.2 w.	1.4 v.v.w.	2.9 w.	1.8 v.w.				
12	3.6 v.w.	0 —	0.7 —	1.2 v.w.	0.1 v.v.w.	1.3 v.w.			
11	0 —	3.5 w.	3.5 w.	1.4 v.w.	4.4 w.+	1.9 v.w.			
10	2.7 v.w.	8.5 w.+	34 m.+	13 m.	12 m.+	23 st.	24 st.		
9	0 —	3.0 w.	9.0 w.+	0.1 v.v.w.	2.1 w.	1.8 v.v.w.	4.4 w.+		
8	0.8 —	1.6 v.w.	0.4 v.w.	1.7 v.w.	0 —	1.6 w.+	2.6 w.		
7	8.3 m.	0 —	2.4 w.	2.8 w.—	2.4 w.	3.6 w.+	1.1 v.v.w.	4.6 m.	
6	52 v.st.	0.8 v.w.	6.4 m.	42 v.st.	6.9 m.	8.1 m.+	25 st.	17 st.	
5	4.4 m.	0.3 v.v.w.	3.1 w.+	5.3 m.	1.7 w.	5.9 m.	2.8 w.	4.5 w.+	
4	0 —	0.4 v.v.w.	0 —	0.6 w.	0.8 w.	0.4 —	3.0 w.+	2.3 w.+	
3	1.2 m.—	6.2 m.	0.1 —	2.6 m.	3.6 m.	2.0 w.	2.1 w.+	0.9 w.	
2	1.6 m.+	52 v.st.	0.8 w.	5.8 st.	44 v.st.	5.5 w.+	9.4 m.—	31 st.	11 st.
1		8.8 st.	0.2 v.v.w.	2.2 m.	5.8 m.	0.4 w.	3.0 w.+	0.6 w.	5.9 m.+
0		1.0 m.	1.4 m.—	1.6 m.—	1.5 w.	1.9 w.	4.1 w.	4.1 m.—	3.0 m.+
—1		1.4 w.	8.3 m.—	0 —	1.2 w.	2.3 w.	3.2 w.+	2.6 w.+	1.2 —
2		0.4 —	51 st.	0 —	3.1 m.	40 v.st.	2.7 w.	6.4 m.—	24 st.
3		0.6 v.w.	5.5 m.—	0.1 —	6.6 m.—	7.6 w.+	0.4 —	7.1 m.—	2.6 v.v.w.
4		1.0 v.v.w.	0.9 w.	0.3 v.v.w.	3.5 w.	2.6 w.	1.6 w.	7.1 m.—	7.2 m.—
5		0 —	1.5 w.	6.6 m.	0.4 —	1.8 w.	2.7 w.	2.9 w.	1.4 w.
6		2.8 m.—	0.2 —	51 st.—	0.2 —	3.1 w.	41 st.	0.5 —	5.5 m.
7		2.7 w.	0.6 v.w.	6.1 m.—	0.2 —	3.6 w.	3.5 w.	0.9 —	6.7 m.
8		0.3 —	6.7 w.	0.5 —	0.4 —	12 w.	1.4 v.w.	1.2 w.	
9		7.9 w.	0.4 —	0.4 —	5.5 v.w.	0.4 —	0.8 w.	1.4 w.	
10		42 m.	3.8 w.	0 —	41 w.+	0.7 —	2.3 w.	32 st.	
11		6.6 w.—	1.9 w.—	2.0 v.v.w.	7.2 w.—	0.1 —	7.4 w.+	7.2 m.+	
12		0.6 —	0 —	8.8 w.—	1.1 —	0.3 —	17 m.		
13		0 —	5.5 w.—	0.1 —	2.1 —	7.2 w.	0.4 —		
14		3.5 v.w.	37 w.+	7.7 w.—	0 —	34 st.—			
15		0.2 —	7.4 w.	3.5 w.—	1.2 —				
16		10 w.	0 —						

As the x-parameters now seemed to be fixed satisfactorily, the z-parameters were determined more accurately from the $h0l$ reflections in the Weissenberg photographs. It proved, however, rather difficult to obtain very satisfactory agreement. The reflections $00l$ with $l = 2 + 4n$, which are weak, could not be explained as depending entirely on oxygen. Therefore, $z_{1Sb} + z_{2Sb}$ could not be exactly 0.25 and $z_{hal.}$ could hardly be exactly $\frac{1}{8}$. However, small variations of z_{1Sb} around 0.05 and z_{2Sb} around 0.20 do not influence the $00l$ reflections very much.

The intensities were calculated for different $z_{hal.}$, and it seemed probable that $z_{hal} < \frac{1}{8}$. Finally the parameters of the oxybromide giving the best calculated intensities were chosen as $z_{1Sb} = 0.051$, $z_{2Sb} = 0.203$ and $z_{Br} = 0.118$.

The calculated and observed intensities of $h0l$ from the Weissenberg photograph of the oxybromide are given in table 2. Considering the general weakening of all reflections in the vicinity of $00l$ the agreement is good.

The calculated and observed intensities of $h1l$ of the oxybromide are given in table 3. Here the agreement seems to be very satisfactory.

Table 4. Intensities of hk0 from Weissenberg photograph of $Sb_4O_5Cl_2$. CuKa radiation. The calculated intensity is given on the left of each column, and the intensity estimated for Patterson analysis on the right.

k	0*k*0		1*k*0		2*k*0		3*k*0		4*k*0		5*k*0		6*k*0		7*k*0	
0			4.6	50	12	60	38	90	3.0	8	24	50	13	17	21	50
1	—	—	1.8	17	2.1	3	0.4	—	1.8	3	2.2	3	0.2	—	5.3	17
2	8.1	100	0	—	1.9	17	12	50	0	—	2.2	8	8.5	22	1.1	5
3	—	—	25	100	9.8	50	8.8	30	33	60	0.6	3	19	50	17	60
4	0	—	0	—	1.7	6	0	—	0.4	3	0.6	3	3.2	15		
5	—	—	10	45	5.0	25	2.3	20	10	50	0.2	15				
6	7.9	22	1.3	1	7.6	30	6.4	40								

Table 5. Intensities of h0l from Weissenberg photograph of $Sb_4O_5Cl_2$. CuKa radiation. The calculated intensity is given on the left of each column, and the intensity estimated for Patterson analysis on the right.

l	00*l*		10*l*		20*l*		30*l*		40*l*		50*l*		60*l*		70*l*	
16	1.8	6	35	80	18	40										
14	0	—	1.0	2	0	—	2.9	7	0	—						
12	33	90	3.0	12	31	80	17	25	20	50	24	50				
10	1.0	5	0	—	0.3	—	0	—	1.1	3	0	—				
8	18	95	45	100	2.4	7	29	50	18	25	21	40	23	50		
6	0.2	5	0	—	0.9	—	0.5	—	0.1	—	3.5	5	1.0	—	1.6	7
4	1.7	40	12	15	31	60	6.7	25	32	95	17	40	22	40	21	50
2	0	—	0.1	—	0.2	—	0.1	3	0.1	2	2.1	5	4.2	7	0.7	—
0			4.6	50	12	60	38	90	3.0	8	24	50	13	17	21	40
—2			0.1	—	1.8	2	0	—	1.0	3	3.4	4	1.2	5	3.3	7
4			45	40	0.4	—	20	15	35	40	5.0	4	31	45	14	17
6			0.1	—	0.1	—	2.9	—	0.2	—	1.4	—	3.6	3	5.3	6
8			2.6	10	53	22	3.6	—	12	6	26	15	4.6	6	27	40
10			0.8	—	0	—	0.2	—	6.7	3	0.1	—	1.6	1	5.8	17
12			11	40	5.9	13	48	50	1.5	5	21	20	30	40		
14			0.2	5	4.4	5	0	—	0.3	—	9.6	15				
16			36	80	18	50	3.5	15								

Variations for the oxychloride gave $z_{1Sb} = 0{,}049$, $z_{2Sb} = 0.203$ and $z_{Cl} = 0.115$ as the most probable values. The calculated and observed intensities of $h0l$ from the Weissenberg photograph are given in table 5. There appeared at first to be a certain disagreement between the observed and calculated intensities of 004 and 104. $I(004)_{calc.} \sim 1.7$ and $I(104)_{calc.} \sim 12$, whereas $I(004)_{obs.}$ is definitely stronger than $I(104)_{obs.}$ in the Weissenberg photograph. This could not be overcome by small variations of the parameters since the indices were low. From the powder photograph (table 8), it was, however found that $I(004)$ must be lower than $I(104)$, in spite of the fact that their $\sin^2\Theta$ coincide with others. There seems to be a general enhancement of the observed intensities of $00l$, and a general weakening in other parts, especially in the neighbourhood of the zone $n0\overline{4}n$ in the Weissenberg photograph. Taking these facts into consideration, the agreement between calculated and observed intensities is fairly satisfactory.

The $0kl$ intensities of the chloride were then calculated. Table 6 shows that the agreement between calculated and observed intensities is quite good.

Table 6. Intensities of 0kl from Weissenberg photograph of $Sb_4O_5Cl_2$. CuKa radiation. The calculated intensity is given on the left of each column, and the intensity estimated for Patterson analysis on the right.

l	$00l$		$01l$		$02l$		$03l$		$04l$		$05l$		$06l$	
0			—	—	8.3	40	—	—	0	—	—	—	7.9	20
1	—	—	1.0	15	9.8	90	1.4	14	0	—	0.2	9	1.9	18
2	0	—	4.8	15	6.2	90	0	2	25	115	0	—	0.1	3
3	—	—	1.9	6	0.7	4	0.2	1	9.6	65	16	85	18	110
4	1.7	50	0.1	—	0	1	29	115	0.2	3	11	85	1.3	4
5	—	—	3.4	6	1.2	4	5.5	60	2.6	14	4.8	40	3.1	40
6	0.2	6	36	40	1.0	3	0.2	3	2.4	14	0.7	—	0.3	—
7	—	—	8.4	40	2.8	6	4.4	14	0	3	0.8	2		
8	18	120	1.1	3	2.3	5	13	60	0.5	3	6.4	40		
9	—	—	0.2	—	3.6	14	0.8	7	2.5	14	20	80		
10	1.0	6	0	—	11	40	0.8	1	28	90	0	1		
11	—	—	0	—	11	40	1.9	7	0.5	2	6.6	80		
12	33	115	1.4	4	9.0	40	8.8	40	0.2	6				
13	—	—	2.0	12	11	30	1.3	13	0.4	6				
14	0	—	18	80	5.0	12	0	—						
15	—	—	4.5	12	0.2	6	2.8	13						
16	1.8	7	0.2	—	1.2	6								

Tables 7 and 8 give the observed and calculated $\sin^2\Theta$ and intensities of the powder photographs of $Sb_4O_5Cl_2$ and $Sb_4O_5Br_2$.

Table 7. Powder photographs of $Sb_4O_5Br_2$. CrKa radiation.

hkl	$\sin^2\Theta$ calc.	$\sin^2\Theta$ obs.	*I* calc.	*I* obs.	*hkl*	$\sin^2\Theta$ calc.	$\sin^2\Theta$ obs.	*I* calc.	*I* obs.
012	0.0791	0.0790	3.0	v.v.w.	$12\bar{3}$	.2837	(.2856)	39	(w.+)
110	.0803		2.4		115	.2858		0.8	
$11\bar{1}$	.0836	.0833	3.2	v.v.w.	123	.3082	.3097	6.7	v.w.
111	.0917	.0916	19	m—	$21\bar{5}$	.3163	(.3166)	3.4	(st.—)
$11\bar{2}$	.1016	—	0.5	—	016	.3163		105	
013	.1159	—	2.2	—	024	.3172		1.3	
004	.1178	.1181	0	st.	$31\bar{1}$	.3201	.3216	0	w.
112	.1179		106		$22\bar{1}$	.3203		8 2	
200	.1224	.1226	2.9	v.v.w.	220	.3211		0.3	
$10\bar{4}$	.1321	(.1321)	61	(st.)	106	.3217		0	
$11\bar{3}$	.1343	—	1.1	—	$11\bar{6}$	.3223		5.6	
$20\bar{2}$	.1355	—	3.0	—	214	.3232		0	
113	.1588	.1588	12	v.w.	310	.3250	(.3268)	3.5	(v.w.)
104	.1647	(.1647)	4.0	(v.w.)	302	.3295	—	0	—
014	.1675	—	0	—	$31\bar{2}$	.3301	—	0	—
202	.1681	—	0.2	—	$12\bar{4}$	.3314	.3320	44	m.
$21\bar{1}$	.1713	(.1711)	18	(w.+)	$22\bar{2}$	.3343	—	0.5	—
210	.1721		3.2		221	.3366	.3381	4.6	v.w.
$11\bar{4}$	.1818	—	1.6	—	$20\bar{6}$	.3399		0	
$21\bar{2}$	.1852	.1850	101	st.—	311	.3446	.3452	4.2	w.
211	.1876	—	0.6	—	$30\bar{4}$	.3447		5.2	
020	.1987	.1987	18	w.—	$31\bar{3}$	.3548	—	0.5	—
021	.2061	.2054	26	w.	$22\bar{3}$	.3632	—	0.5	—
$20\bar{4}$	.2076	—	0.1	—	124	.3641	—	0.7	—
$21\bar{3}$	.2139	(.2139)	10	(v.w.)	222	.3670	.3682	24	w.+
114	.2148		0.6		116	.3714	—	1.8	—
212	.2178	—	1.9	—	312	.3791	.3807	12	w.
022	.2282	(.2278)	9.3	(m.—)	025	.3838	—	1.1	—
120	.2293		1.5		$21\bar{6}$	.3895	—	0.5	—
$12\bar{1}$	.2326	(.2339)	0	(v.v.w.)	$12\bar{5}$	3940	(.3944)	39	(w.+)
015	.2338		7.4		$31\bar{4}$	.3944		0.5	
					215	.3981	—	6.7	—
121	.2408	—	0	—	$22\bar{4}$	.4068	—	0.2	—
$11\bar{5}$	.2449	—	0	—	223	.4122	(4129)	34	(m.—)
$12\bar{2}$	.2507	.2514	9.5	v.w.	017	.4125		17	
$21\bar{4}$	.2578	(.2579)	1.7	(v.v.w.)	$11\bar{7}$	.4145		5.8	
213	.2632	(.2628)	0.2	(w.)	313	.4284	(.4292)	4.4	(w.)
023	.2654	(.2671)	0.2	(v.w.)	125	.4349	—	8.7	—
006	.2665		0.8		206	.4380	(.4396)	0.8	(v.w.)
122	.2671		2.3		304	.4429	(.4441)	7.4	(v.w.)
$10\bar{6}$	.2726	—	0.1	—	$31\bar{5}$	.4487	(.4486)	13	(w.)
204	.2736	(.2760)	50	(st.)	031	.4545	—	1.2	—
300	.2753		64		$22\bar{5}$	.4653	—	2.7	—
$30\bar{2}$	.2804	—	0	—	026	.4653	—	3.4	—

hkl	$\sin^2\Theta$ calc.	$\sin^2\Theta$ obs.	*I* calc.	*I* obs.	*hkl*	$\sin^2\Theta$ calc.	$\sin^2\Theta$ obs.	*I* calc.	*I* obs.
$30\bar{6}$	.4683	(.4694)	7.1		$23\bar{1}$	.5680	.5688	26	
$32\bar{1}$	.4691		27	(w.)	230	.5689		20	w.
$12\bar{6}$	.4713		15		315	.5720	—	10	—
117	.4717	—	0	—	323	.5778	(.5789)	1.9	
$10\bar{8}$	.4718	—	0.1	—	$13\bar{4}$	.5786		2.2	(v.v.w.)
224	.4723	.4739	47	m.	$21\bar{8}$	.5788		13	
$13\bar{1}$	.4736		0.1		$23\bar{2}$	.5819	—	7.4	—
008	.4739		11		231	.5845	—	2.2	—
320	.4740		47		118	.5860	—	3.2	—
032	.4767	—	1.5	—	217	.5919	.5918	4.6	v.v.w.
$21\bar{7}$	.4776	.4789	1.1	w.	$41\bar{4}$	.5919		6.7	
130	.4777		39		$32\bar{5}$	.5968	—	9.2	—
$32\bar{2}$	.4791		18		$31\bar{7}$	.6005	—	12	—
131	.4818		25		412	.6024	(.6030)	88	(m.—)
$40\bar{2}$	.4863	—	0.4	—	$23\bar{3}$	.6106	.6117	31	w.
216	.4877	.4894	13	w.—	134	.6117		31	
400	.4894		1.3		232	.6149	—	0.5	—
314	.4925	—	1.2	—	306	.6160	—	0.4	—
321	.4937	.4944	20	w.—	127	.6198	.6198	28	v.w.
$13\bar{2}$	.4991	(.4999)	4.0	(w.—)	$22\bar{7}$	.6250	.6250	30	w.—
$32\bar{3}$	.5038	—	1.7	—	035	.6310	.6320	26	v.v.w.
033	.5137	—	0.4	—	226	.6364	—	7.9	—
132	.5155	.5178	8.2	m.	$13\bar{5}$	.6410	(.6423)	1.4	
$31\bar{6}$	.5179		100		$11\bar{9}$	.6413		16	
126	.5204	—	13	—	324	.6419		1·5	(v.w.)
$11\bar{8}$	.5215	—	0.7	—	$41\bar{5}$	.6419		0.8	
018	.5236	—	1.6	—	$30\bar{8}$	.6493	—	5.0	—
322	.5284	—	12	—	$23\bar{4}$	.6540	(.6542)	45	
$20\bar{8}$	.5292	.5305	69	v.w.	413	.6559		6.3	(w.)
$41\bar{1}$	.5307		2.2		$40\bar{6}$	.6570	—	0	—
$13\bar{3}$	.5310		6.7		233	.6601	—	0	—
$41\bar{2}$	.5363	.5357	6.1	w.	208	.6613	—	4.9	—
108	.5364		61		316	.6656	(.6662)	83	
$22\bar{6}$	.5373		10		$32\bar{6}$	.6657		3.0	(w.+)
410	.5398	—	2.9	—	$12\bar{8}$	.6687		4.1	
$40\bar{4}$	.5423	(.5423)	58	(w.+)	028	.6711	—	1.9	—
$32\bar{4}$	.5427		0.4		404	.6745	.6744	17	v.w.
225	.5469	.5470	33	v.v.w.	019	.6779	—	0	—
402	.5528	—	0.7	—	$42\bar{1}$	.6795	—	2.5	—
133	.5558	(.5565)	24	(v.w.)	135	.6823	(.6845)	4.4	(v.w.)
$41\bar{3}$	.5567		14		$42\bar{2}$	.6851		6.3	
027	.5603	—	2.3	—	420	.6886	—	0.8	—
$12\bar{7}$	.5620	—	0.2	—	$21\bar{9}$	.6961	—	0.8	—
$41\bar{1}$	.5637	.5641	12	w.	$31\bar{8}$	.6989	(.6994)	1.0	(v.v.w.)
034	.5645		49		$42\bar{3}$	.7055	.7053	22	v.w.
					$41\bar{6}$	.7066		0.4	

Table 8. Powder photographs of $Sb_4O_5Cl_2$. CrKa radiation.

hkl	$\sin^2\Theta$ calc.	$\sin^2\Theta$ obs.	*I* calc.	*I* obs.	*hkl*	$\sin^2\Theta$ calc.	$\sin^2\Theta$ obs.	*I* calc.	*I* obs.
012	0.0790	(0.0790)	9.5	(m.—)	$32\bar{4}$	.5771	—	1.6	—
110	.0842	.0838	3.5	v.w.	118	.5826	—	1.9	—
$11\bar{1}$	.0874	(.0876)	5.8	(w.)	$13\bar{4}$	.5858	—	0.1	—
111	.0954	.0954	17	m.+	$23\bar{1}$	.5872	(.5879)	14	(w.)
$11\bar{2}$	.1052	.1054	7	w.	230	.5879		20	
013	.1152	.1157	3.9	v.w.	$41\bar{1}$	.5891	—	5.6	—
004	.1158		1.7		$21\bar{8}$	.5894	—	5.6	—
112	.1210	.1212	68	v.st.	$41\bar{2}$	.5950	.5949	24	m.—
$10\bar{4}$	.1342	.1342	45	st.	410	.5978	—	3.7	—
200	.1366	(.1366)	12	(st.)	217	.6000	(.6008)	7	(m.—)
$11\bar{3}$	.1374		2.6		$40\bar{4}$	.6003		35	
$20\bar{2}$	.1498	.1502	1.9	v.v.w.	$31\bar{5}$	.6003		8.2	
113	.1612	.1612	11	w.	$23\bar{2}$	.6010		0.8	
104	.1658	(.1658)	12	(m.)	231	.6032	—	1.8	—
014	.1659		0.2		402	.6088	—	0.1	—
202	.1814	.1813	0.2	v.v.w.	323	.6101	—	3.4	—
$11\bar{4}$	.1842	—	0.2	—	$41\bar{3}$	.6154	.6152	20	w.
$21\bar{1}$	.1860	.1861	14	m.	134	.6178	.6198	33	w.
210	.1866		4.4		127	.6197		20	
$21\bar{2}$	.1998	.2001	65	st.	411	.6211		6.7	
020	.2001		8.1		$23\bar{3}$	.6295	.6319	13	w—
211	.2018	—	0.4	—	$32\bar{5}$	.6307		16	
021	.2073	.2075	20	w.	$31\bar{7}$	.6312		11	
114	.2158	—	0.7	—	232	.6330		0	
$20\bar{4}$	.2208	—	0.4	—	035	.6331		11	
$21\bar{3}$	.2281	.2290	9.3	w.	$22\bar{7}$	.6384	.6387	22	v.w.
022	.2290		12		$11\bar{9}$	.6385		15	
015	.2310		7.0		019	.6403	—	0.3	—
212	.2314		0.6		306	.6424	—	0.5	—
321	.5278	(.5274)	11	(v.v.w.)	226	.6476	—	7.7	—
108	.5325	.5322	45	v.v.w.	$13\bar{5}$	.6493	—	3.5	—
$32\bar{3}$	.5381	.5394	6.5	w.	$41\bar{4}$	.6504	—	0.6	—
$13\bar{3}$	.5388		10		412	.6589	.6590	44	m.
$20\bar{8}$	.5393		53		028	.6667	—	4.8	—
$40\bar{2}$	.5449	—	1	—	208	.6672	—	2.4	—
400	.5477	(.5481)	3.0	(w.+)	$12\bar{8}$	.6690	—	0.1	—
$31\bar{6}$	.5485		67		$41\bar{5}$	.6700	—	1.4	—
$22\bar{6}$	.5517	—	15	—	$23\bar{4}$	.6725	.6725	56	w.
027	.5574	—	5.8	—	324	.6731		0.2	
225	.5595	(.5593)	22	(v.w.)	233	.6774	—	0.7	—
322	.5616	—	9.8	—	$30\bar{8}$	.6784	—	3.7	—
133	.5628	—	11	—	135	.6853	—	3	—
$12\bar{7}$	.5637	—	2	—	316	.6925	.6918	43	m.
034	.5675	.5675	58	v.w.	$32\bar{6}$	.6988	—	0.1	—

hkl	$\sin^2\Theta$ calc.	$\sin^2\Theta$ obs.	*I* calc.	*I* obs.	*hkl*	$\sin^2\Theta$ calc.	$\sin^2\Theta$ obs.	*I* calc.	*I* obs.
$2\bar{1}9$	.7052	—	1	—	$10\bar{1}0$	.7228	—	0.8	—
119	.7105	(.7108)	9.3	(v.v.w.)	$13\bar{6}$	.7235	—	0	—
413	.7114		2.9		404	.7283	.7285	32	
036	.7132	—	0.5	—	$31\bar{8}$	.7285		1.8	w.+
$40\bar{6}$	.7140	—	0.2	—	0010	.7286		1	
218	.7173	—	2	—					

The intensities were calculated from $I \sim p\left[\frac{F}{4f_{Sb}}\right]^2$ for powder photographs, where p is the number of cooperating planes, and from $I \sim \left[\frac{F}{4f_{Sb}}\right]^2$ for Weissenberg photographs. Due consideration was given to the variation of $\frac{f_A}{f_{Sb}}$ with Θ, except in the Weissenberg photograph of *hll* of the oxybromide, where the average values $\frac{f_{Br}}{f_{Sb}} = 0.645$, $\frac{f_O}{f_{Sb}} = 0.1$ were used. In the lists of the powder photographs, the limits between the angular ranges of the three different focusing cameras are marked. Reflections systematically absent are omitted. The β-reflections have been omitted. If a tabulated reflection coincides with a β-reflection, the $\sin^2\Theta$ and the intensity of the resulting line are given in brackets. The observed intensities are indicated as follows: vst = very strong, st = strong, m = medium, w = weak, vw = very weak and vvw = very,very weak.

The atomic coordinates of $Sb_4O_5Cl_2$ and $Sb_4O_5Br_2$ are given in table 9.

Table 9. Atomic coordinates of $Sb_4O_5Cl_2$ and $Sb_4O_5Br_2$.

	$Sb_4O_5Cl_2$			$Sb_4O_5Br_2$		
	x	*y*	*z*	*x*	*y*	*z*
4 Sb_1	0.186±0.005	0.225±0.005	0.049±0.003	0.174±0.005	0.225±0.005	0.051±0.004
4 Sb_2	0.796±0.005	0.113±0.005	0.203±0.003	0.806±0.005	0.125±0.005	0.203±0.004
4 X	0.51 ±0.01	0.703±0.01	0.115±0.005	0.51 ±0.01	0.705±0.01	0.118±0.005
2 O_1	0	$\frac{1}{2}$	0	0	$\frac{1}{2}$	0
	0	0	$\frac{1}{2}$	0	0	$\frac{1}{2}$
4 O_2	0.13	0.35	0.185	0.13	0.37	0.187
4 O_3	0.07	0.05	0.915	0.08	0.04	0.916

DISCUSSION OF THE STRUCTURE

Projections of the structure of the oxychloride on the *xz*-plane and on the *yz*-plane are shown in fig. 6. The *yz*-projection is an orthogonal projection of the cell for $-\frac{1}{2} \lessapprox x \lessapprox +\frac{1}{2}$ on $x = 0$. It shows that the antimony atoms form zig-zag chains in two layers, with a distance of 2.4 kX between

the layers. The oxygen atoms enter between these layers, so that Sb — O chains seem to be formed, which are held together by the oxygen in 2(c), thus forming an Sb — O layer. This is more obvious from fig. 5, where the halogens are omitted. Between these layers, there are layers of halogen. As has been mentioned above, $Sb_4O_5Cl_2$ forms two types of crystals, one of which forms thin plates. In order to check the structure, suitable plates were selected and rotation photographs taken around the three axes. They showed that

Table 10. Interatomic distances in $Sb_4O_5X_2$. kX units.

		$Sb_4O_5Cl_2$				$Sb_4O_5Br_2$		
Within Sb—O chain	Sb_1—2O	2.02	2.06			2.03	2.06	
	Sb_2—2O	2.03	2.06			1.98	2.04	
	Sb_1—Sb_2	3.88	3.83			3.88	3.87	
	O_2—O_3 at »angle»		3.21			3.17		
	O_2—O_3	3.93				3.98		
Between atoms in adjacent Sb—O chains	Sb_1—O	2.22				2.25		
	Sb_2—O	2.44				2.51		
	Sb_1—Sb_1	3.43				3.41		
	Sb_1—Sb_2	3.44				3.42		
	Sb_2—Sb_2	3.71				3.72		
	O_2—O_3	2.67				2.78		
	O_3—O_3	2.61				2.65		
Sb_1—3O (4O)	1.89	2.02	2.06	(2.22)	1.89	2.03	2.06	(2.25)
Sb_1—3X	3.11	3.22	3.39		3.30	3.35	3.51	
Sb_2—2O (3O)	2.03	2.06	(2.44)		1.98	2.04	(2.51)	
Sb_2—X (3X)	2.91	(3.33	3.62)		3.03	(3.42	3.65)	
O_1—2Sb_1	1.89				1.89			
O_2—2Sb (3Sb)	2.02	2.03	(2.44)		2.03	1.98	(2.51)	
O_3—2Sb (3Sb)	2.06	2.06	(2.22)		2.04	2.06	(2.25)	
X—Sb (6Sb)	2.91	(3.11	3.22	3.33)	3.03	(3.30	3.35	3.42
		3.39	3.62)			3.51	3.65)	
X—X (4X)	3.72	(4.33	4.46	4.46)	3.79	(4.37	4.40	4.40)
X—3O (5O)	2.98	3.21	3.37	(3.51	3.09	3.28	3.39	(3.65
		3.74)				3.94)		
O_1—4X	3.51	3.74			3.65	3.94		
O_2—2X	3.21	3.37			3.28	3.39		
O_3—X	2.98				3.09			
O_1—4O (6O)	2.63	2.64	(3.09)		2.62	2.70	(3.06)	
O_2—2O (3O)	2.64	2.67	(3.21)		2.62	2.78	(3.17)	
O_3—3O (5O)	2.61	2.63	2.67	(3.09	2.62	2.70	2.78	(3.06
		3.21)				3.17)		

the y-and the z-axes lie in the plane of the plates, which supports the proposed structure.

The interatomic distances are given in table 10. It seems rather difficult to make a definite statement on the nature of the antimony bonds, as the oxygen positions, because of the method in which they have been determined are a little uncertain. Now there are two different kinds of antimony atoms, Sb_1 and Sb_2.

Sb_2 is adjacent to two oxygen atoms at 2.03 and 2.06 kX and a third oxygen atom at 2.44 kX. Even considering the uncertainty of the oxygen positions, the third distance seems to be too large to indicate an Sb — O bond. Sb_2 is also close to three chlorine atoms at distances 2.91, 3.33 and 3.62 kX (2.91 kX is the shortest Sb — Cl distance in the structure). This seems to indicate that Sb_2 is bound to two oxygen atoms and one chlorine atom at the distance 2.91 kX. This would give angles O — Sb — O 103°, O — Sb — Cl 84° and 71°. However, 2.91 kX for the distance Sb_2 — Cl seems rather large for a covalent bond, as the observed Sb — Cl distance in $SbCl_3$ is 2.37 kX [1]. Sb_1 has four oxygen atoms at distances 1.89, 2.02, 2.06 and 2.22 kX as nearest neighbours. Sb_1 is also fairly close to three chlorine atoms at 3.11, 3.22, 3.39 kX. If it is assumed that Sb_1 is bound only to the nearest three oxygens (1.9—2.1 kX) valence angles of 83°, 84° and 149° are obtained, but 149° seems to be too great a distorsion of a valence angle expected to be 90°—100° (*cf.* p. 178). If the four oxygen atoms are considered the angles O—Sb—O for nearest neighbours of oxygen are 83°, 84°, 79°, 75°, and the »diagonal» angles 97° and 149°. The angles 97°, 83°, 84°, 79°, and 75° are of about the same magnitude as the valence angles of antimony. We can thus combine the »diagonal» angle 97° with two of the other four angles and obtain two possible valence configurations. One would have the angles 97°, 83°, and 75°, and the other the angles 97°, 84°, and 79°. From these values there does not seem to be any reason to prefer one set of angles before the other, so here the nature of the bonds has to be left open. In $Sb_4O_5X_2$ the Sb—X distances seem to be rather large. This is perhaps not so objectionable, as this is also the case for the Me—X distances in the layer structure Me_2O_2X [8]. In the latter structure the distances X—O are also a little large. In $Sb_4O_5Cl_2$, however, there seems to be contact between halogen and oxygen.

Within an antimony-oxygen chain the Sb—O—Sb angles are 147° and 137°.

It seems as if the antimony bonds in $Sb_4O_5X_2$ may be regarded as intermediate between covalent and ionic binding.

The fact that antimony and oxygen seem to form chains in the structure of $Sb_4O_5X_2$, may perhaps be of interest when considering the further hydrolysis of $SbCl_3$. According to Cooke [11] and Lea and Wood [12] needle-shaped crystals

are formed on further hydrolysis, Lea and Wood assigned to them the formula $Sb_4O_3(OH)_5Cl$. Crystal needles have also been obtained in the present investigation. A preliminary analysis does not seem to agree with Lea and Wood's formula, but there might of course have been impurities in the present preparations consisting of amorphous or microcrystalline material. It seems clear that they contain some water and less Cl than $Sb_4O_5Cl_2$. On further hydrolysis the orthorhombic modification of Sb_2O_3, containing chains of $(Sb_2O_3)\infty$ is formed, although this is normally the high temperature modification [15].

A determination of the crystal structure of the needle-shaped oxychloride seems to be of interest, and will if possible be carried out. An investigation of these and other antimony oxyhalides may perhaps give further knowledge regarding the oxygen positions in these compounds ($Sb_4O_5X_2$).

SUMMARY

The isomorphous compounds $Sb_4O_5Cl_2$ and $Sb_4O_5Br_2$ have been investigated by X-ray crystallographic methods. They are monoclinic, space group C_{2h}^5, and the unit cell contains two formula units. Cell dimensions:

$a = 6.229$ $b = 5.107$ $c = 13.50$ kX $\beta = 97.27°$ for $Sb_4O_5Cl_2$
$a = 6.593$ $b = 5.133$ $c = 13.43$ kX $\beta = 97.89°$ for $Sb_4O_5Br_2$

The atomic coordinates are given in table 9.

These compounds form a new structure type. Projections of the structure are given in fig. s 5 and 6. It is a layer structure, in which antimony-oxygen sheets are connected by single halogen sheets. In the antimony-oxygen sheets there seem to be zig-zag chains of antimony and oxygen. There is, of course, some uncertainty in the oxygen positions. Further investigations of the antimony oxyhalides may perhaps give a more complete picture of the coordination of antimony.

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Potentiometric Study on the First Ionization of Carbonic Acid in Aqueous Solutions of Sodium Chloride

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In a recent investigation by the author on barium carbonate [1] the values of the ionization constants of carbonic acid in sodium chloride solutions were required for the numerical treatment of the solubility equilibrium. This led the author to study the ionization of carbonic acid in sodium chloride solutions. The experiments for determination of the first ionization constant are described in this paper. The results on the second ionization were published earlier [2].

The ionization equilibrium in carbonic acid solution is given by

$$CO_2 + H_2O \rightleftharpoons H_2CO_3 \rightleftharpoons H^+ + HCO_3^- \tag{1}$$

The first ionization constant is defined by

$$\frac{[H^+][HCO_3^-]}{[CO_2]} = K_1 \tag{2}$$

and the carbon dioxide molality is given by

$$[CO_2] = Sp_{CO_2} \tag{3}$$

where p_{CO2} is the partial pressure of carbon dioxide and S the Henry's law constant. The elimination of $[CO_2]$ from equations (2) and (3) leads to the expression

$$[HCO_3^-] = \frac{K_1 S p_{CO_2}}{[H^+]} \tag{4}$$

To obtain the buffer capacity in the usual manner a small quantity of sodium hydroxide is added and then titrated with hydrochloric acid. Near the inflection point the following relation exists

$$y = c_A - c_B = [H^+] - [HCO_3^-] \tag{5}$$

where c_A denotes the concentration of hydrochloric acid and c_B that of sodium hydroxide. From (4) and (5) is obtained

$$y = c_A - c_B = [H^+] - K_1 S p_{CO_2} / [H^+] \tag{6}$$

The first derivative of y with respect to pH is

$$P = dy/dp\mathrm{H} = 2.303 \left\{[H^+] + K_1 S p_{CO_2}/[H^+]\right\} \tag{7}$$

When the second derivative of y is put equal to zero it follows that at inflection point

$$[H^+] = \sqrt{K_1 S p_{CO_2}} \tag{8}$$

Introducing this equation into equation (7) we obtain for buffer capacity at inflection point the expression

$$P = 4.606 \sqrt{K_1 S p_{CO_2}} \tag{9}$$

Solving this equation for K_1 the result is

$$K_1 = \frac{4.714 \cdot 10^{-2} P^2}{S p_{CO_2}} \tag{10}$$

By means of equation (10) when the quantity P and the constant S are known, it is possible to calculate the ionization constant K_1. Equation (9) represents a very simple individual form of the general equation of the buffer capacity at inflection point [3].

EXPERIMENTAL

The temperature of the water bath in which the measurements were carried out was kept constant with an accuracy of $\pm$ 0.01°. A hydrogen electrode was used as indicator electrode. The titration vessel used was similar to that described earlier by the

present author [1]. The gas mixture entering the titration vessel from a tank filled with tank hydrogen and carbon dioxide contained 16.60 % carbon dioxide by volume. The hydrochloric acid solution used in the determination of the buffer capacity was 0.01920 *N*. Kahlbaum's chemicals for analysis were used. The potential jumps were measured with a Leeds & Northrup K type potentiometer.

For further experimental details the reader is referred to the earlier papers of the author [4].

RESULTS

Table 1 includes the results of the measurements. The ionic strength μ in this table is defined by $\mu = \frac{1}{2}\Sigma m_i z_i^2$, where m_i denotes molality of ion indicated. The buffer capacity P of carbonic acid solution (or, in other words, the reciprocal of the maximum slope of the titration curve) was calculated from the potentiometric data with the aid of equations [4]

$$P = 2.303\ \Delta a C R T / \Delta E F V m_0 \tag{11}$$

and

$$\Delta E = \Delta m + \frac{1}{6}\{(\Delta m - \Delta_1) + (\Delta m - \Delta_2)\} - \frac{(\Delta m - \Delta_1)\ (\Delta m - \Delta_2)}{2\{(\Delta m - \Delta_1) + (\Delta m - \Delta_2)\}} \tag{12}$$

In these equations the denotations are:

C the normality of hydrochloric acid solution,
Δa the small increment of hydrochloric acid solution in ml,
R the gas constant,
T the absolute temperature,
F Faraday charge,
m_0 the amount of water in the titrated solution at the inflection point in grams,
Δ_1, Δm, Δ_2 successive changes of potential produced by the addition of Δa ml of hydrochloric acid solution (Δm is the maximum change).

The partial pressure p_{CO_2} of carbon dioxide expressed in atm was calculated from barometric pressure, the composition of the gas mixture (16.60 % carbon dioxide) and the vapor pressure of water. The vapor pressure of the sodium chloride solution was obtained from »International Critical Tables» [5]. The Henry's law constant S is obtained from a recent paper of Harned and Davis [6].

Table 1. The calculation of the first ionization constant of carbonic acid in sodium chloride solutions.

t	μ	$P \cdot 10^4$	p_{CO_2}	$S \cdot 10^2$	pK_1
5	0.00312	2.800	0.1633	6.40	6.4506
»	0.0142	2.939	0.1645	6.39	6.4120
»	0.143	3.448	0.1648	6.16	6.2584
»	1.065	3.830	0.1631	4.85	6.0584
»	3.135	2.774	0.1619	3.10	6.1410
15	0.00312	2.607	0.1633	4.56	6.3662
»	0.0142	2.786	0.1640	4.55	6.3095
»	0.143	3.256	0.1643	4.40	6.1600
»	1.065	3.641	0.1645	3.50	5.9646
»	3.135	2.625	0.1608	2.31	6.0547
25	0.00312	2.477	0.1631	3.44	6.2878
»	0.0142	2.588	0.1620	3.43	6.2442
»	0.143	3.065	0.1621	3.32	6.0851
»	1.065	3.369	0.1618	2.69	5.9105
»	3.135	2.525	0.1622	1.81	5.9889
35	0.00312	2.236	0.1582	2.68	6.2542
»	0.0142	2.354	0.1577	2.67	6.2075
»	0.143	2.816	0.1574	2.59	6.0322
»	1.065	3.056	0.1550	2.13	5.8762
»	3.135	2.320	0.1591	1.45	5.9574
45	0.00312	2.011	0.1491	2.18	6.2317
»	0.0142	2.120	0.1492	2.19	6.1861
»	0.143	2.530	0.1490	2.12	6.0199
»	1.065	2.798	0.1500	1.73	5.8477
»	3.135	2.112	0.1520	1.18	5.9322

The thermodynamic ionization constant is calculated with the aid of equation

$$pK_1 = pK_{1,0} - \frac{2\,A\,\sqrt{\mu}}{1+\sqrt{\mu}} + B\mu + D\mu^2 \qquad (13)$$

in which the constant A is calculated from

$$A = 0.4883 + 0.75545 \cdot 10^{-3}\,t + 0.1743 \cdot 10^{-5}\,t^2 + 0.11665 \cdot 10^{-7}\,t^3 \qquad (14)$$

obtained by Scatchard[7] on the basis of the recent tables of Birge[8]. The values of $pK_{1,0}$, B and D, evaluated by the method of least squares, are compiled in table 2. With these values pK_1 may be computed with a maximum deviation of 0.0107 and average deviation of 0.0036 from the pK_1-values in table 1. The values of the thermodynamic ionization constant may be calculated from

$$pK_{1'0} = 6.5720 - 0.012173\,t + 0.00013329\,t^2 \qquad (15)$$

Table 2. The thermodynamic first ionization constant of carbonic acid and the parameters of equation (13).

t	$pK_{1,0}$	$2A$	B	D
5	6.5140	0.9842	0.02401	0.0183
15	6.4210	1.000	0.03037	0.0183
25	6.3489	1.017	0.06524	0.00861
35	6.3104	1.035	0.07782	0.00671
45	6.2939	1.054	0.07676	0.00716

The maximum and average deviation of the values calculated by equation (15) from those in table 2 is 0.0021 and 0.0012 respectively. The constants B and D may be calculated satisfactorily by

$$B = 0.01660 + 0.01530\ t \tag{16}$$

and

$$D = 0.02028 - 0.000339\ t \tag{17}$$

DISCUSSION

The values of the thermodynamic constant obtained in this investigation agree excellently with the recent results of Harned and Bonner [9]. The maximum and average deviation of $pK_{1,0}$-values of this paper from those of Harned and Bonner is 0.0048 and 0.0026 respectively. In sodium chloride solutions, however, the maximum and average deviation of pK_1-values increases as the ionic strength rises, being at 1 M solution about 0.04 and 0.02 respectively. The values of this paper are smaller than the values of Harned and Bonner.

The greatest error in the method used in the present investigation arises from the measurement of the potential jumps. An accuracy better than 0.5 % is available. This causes an error of less than 1 % in the ionization constant. The other errors are of the same order of magnitude as in the method used by Harned and Bonner.

The free energy, heat content, heat capacity and entropy of ionization may be derived in the usual manner from the ionization constants obtained above. These calculations are not performed in this connection because it is the purpose of the author to complete the measurements in the future.

SUMMARY

The buffer capacity of carbonic acid solutions with added sodium chloride has been determined up to a concentration of 3 M salt and at temperatures of 5°, 15°, 25°, 35° and 45°.

From these potentiometric data and from the solubility of carbon dioxide in these solution the first ionization constant of carbonic acid has been calculated over the above range of concentration and temperature.

Equations for computing the ionization constant at temperatures from 5° to 45° and at ionic strength from 0 to 3 *M* are given.

Equation for computing of the thermodynamic first ionization constant as function of temperature is given.

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The Structure of MARQUIS' Nitroacetin

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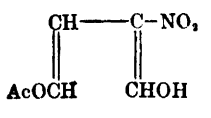
In a paper by Rinkes[1] the fairly well known rules of nitration in the furan series have been reviewed. According to these rules, substitution, if possible at all, takes place in α-position. Even if both α-positions are occupied, the nitro-group is capable of removing other groups, *e. g.* carboxylic acid groups and sulphonic acid groups. If both α-positions are occupied with irreplaceable groups, β-substitution may in some cases take place. The only occurrence of β-nitration of a furan derivative with a replaceable α-group is the nitration of 5-methylfuroic acid which yields a small amount of 4-nitro-5-methylfuroic acid together with the expected 2-nitro-5-methylfuran[2].

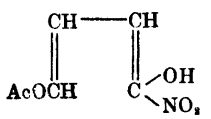
It has been shown, especially by the investigations of Marquis, Gilman, Rinkes and Johnson, that α-nitration of the furans in some cases proceeds through an intermediate addition product. Freure and Johnson[3] have suggested that this is *always* the fact, but that the preliminary addition product usually has not been isolated because of its instability. There is much experimental evidence in favour of this theory and we also believe it to be true.

Several structures have been proposed for the addition products. This can be exemplified by nitroacetin, the addition product, formed when furan is nitrated in acetic anhydride at — 5° C. This compound has been written as follows (I—V).

The formula I, originally proposed by Marquis[4], was altered into II by Rinkes[5] and by Gilman and Wright[6] because nitroacetin when treated with



I



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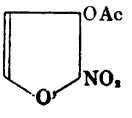
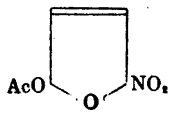
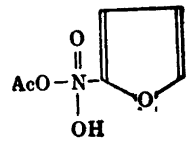


III



IV



V

pyridine yields α-nitrofuran. The formulas III and IV, advocating 1,2- and 1,4-addition, are due to Freure and Johnson. V is set forth by Gilman and Wright. Several oxonium formulas have also been proposed (see Gilman and Wright).

The oxonium formulas are incompatible with the solubility properties of nitroacetin. Therefore only the formulas II—V have to be taken into consideration. From a discussion given by Freure and Johnson in their paper it is evident that either formula III or formula IV is the correct one. Accordingly nitroacetin is formed by addition of the radicals $—NO_2$ and $—OOC \cdot CH_3$, presumably formed by the cleavage of a mixed anhydride of nitric acid and acetic acid (*cf.* Michael [7]) to furan. Freure and Johnson did not take any final decision between the two formulas, but seem to be inclined to IV [8].

Already Marquis, who first synthesized nitroacetin, observed that it was easily hydrolyzed to malealdehyde, nitrous acid, and acetic acid. It seems strange to us, that this formation of malealdehyde has never been used as a decisive proof of the structure of nitroacetin, as the formation of this aldehyde, in our opinion, can only be explained by formula IV.

Hydrolysis of I should lead to malaldehyde (2-hydroxy-1,4-butanedial), II should yield succinic acid aldehyde, III malaldehyde and V succinic acid aldehyde.

We find the formation of malealdehyde by the hydrolysis of nitroacetin an unequivocal proof that the formula IV must be ascribed to nitroacetin.

Meanwhile Wohl and Mylo [9] prepared malealdehyde starting from acetylene, and these authors obtained a bis-phenylhydrazone and a dioxime with melting-points entirely different from those reported by Marquis. Wohl and Mylo suggested that Marquis' compounds might be derivatives of fumaraldehyde, in spite of the fact, that Marquis prepared pyridazine by treating nitroacetin with hydrazine. It is evident, that pyridazine can only be derived from the *cis*-form of butenedial, malealdehyde, and not from the *trans*-form, fumaraldehyde. If therefore the suggestion of Wohl and Mylo is correct, a *cis-trans*-rearrangement of primary formed malealdehyde must have taken place during the formation of the two derivatives first mentioned, but not during the formation of pyridazine.

We have prepared several derivatives of malealdehyde, synthesized according to Wohl and Mylo. Of these only the bis-phenylhydrazone, the bis-methylphenylhydrazone and the bis-diphenylhydrazone were suitable for characterization by melting points, crystallographic properties and solubility. We have demonstrated all three products to be identical with the corresponding compounds obtained by hydrolysis of nitroacetin.

As to the bis-phenylhydrazone, the only one of the above named derivatives hitherto prepared, we found the melting point 171° on the Kofler stage, 180—184° in a tube. Wohl and Mylo report 198—199° (tube), Marquis 236—237° (bloc Maquenne). In spite of several preparations we never isolated any compounds melting higher than 171° (Kofler). The difference between our melting point and the one reported by Wohl and Mylo may be due to an experimental error, but the melting point of Marquis' compound is so high, that his product must have been an isomer of malealdehyde-bisphenylhydrazone. It is of course impossible to deduce the structure of this isomer without further experiments.

All other properties of our product were in agreement with those found by Marquis and by Wohl and Mylo.

The identification of malealdehyde proves that the formula for nitroacetin is the formula IV of Freure and Johnson. We believe, that an analogous structure must be ascribed to all similar addition-products. α-Nitration of furans thus proceeds through a 1,4-addition, followed by a 1.4-elimination of acetic acid. This mechanism of the nitration process corresponds to the mode of action earlier discussed for α-substitutions in the furan series[3, 6, 10]. *Cf.* also Shephard and Johnson[11].

The hydrolytical method employed here to prove the structure of nitroacetin is very convenient because of the ease, with which the carbonyl compounds formed by the reaction may be identified. We propose that the method should find general application in investigations on the constitution of similar 2,5-dihydrofurans.

EXPERIMENTAL

Hydrazones from malealdehyde tetraethylacetal

Malealdehyde tetraethylacetal was obtained from acetylene according to the method of Wohl and Mylo *(l. c.)* (b. p. 132—133°/16 mm). For preparation of the hydrazones, about 200 mg of acetal were boiled with 3 ml 0.01 *N* sulfuric acid and 2 ml of alcohol for 45 seconds. After cooling, a solution of the corresponding hydrazine in diluted acetic acid (12 ml) was added. The hydrazones precipitated immediately. The mixture was allowed to stand for some hours, then the precipitate was filtered off, washed thoroughly with water and dried *in vacuo* over calcium chloride. The yields of the crude hydrazones amounted to about 80 %.

Bis-phenylhydrazone. The crude product was dissolved in boiling acetone (solubility 5.3 g in 100 ml). An equal amount of benzene was added and the solution evaporated rapidly under atmospheric pressure until a large crop of crystals had settled. After cooling the precipitate was filtered off and washed with benzene. Yield of recrystallized proportionate to crude product 70 %. Yellow plates, m. p. 171° (Kofler stage), 180° (tube) *.

Ebullioscopic molecular weight determination in acetone with the Rieche apparatus.

$C_{16}H_{16}N_4$	Calc.	C 72.75	H 6.06	N 21.21	Mol. wt. 264
	Found	» 72.56	» 6.14	» 20.95	» » 273

Bis-methylphenylhydrazone. The crude product was recrystallized from acetone-benzene. Yield of recrystallized proportionate to crude product 50 %. This hydrazone is more soluble in benzene than is the bis-phenylhydrazone and it is better to recrystallize from acetone-alcohol as described below for the derivative prepared from nitroacetin. Yellow plates, m. p. 173° (Kofler stage and tube).

$C_{18}H_{20}N_4$ (292)	Calc.	C 73.97	H 6.85	N 19.18
	Found	» 73.50	» 6.85	» 18.87

Bis-diphenylhydrazone. This hydrazone is still more soluble in benzene than the former and can only be recrystallized from acetone-benzene in a 30 % yield. The crystals contain crystal-benzene which is removed by drying *in vacuo* over paraffin. A good yield (80 %) is obtained by recrystallizing from acetone-alcohol, and this product holds no crystal-alcohol. Yellow plates, m. p. 177° (Kofler stage), 180° (tube).

$C_{28}H_{24}N_4$ (416)	Calc.	C 80.77	H 5.77	N 13.46
	Found	» 80.07	» 5.80	» 13.41

Hydrazones from nitroacetin

Nitroacetin was prepared from 2.2 g of furan following Marquis' directions *(l. c)*. The yellow oil was transferred to a 25 ml volumetric flask which was filled with methanol to the mark.

Bis-phenylhydrazone. 5 ml of the methanolic solution of nitroacetin was diluted with methanol (50 ml), water (50 ml), and acetic acid (10 ml). 3.2 g of phenylhydrazine was added, and the mixture boiled for several minutes. After addition of another 50 ml of water, boiling was continued for about half a minute, and the mixture allowed to stand over night. The precipitate was filtered off, washed with water and dried in vacuo over calcium chloride. Yield 32 % proportionate to furan (Marquis reports 21 %). The crude product was recrystallized 3 times from acetone-benzene as described above. Yield of recrystallized to crude product 61 %. Yellow plates, m. p. 170° (Kofler stage), 184° (tube).

$C_{16}H_{16}N_4$ (264) Calc. N 21.21 Found N 21.26

* All melting-points are corrected.

A careful crystallographic investigation in polarized light showed that the substance was in every respect identical with the hydrazone prepared from malealdehyde tetraethylacetal.

The boiling point of a saturated solution of one of the hydrazones in acetone was not altered by the addition of the other hydrazone. The bis-phenylhydrazone was prepared several times under various conditions including those described by Marquis, but no product melting higher than 171° could be isolated.

Bis-methylphenylhydrazone. Prepared in the same manner as the phenyl derivative. Yield of crude product 37 %. Recrystallization from acetone-alcohol. Yield of recrystallized to crude product 78 %. Yellow plates, m. p. 176° (Kofler stage and tube).

$C_{18}H_{20}N_4$ (292) Calc. N 19.18 Found N 19.06

Bis-diphenylhydrazone. Prepared as the phenyl derivate, yield 28 %. Recrystallized from acetone-alcohol. Yield of recrystallized to crude product 75 %. Yellow plates, m. p. 173—177° (Kofler stage), 179° (tube).

$C_{28}H_{24}N_4$ (416) Calc. N 13.46 Found N 13.67

The bis-methylphenylhydrazone and the bis-diphenylhydrazone exhibited properties in every respect identical with those found for the corresponding derivatives prepared from malealdehyde tetraethylacetal.

SUMMARY

The formula of Marquis' nitroacetin, the nitroacetate prepared by the action of nitric acid on furan in acetic anhydride, is established with certainty by identification of the malealdehyde formed when nitroacetin is hydrolyzed. This formula is identical with one of two alternatives proposed earlier by Freure and Johnson. The mechanism of α-nitration in the furan series is discussed. The hydrolytical method employed is recommended for general application.

All analyses have been made in the most careful way by our colleague Mr. O. Rosenlund Hansen.

During the investigation we have received financial aid from *Tuborg Fondet* (N. Clauson-Kaas) and *Det teknisk-videnskabelige Forskningsraad* (J. Fakstorp).

We wish to thank the director of the Chemical Laboratory of the University of Copenhagen, Professor Dr. A. Langseth, for his kind interest in our work.

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The Hydroxylation of Furan with Osmium Tetroxide

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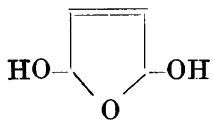
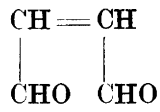
Hydroxylation of furan should yield 2,5-dihydroxy-2,5-dihydrofuran (I), provided 1.4-addition of the hydroxy groups takes place. In order to ascertain this, furan was oxidized in alcoholic solution with osmium tetroxide and hydrogen peroxide according to Milas and Sussman[1]. These authors used an anhydrous solution of hydrogen peroxide in tert. butanol. We have employed a mixture of 30 % aqueous hydrogen peroxide and methanol. This reagent, which is cheaper and easier to prepare than that of Milas and Sussman, proved to be perfectly stable at 0° in the presence of osmium tetroxide.

In aqueous methanol 2,5-dihydroxy-2,5-dihydrofuran is probably easily hydrolyzed to yield hydrates of malealdehyde (II). Consequently no attempt was made to isolate the dihydrofuran but phenylhydrazine added directly to the reaction mixture in order to obtain the bis-phenylhydrazone of malealdehyde.

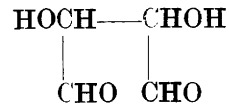
When equimolar amounts of furan and hydrogen peroxide were allowed to interact at 0° all hydrogen peroxide had disappeared after 40 hours. Addition of phenylhydrazine yielded a precipitate of yellow crystals, which turned out to be a mixture of approximately one part of mesotartaraldehyde bis-phenylhydrazone and two parts of malealdehyde bis-phenylhydrazone. The hydrazones were separated by fractionate crystallization from acetone-benzene and identified by analysis and melting-point determination; the total yield was 38 % proportionate to furan and 47 % proportionate to hydrogen peroxide.



I



II



III

The tartaraldehyde derivative, which has been prepared earlier by Wohl and Mylo[2], is reported to form yellow crystals just as the corresponding bis-phenylhydrazones of malealdehyde or glyoxal. However in these compounds all double bonds are conjugated. Inasmuch as conjugation is interrupted in tartaraldehyde bis-phenylhydrazone, this compound should be colourless, *e. g.* as succinaldehyde bis-phenylhydrazone. Still our product also had a strong yellow colour, but after three recrystallizations from acetone-benzene perfectly colourless crystals were obtained. The yellow colour of Wohl and Mylo's hydrazone, as well as of our crude product, is undoubtedly due to a small amount of malealdehyde bis-phenylhydrazone. This is in agreement with the fact that the tartaraldehyde tetraethylacetal used by Wohl and Mylo for the preparation of their hydrazone was obtained by hydroxylation of malealdehyde tetraethylacetal.

The hydroxylation of furan had thus taken the expected course, although some of the malealdehyde had been further hydroxylated to mesotartaraldehyde (III).

After standing at room temperature for one week, the reaction mixture furnished exclusively mesotartaraldehyde bis-phenylhydrazone on addition of phenylhydrazine. The yield was exactly the same as that originally obtained of this hydrazone. The malealdehyde had accordingly been completely destroyed by standing, the tartaraldehyde had not been affected. At 0° malealdehyde is somewhat more stable. The stability of the aldehydes, in whatever form they may exist in the reaction mixture, corresponds to that reported by Wohl and Mylo for malealdehyde and tartaraldehyde.

When furan reacts with twice the equimolar amount of hydrogen peroxide, the total yield of the hydrazones isolated from the reaction mixture is increased to 59 % (proportionate to furan). The yield proportionate to hydrogen peroxide is 43 %. The ratio mesotartaraldehyde to malealdehyde bis-phenylhydrazone is now approximately 1 : 1.4.

No attempt was made to examine the other reaction products in order to find out what the rest of the hydrogen peroxide had been used for. Actually there was an evolution of oxygen, but far from enough to account for all the hydrogen peroxide.

In conclusion the effect of different admixtures on the hydroxylation reaction was investigated. Addition of acetic acid in small amounts did neither affect the velocity nor the course of reaction. Small amounts of sulfuric acid caused an almost complete stop of the reaction. Pyridine (*cf.* Crigee[3]) had a very marked influence; velocity and yield were decreased and the course of reaction was also affected. No appreciable amount of mesotartaraldehyde was formed, and only the bis-phenylhydrazone of malealdehyde could be

isolated. It is possible that the addition of pyridine or similar tertiary amines may find general application for determining the course of hydroxylation reactions with osmium tetroxide.

EXPERIMENTAL

Preparation of reagent

6.00 ml of 30 % (9.7 *M;* determined by titration) hydrogen peroxide, technical grade, were mixed with methanol (18 ml) in a 25 ml volumetric flask and the mixture cooled to 0°. 0.40 ml of a solution of osmium tetroxide in tert.-butanol (0.62 %) were added and the flask filled to the mark with methanol. The molarity was determined by iodometric titration to 2.324. After standing for four days at 0°, *M* was determined to 2.302 which proves the stability of the reagent at this temperature.

The hydrogen peroxide (9.7 *M)* and the osmium tetroxide solution (0.62 %) used here were also employed in all the following experiments.

Hydroxylation of furan (furan:hydrogen peroxide = 1: 1.06)

Freshly distilled furan (2.111 g), methanol (18 ml) and hydrogen peroxide (3.36 ml) were mixed in a 25 ml volumetric flask and cooled to 0°. 0.40 ml of osmium tetroxide solution were added and the flask filled to the mark with methanol. After standing for about 40 hours at 0°, all hydrogen peroxide had disappeared. The volume of the reaction mixture had decreased a little, and the flask was anew filled with methanol. 2.00 ml were added to a solution of phenylhydrazine (0.50 ml) in ethanol (1 ml) and glacial acetic acid (0.1 ml). The mixture solidified almost immediately to a mass of orange yellow crystals. After standing for some hours, the crystals were filtered off and washed with ethanol (7 ml) and benzene (6 ml). Yield 243 mg. From the mother liquor another 18 mg were obtained; total yield 261 mg.

The crystals were dissolved in boiling acetone (30 ml). 30 ml of benzene were added, the solution evaporated under atmospheric pressure to 20 ml, cooled rapidly and filtered. The lemon yellow crystals were washed with benzene. Yield 80 mg = 11 %, calculated as tartaraldehyde bis-phenylhydrazone ($C_{16}H_{18}O_2N_4$).

$C_{16}H_{18}O_2N_4$ (298) Calc. N 18.79 Found N 18.78

Further evaporation of the mother liquor to about 5 cc. gave another crop of crystals, which was filtered off and washed with a small amount of benzene. Yellow plates, yield 156 mg = 24 %, calculated as malealdehyde bis-phenylhydrazone ($C_{16}H_{16}N_4$).

$C_{16}H_{16}N_4$ (264) Calc. N 21.21 Found N 21.55

About twice as much malealdehyde as mesotartaraldehyde has been formed by the reaction. This corresponds to a 38 % yield of the crude mixture of the hydrazones (261 mg) proportionate to furan and a 47 % yield proportionate to hydrogen peroxide.

It is evident that nitrogen determination is not a very fine method to control the purity of the hydrazones. The analyses have been carried out only to get a rough picture

of the proportion in which the two aldehydes are formed by the reaction. The pure bis-phenylhydrazones were obtained by recrystallizing each of the two fractions (80 mg and 156 mg) once or twice more from acetone-benzene. The melting point (Kofler stage, corrected) of the tartaraldehyde fraction hereby rose from 187—189° to 195° (Wohl a. Mylo 197.5 [2]), of the malealdehyde fraction from 162—165° to 172° (171° has earlier been found [4]). Further recrystallizations did not alter the melting points. Both of the two crude fractions had a strong yellow colour but the tartaraldehyde fraction was perfectly colourless after three recrystallizations (N 18.58 %).

Stability of the aldehydes

After standing for a week at 0°, 2.00 ml of the reaction mixture only yielded 188 mg of crude hydrazones. When standing for a week at room temperature, the yield was 78 mg. The melting point (185°) and a nitrogen determination (N 18.90 %) showed that the product consisted mainly of tartaraldehyde derivative. Accordingly, when recrystallized once in a 70 % yield, the melting point was raised to 194°.

Hydroxylation of furan (furan: hydrogen peroxide = 1: 1.96)

2.094 g of furan, 6.20 ml of hydrogen peroxide and 0.4 ml of osmium tetroxide solution were mixed with methanol in a 25 ml volumetric flask as described above. The hydrogen peroxide had disappeared after 48 hours. The total yield of crude product from 2.00 ml of the reaction mixture was now 403 mg, consisting of 1 part of tartaraldehyde bis-phenylhydrazone (N 18.84 %) and 1.4 parts of malealdehyde bis-phenylhydrazone (N 21.08 %). This corresponds to a 59 % yield proportionate to furan and a 43 % yield proportionate to hydrogen peroxide.

Effect of admixtures on the reaction

The reaction described above (furan: hydrogen peroxide = 1 : 1.96) was repeated three times with the addition of 0.5 ml of glacial acetic acid, 0.5 ml of concentrated sulfuric acid and 0.2 ml of pyridine respectively.

Acetic acid had no influence on the reaction and the hydrazones were isolated in exactly the same yields as above.

Addition of sulfuric acid arrested the reaction almost completely. After two days only 10 % of the hydrogen peroxide had been consumed.

Pyridine also markedly decreased the velocity of the reaction. After two, five and twelve days the comsumption of hydrogen peroxide was 33 %, 50 % and 75 % respectively. At this time 2.00 ml of the reaction mixture were precipitated with phenylhydrazine. The crude hydrazone melted at 167—170° and contained 21.28 % of nitrogen; it must therefore consist mainly of malealdehyde bis-phenylhydrazone. The yield was only 8 % proportionate to furan.

In another experiment, equimolar amounts of furan and hydrogen peroxide were allowed to react in the presence of osmium tetroxide and pyridine. The hydrazone was precipitated after 24 hours in a 15 % yield (m. p. 168—169°; N 21.16 %). Neither of the two products from these preparations could be divided into different fractions by recrystallization. Hence, no detectable amount of tartaraldehyde is formed by the reaction.

SUMMARY

Furan has been oxidized in methanolic solution with 30 % aqueous hydrogen peroxide in the presence of osmium tetroxide. The bis-phenylhydrazones of malealdehyde and mesotartaraldehyde were isolated by addition of phenylhydrazine to the reaction mixture. The influence of small amounts of acetic acid, sulfuric acid and pyridine on the course of reaction has been studied.

All analyses have been made in the most careful way by our colleague Mr. O. Rosenlund Hansen.

During the investigation we have received financial aid from *Tuborg Fondet* (N. Clauson-Kaas) and *Det teknisk-videnskabelige Forskningsraad* (J. Fakstorp).

We wish to thank the director of the Chemical Laboratory of the University of Copenhagen, Professor Dr. A. Langseth, for his kind interest in our work.

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Velocity of Dissociation and Viscosity of Alkaline Polymetaphosphates

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The investigations of Lamm and Malmgren[1,2] into the relationships between alkaline metaphosphates in aqueous or in rhodanide solution respectively have proved that these substances are highly polymerized and that they very quickly dissociate, according to their composition and the solvent. Experiments with the ultracentrifuge showed that the solutions become polydispersed very soon, assuming that this has not already taken place during production; but it was impossible to get definite quantitative results.

Attempts were therefore made to follow the course of dissociation by measuring the viscosity. Here — as with every total characteristic of a solution — only summary effects are obtained; but since the viscosity can be measured accurately and changes considerably in the present instance, it might be expected that the theoretical analysis of the time curve would yield some information.

EXPERIMENTAL WORK

Measurements were taken at a temperature of 20° C with the Ostwald viscosimeter. This had a water outflow time of about 200 seconds in the case of table 1, so that the outflow times of these solutions fell from about 800 to about 340 seconds; they are therefore accurate at least initially to with inless than 1 ‰. The surface tension correction[3] could be ignored since it is very small and practically constant for the given total concentration.

For the purpose of the experiments the mixed salt $K_2Na(PO_3)_3$ was employed. It dissolves only very slowly in water, and before its complete dissolution noticeable dissociation may already have occurred. Therefore in the first experiments at different concentrations the zero point of dissociation time and consequently the particularly important changes in the early stage were uncertain. On that account no details are given here. The following conclusions could be deduced.

The increment of viscosity * $\varrho = \frac{\eta - \eta_0}{\eta_0}$ changes almost uniformly as a function of the time at different total concentrations a (gram per kilogram solution). The reactions of dissociation are therefore analogous. The intrinsic viscosities $Wa = \varrho_0 / a$ at $\tau = 0$ depend only to a small degree on the total concentration; therefore ϱ is the sum of the partial values of the dissociation products. As an example some typical values are given below.

a	ϱ_0	ϱ_0 / a
1.58	9.26	5.9 **
0.790	5.10	6.5
0.343	2.21	6.4
0.201	1.26	6.3
0.040	0.26	6.5

In taking the more exact measurements of table 1 the solution was filtered after a quarter of an hour, without waiting until the substances had been dissolved completely, and the concentration was then tested. The result of the phosphate analysis *** showed that the proportion between sodium and potassium changes slightly when fractionally dissolved, but this circumstance is of no particular importance.

The duration τ of the experiment was counted in hours from the moment of adding the water to the solid substance, and as a starting point for each measurement the average value between the beginning and end of the outflow was selected. By this means the important initial values are accurately determined to within 1 to 2 minutes.

Table 1 gives the time values τ and the increments of viscosity ϱ as they were determined. ϱ is deduced from the relative outflow time and the specific gravity of the solution. ($a = 0.660$, $^0/_{00}$ $s/s_0 = 1.00465$).

Table 1. Change of the increment of viscosity (ϱ) with age (τ hours) of a solution of $K_2Na(PO_3)_3$.

τ	ϱ	τ	ϱ
0.76	3.046	24.08	2.456
1.02	3.037	24.30	2.451
1.27	3.031	24.51	2.448
1.77	3.018	25.62	2.426
2.68	2.988	27.10	2.399
3.50	2.963	28.10	2.378
4.20	2.939	29.10	2.362
5.20	2.911	48.58	2.063

* We avoid the expression »specific viscosity» used in scientific literature, because the term specific values is understood to mean such values which refer to the unit of the quantity of substance.

** Probably too small because dissociation had already advanced too far.

*** For this information the author is indebted to fil.mag. H. Malmgren.

τ	ϱ	τ	ϱ
50.37	2.037	143.4	1.290
51.82	2.022	146.0	1.276
52.92	2.008	148.9	1.259
73.92	1.780	167.5	1.160
74.17	1.778	171.6	1.140
75.83	1.761	172.8	1.135
95.80	1.596	192.0	1.038
97.37	1.586	197.9	1.006
98.90	1.576	217.0	0.918
101.12	1.557	220.9	0.900
119.83	1.429	244.1	0.793
122.98	1.412	263.9	0.714
125.0	1.398		

THEORY

According to the above mentioned results the increment of viscosity ϱ at the time τ is expressed as an algebraic sum of the products of the intrinsic viscosities W_a, W_b . . . of the components and their concentrations respectively. If the substance dissociates in several stages and if the part x of the initial concentration a has dissociated after τ hours — and furthermore in the same time the part y of x has dissociated etc., — then the following equations will apply if three stages only are considered:

$$\text{a)}\quad \begin{aligned}\varrho &= W_a\,(a-x) + W_b\,(x-y) + W_c\,(y-z) + W_d \cdot z \\ &= W_a \cdot a - (W_a - W_b)\,x - (W_b - W_c)\,y - (W_c - W_d)\,z \\ &= \varrho_0 - \Delta_1 x - \Delta_2 y - \Delta_3 z\end{aligned}$$

$$\text{b)}\quad \frac{d\varrho}{d\tau} = -\Delta_1 \frac{dx}{d\tau} - \Delta_2 \frac{dy}{d\tau} - \Delta_3 \frac{dz}{d\tau}$$

As a second fundamental assumption which needs hardly to be verified, it may be assumed that any dissociation whatever progresses as a first class reaction without any inverse reaction.

The following relations for different schemes of dissociation are thus obtained.

First case. The originally homogeneous polymeric substance A may dissociate into a stable product B which for its part may consist of particles of the same or different size *.

* If the particles vary in size, the coefficient W_b for stage B is expressed as a sum of several terms, which is indivisible but does not need to be divided. The same applies to all subsequent stages.

The velocity equation will then be

1a) $\frac{dx}{d\tau} = k_a\ (a - x)$ with the integral 1c) $x = a - a \cdot e^{-k_a \tau}$

and the viscosity may be written

1b) $\varrho = \varrho_0 - \Delta_1 x$

From the differentiation of 1b follows *

1d) $-\frac{d^2\varrho}{d\tau^2} \Big/ \frac{d\varrho}{d\tau} = k_a$

Second case. A may dissociate in two parallel processes into two stable products B_1 and B_2. Each of them may be composed either of equal or unequal parts.

Instead of one velocity equation the two following are obtained:

2a) $\frac{dx_1}{d\tau} = k_1 \cdot (a - x)\ \frac{dx_2}{d\tau} = k_2\ (a - x)$

where $x_1 + x_2 = x$. If $x_1 = n \cdot x$ and $x_2 = (1 - n)\ x$, it follows that $k_1 : k_2 = n : (1 - n)$; but as n is unknown, it is impossible to separate the two constants, and only their sum $k_0 = k_1 + k_2 = \frac{1}{a-x} \cdot \frac{dx}{d\tau}$ can be obtained as in the first case.

The friction is expressed by the term

2b) $\varrho = W_a\ (a - x) + W_{b1}\, nx + W_{b2}\, (1 - n)\ x = \varrho_0 - \Delta_1 x$

also as above. $(\Delta_1 = W_a - W_{b1}\, n - W_{b2} + W_{b2}\ n)$

Therefore this case and every subsequent analogous case can be treated as if there were only one dissociation process.

Third case. A may dissociate first into B and B into a stable product C. It is preliminary assumed that B consists of equal parts. For the first dissociation equation 1a applies; for the second

3a) $\frac{dy}{d\tau} = k_b\ (x - y)$

---

* If therefore ϱ can be expressed by an empirical series

$$\varrho = \varrho_0 + \alpha.\tau + \beta.\tau^2 + 8.\tau^3 + \varrho.\tau^4$$

then case 1 requires that

$$k_a = -\frac{2\beta}{\alpha} = -\frac{3\gamma}{\beta} = -\frac{4\varrho}{\gamma}$$

and the friction equation will be

$$3b) \quad \varrho = W_a\ (a - x) + W_b\ (x - y) + W_c y = \varrho_0 - \Delta_1 x - \Delta_2 y$$

The integral of 3a is

$$3c) \quad y = a + a \cdot \frac{k_b}{k_a - k_b} \cdot e^{-k_a \tau} - a \frac{k_a}{k_a - k_b} \cdot e^{k_b \tau}$$

If we differentiate 3b repeatedly and substitute x and y (according to 1c and 3c), we get in abridged form $\frac{d\varrho}{d\tau} = d_1$ and $\frac{d^2\varrho}{d\tau^2} = d_2 \ldots .$

$$3d) \quad -k_b = \frac{d_3 + k_a d_2}{d_2 + k_a d_1}$$

Fourth case. A may dissociate in one reaction into two unequal parts B_1 and B_2, which in turn change into stable final products, $B_1 \rightarrow C_1$ and $B_2 \rightarrow C_2$.

The equation 1a applies as before, but 3a is split into two formulae

$$4a) \quad \frac{dy_1}{d\tau} = k_{b1}\ (x_1 - y_1) \qquad \frac{dy_2}{d\tau} = k_{b2}\ (x_2 - y_2)$$

If we put $x_1 = n.x$, $x_2 = (1 - n)\ x$, as above, the integrals will be

$$4c) \quad y_1 = n\ (a + a \cdot \frac{k_{b1}}{k_a - k_{b1}} e^{-k_a \tau} - a \frac{k_a}{k_a - k_{b1}} e^{-k_{b1} \tau})$$

$$y_2 = (1 - n)\ (a + a \cdot \frac{k_{b2}}{k_a - k_{b2}} \cdot e^{-k_a \cdot \tau} - a \cdot \frac{k_a}{k_a - k_{b2}} \cdot e^{-k_{b2} \tau})$$

where n remains unknown as before. The friction equation is

$$4b) \quad \varrho = \varrho_0 - \Delta_1 x - \Delta_2 y_1 - \Delta_3 y_2$$

where $\Delta_1 = W_a - W_{b1}\ n - W_{b2}\ (1 - n)$, $\Delta_2 = W_{b1} - W_{c1}$, $\Delta = W_{b2} - W_{c2}$ and after repeated differentiation as above it is found that *

$$4d) \quad -k_{b2} = \frac{d_4 + (k_a + k_{b1})\ d_3 + k_a \cdot k_{b1}\ d_2}{d_3 + (k_a + k_{b1})\ d_2 + k_a \cdot k_{b1} \cdot d_1}$$

* If one of the two first dissociation products should be stable, case 4 is reduced to the foregoing case 3.

Fifth case. A may dissociate only into equal parts B, these further into C and C, finally, into a stable product D.

Besides the velocity equations 1a and 3a we have here

$$5a) \quad \frac{dz}{d\tau} = k_c\,(y - z)$$

with the integral

$$5c) \quad z = a - a \cdot \frac{k_b \cdot k_c}{(k_b - k_c)\,(k_c - k_a)} \cdot e^{-k_a\tau} + a\frac{k_a \cdot k_c}{(k_b - k_a)\,(k_c - k_b)} c^{-k_b \cdot \tau} - a \cdot \frac{k_a \cdot k_b}{(k_c - k_a)\,(k_c - k_b)} e^{-k_c\tau}$$

If we introduce this into the friction equation

$$5b) \quad \varrho = \varrho_0 - \Delta_1 x - \Delta_2 y - \Delta_3 z$$

(here again $\Delta_1 = W_a - W_b$, $\Delta_2 = W_b - W_c$, $\Delta_3 = W_c - W_d$)

and then differentiate as above, it follows that

$$5d) \quad -k_c = \frac{d_4 + (k_a + k_b)\,d_3 + k_a \cdot k_b \cdot d_2}{d_3 + (k_a + k_b)\,d_2 + k_a \cdot k_b \cdot d_1}$$

From the foregoing five examples, the number of which could be increased indefinitely, the relation between the differential quotient of ϱ and the velocity constants becomes quite clear. The greater the number of separate reactions, the more time terms will be needed for the calculation, and consequently the more accurate the measurements must be. A formula including the fourth differential quotient of ϱ as in case 4 and 5 would seem to be the limit of what is obtainable by viscosity measurements at the present time.

More important than the velocity factors discussed in the above mentioned deductions are the intrinsic viscosities W_b, W_c etc. (see equation a). From the velocity formulae (see equation b) it follows that in the limit case $\tau = 0$ all the first differential quotients of the concentrations become zero, except $dx/d\tau$, which acquires the value $a \,.\, k_a$. Thus irrespective of the number of stages, the following will always apply:

$$6)\quad \frac{1}{\varrho_0}\cdot \lim_{\tau=0}\frac{d\varrho}{d\tau} = -\Delta_1\cdot\frac{a}{\varrho_0}\cdot k_a$$

In the second derivation all the terms for $\tau = 0$ are eliminated, except $d^2x/d\tau^2$ and $d^2y/d\tau^2$, and the following limit value is obtained:

$$7)\quad \frac{1}{\varrho_0}\cdot \lim_{\tau=0}\frac{d^2\varrho}{d\tau^2} = \Delta_1\frac{a}{\varrho_0}\cdot k_a^2 - \Delta_2\frac{\pi}{\varrho_0}\cdot k_a\cdot k_b$$

likewise independent of all that follows the second stage. If k_a and Δ_1 are known, we get from this relation a value for $\Delta_2 = W_b - W_c$ (see case 3), irrespective of whether the dissociation product C is stable or dissociates further *.

RESULTS

According to formula 1d the same value for **) $k_a = -\frac{d_2}{d_1} = -\frac{d_3}{d_2}\ldots$ should be obtained all along the curve, if only the single dissociation A → B were to take place. Calculations of the differential quotients for different lengths of the curve show that this is not the case. At most the subsequent reactions approximately can be neglected up to $\tau = 50$. For longer times they must be taken into consideration, but here only case 3, A → B → C, is discussed in detail.

Up to $\tau = 100$, ϱ can be expressed by the empirical series $\varrho = 3.065 - 3.11_9\cdot 10^{-2}\cdot\tau + 2.95_5\cdot 10^{-4}\cdot\tau^2 - 1.88\cdot 10^{-6}\cdot\tau^3 + 0.548\cdot 10^{-8}\cdot\tau^4$ wherefrom the differential quotients d_1, d_2 etc. can be easily deduced for each time; thus — according to formula 6 —

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* Given case 5, the relation

$$8)\quad \lim_{\tau=0}\frac{d^3\varrho}{d\tau^3} = -\Delta_1 a k_a^3 + \Delta_2 a\cdot k_a\cdot k_b\ (k_a + k_b) - \Delta_3 a\cdot k_a\cdot k_b\cdot k_c$$

would be formed, where k_c and $\Delta_3 = W_c - W_d$ also appear. Given case 3 only, ($k_c = 0$), the last term in formula 8 disappears.

** If the fundamental assumption stated above is not fulfilled, namely that at the time $\tau = 0$ only one homogeneous highly polymerized substance A is present, but if in consequence of a very quick reaction at the given time a part b of the total concentration a is already transformed, we have to substitute in all relations for the term a the constant but unknown term $a - b$. This does not entail any alteration in the formulae, but in the limit value $\varrho_0 = W_a\cdot a$ and in the differential quotients of ϱ.

Incidentally though perhaps unnecessarily, it may be mentioned that there is no change in all these considerations if the molecular concentrations are introduced in place of the weight concentrations employed here. The molecular weights will then appear everywhere as constant but unknown factors.

$$\frac{1}{\varrho_0} \cdot \lim_{\tau = 0} \frac{d\varrho}{d\tau} = -\Delta_1 \frac{a}{\varrho_0} \cdot k_a = -\ 1.02.10^{-2}$$

Since $\Delta_1 = W_a - W_b$ cannot be greater than $W_a = \frac{\varrho_0}{a} \frac{3.065}{0.660} = 4.65$, k_a must be greater than $1.02 \cdot 10^{-2}$. According to formula 3d the second velocity constant k_b is calculated by some selected values for k_a $1.02 \cdot 10^{-2}$, further according to formula 6, $\Delta_1 = \frac{1.02 \cdot 10^{-2} \cdot 4.65,}{k_a}$ from which follows $W_b = W_a - \Delta_1$ and finally Δ_2 according to formula 7. Thus the following table is obtained *.

Table 2. Velocity constants and intrinsic viscosities deduced from table 1.

$10^2 k_a$	$10^2 k_b$	Δ_1	W_b	Δ_2	W_c
2.00	1.62	2.36	2.29	0.16	2.13
2.20	1.82	2.15	2.50	0.36	2.14
2.50	1.86	1.89	2.76	0.61	2.15
3.00	1.88	1.58	3.08	0.92	2.16
4.00	1.88	1.18	3.47	1.32	2.15
5.00	1.89	0.95	3.70	1.56	2.14
6.00	1.89	0.79	3.86	1.71	2.15

Without placing any value on the absolute magnitude of the velocity coefficients the intrinsic viscosities obtained may be considered. They show the expected sequence $W_a > W_b > W_c$, but are of the same order of magnitude, which means that the fragments formed are not much smaller than the original substance A, *i. e.* they are still highly polymerized. This result could be extended by including case 5 in the calculation, in which case values of ϱ corresponding to longer times would be required.

Such a calculation based on formula 5d is not only very involved, but also far more liable to error than that carried out above. A satisfactory result, however, with respect to order of magnitude may be obtained by another method. Since the value of k_a is apparently very high, $(a - x)$ is reduced to such an extent about the middle of the experimental series that it can be practically ignored *.

* Values of $k_a < 2 \cdot 10^{-2}$ were not taken into consideration since it follows from them that $k_b > k_a$, which would mean that the first stage A $\rightarrow$ B does not come into question and A $\rightarrow$ C takes place directly. Further Δ_2 would also become negative, *i. e.* $W_c > W_b$, which is likewise excluded.

The curve then runs on as if A had not been present from the outset; hence the dissociation B → C → D may be treated according to case 3, any possible subsequent stages being disregarded. Above $\tau = 160$, it is thus found that $k_b = 1.88 \cdot 10^{-2}$ (see table 2), $k_c \approx 0.5 \cdot 10^{-2}$. The concentrations of the components can be calculated according to the formulae 1c, 3c and 5c, wherefrom formula a gives the values for W_b, W_c and W_d.

By this means it was found that $\Delta_1 = 1.22$, $\Delta_2 = 1.15$, $\Delta_3 = 1.0$, thus $W_a = 4.65$, $W_b = 3.43$, $W_c = 2.3$, $W_d \approx 1.2$, approximately equal to the values in table 2.

Accordingly W_d is likewise still so large that it could belong to a very high polymer.

As already mentioned, no importance should be attached to the absolute values of the intrinsic viscosities and dissociation constants obtained. The following results, however, may be considered reliable.

SUMMARY

The theoretical analysis of the temporary change in viscosity shows that the polymerized alcaline metaphosphates dissociate in a series of successive reactions, each of which may consist of several side reactions. Two stages of this series could be definitely isolated and the third with some probability, with respect to possible further stages the experiments yield no results. The first dissociation proceeds very quickly, but in the following the velocities decrease noticeably. In the same way the intrinsic viscosities decrease, but their values show that the dissociation products are still highly polymerized.

The final state is by no means reached even at the end of a month, and a freshly prepared solution rapidly passes into a polydispersed system even if it was highly polymerized in a uniform manner at the outset.

The author again takes the opportunity of cordially thanking Professor The Svedberg for his constant friendly interest.

The author is also indebted to fil.mag. H. Malmgren, whose experiments were the starting point for the present investigation.

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* If $k^4 = 4.0 \cdot 10^{-2}$, A is dissociated to more than 99 % at $\tau = 160$.

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The Degradation of Potato Starch by Pancreatic Amylase

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It is generally assumed that the fermentable sugar formed by the action of pancreatic amylase on starch is maltose. Preliminary investigations (1934) did not agree with this assumption. This work (1940) was carried out to get more information about the enzymic degradation products in general and about the fermentable sugars in particular. For this purpose the action of pancreatic amylase on starch paste was interrupted at different degrees of hydrolysis and the degradation products formed were fermented by *Saccharomyces cerevisiae*. Results calculated from gravimetric, reductometric and polarimetric measurements before and after fermentation are collected in the following table. The difference between fermentable and unfermentable carbohydrates is well defined. Fermentation stops abruptly. The weight of unfermentable carbohydrates decreases during hydrolysis to about 25 % TMG, the rest being dextrins which are attacked very slowly. Similar phenomena are observed with α-β-malt amylases. During hydrolysis the reduction power of the unfermentable components (% TMR) increases, remains constant and, finally, decreases. RM follows a similar curve. The weight of fermentable carbohydrates increases to about 75 %, then remaining constant although the hydrolysis, measured in % TMR, continues. Until a degradation to about 60 % TMR, RM of fermentable carbohydrates is constant = 88. This result indicates that *considerable quantities of fermentable oligosaccharides with a lower reduction power than maltose are formed.*

The degradation products of unfermentable carbohydrates may be a) unfermentable, b) mixtures of fermentable and unfermentable or c) fermentable carbohydrates. Hydrolysis of fermentable carbohydrates can only yield d) fermentable sugars. At every stage of degradation all 4 reactions are possible. The present results indicate that all of them occur, however, more or less dominantly. A detailed study of the table leads to the conclusion that in the course of degradation the processes a), b), c) and d) dominate in the

Table 1. Potato starch paste and pancreatic amylase. Starch paste 3 %, pH = 6.9, 20°, [Cl⁻] = 0.005.

Before fermentation	Unfermentable			Fermentable			
% TMR	% TMG	% TMR	RM	% TMG	% TMR	RM	$[\alpha]_D^{20}$
18.1		4.9			13.2		
26.1	79.7	8.3	10	20.3	17.8	88	
32.7	74.1	9.7	13	25.9	23.0	89	
39.1	67.6	10.3	15	32.4	28.8	89	
48.9	56.0	10.3	18	44.0	38.6	88	
53.7	49.9	10.0	20	50.1	43.7	87	
59.8	43.9	10.3	23	56.1	49.5	88	
64.0	38.8	9.1	23	61.2	54.9	90	
67.9	32.1	7.1	22	67.9	60.8	90	
69.7		5.3			64.4		
71.2	29.9	4.6	15	70.1	66.6	95	154°
71.6	28.6	4.8	17	71.4	66.8	94	151°
81.5	24.6	6.7	27	75.4	74.8	99	138°
82.9	24.8	6.5	26	75.2	76.4	102	141°
86.3	25.3	6.3	25	74.7	80.0	107	141°
91.8	24.3	6.4	26	75.7	85.4	113	134°

% TM = Percentage of theoretically obtainable maltose.
R = Reductometrical determination. Bertrands method.
G = Gravimetrical determination.
RM = Reduction power; maltose = 100.

mentioned sequence. Generally, it can be concluded that the large molecules are hydrolyzed at first — % TMR of unfermentable increases. The chains become shorter — % TMR of unfermentable is constant. Finally the chains become so short that both fractions are fermentable — % TMR of unfermentable decreases. When process c) has practically finished, reaction d) starts. The latter manifests itself as an increase in RM and a decrease in $[\alpha]_D^{20}$ of the fermentable carbohydrates. Also the following investigations on starch degradation (% TMR) as a function of time (fig. 1) indicate that a primary process has practically finished at about 65 % TMR, while a secondary process starts, running 2—3000 times more slowly. A comparison of the table and the diagram leads to the conclusion that the primary very rapid process consists of reaction a), b), c), while the secondary, very slow process, must be reaction d).

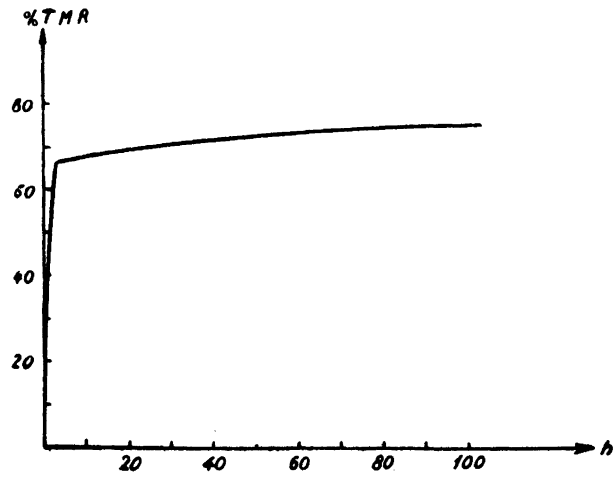

Fig. 1. Degradation of potato starch paste by pancreatic amylase as a function of time. Starch paste 3 %, pH = 7.2, 37°, [Cl⁻] = 0.005.

SUMMARY

Experiments on the degradation of potato starch by pancreatic amylase performed in 1940, however, not published in view of the occupation of our country, offer information on the structure of starch. The main result is that considerable quantities of fermentable oligosaccharides are formed which have a lower reduction power and a higher specific rotation than maltose.

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The Formation of Trisaccharides during Degradation of Potato Starch by Pancreatic Amylase

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In 1941, Smits van Waesberghe [1] showed that during degradation of potato starch by bacterial amylase apart from glucose and maltose small quantities of a third fermentable sugar were formed, presumably a trisaccharide. The separation was carried out by fermentation with *Zygosaccharomyces marxianus*, *Saccharomyces uvarum* and *Saccharomyces cerevisiae*. The first only ferments glucose, the second glucose and maltose and the third all three sugars. Our preceding work was interrupted because we were unable to separate the fermentable sugars. It was resumed in 1944—45 when we got cultures of *S. uvarum* and *Z. marxianus*.

It may be concluded from the table in the preceding paper that the unknown carbohydrates must be present in maximum amount when the difference between % TMG and % TMR of fermentable carbohydrates is greatest. This is the case at a degradation of about 65 % TMR. Experiments confirmed our assumption; only the experiments in the following table will be mentioned; others gave similar results. *S. uvarum* does not attack the unknown carbohydrates which are fermented by *S. cerevisiae*. The degradation products of starch hydrolyzed to 64 % TMR contain no glucose, about 33 % disaccharides, about 31 % trisaccharides and about 36 % dextrins. The RM of the unknown sugars indicates that we have to deal with trisaccharides. They can easily be separated from lower molecular sugars; a separation from higher molecular components involves great difficulties. By precipitations and extractions with methyl- and ethylalcohol of different concentrations we

* This and the preceding paper were read in a joint session of Swedish and Danish chemists at Lund, 11. 5. 1946.

We wish to express our best thanks to the Management of the Tuborg Breweries for excellent working conditions.

Table 1. Degradation products of potato starch by pancreatic amylase. Starch paste 3 %, pH = 6.9, 20°, [Cl⁻] = 0.005. Degree of hydrolysis 63.8 % TMR.

	% TMG	% TMR	RM	$[\alpha]_D^{20}$
Zygosaccharomyces marxianus				
Glucose	0.0	0.2	—	—
Saccharomyces uvarum				
Glucose + disaccharides	32.8	34.4	105	138°
Saccharomyces cerevisiae				
Glucose + disaccharides + trisaccharides	63.3	56.9	90	150
Trisaccharides	30.5	22.5	74	162°
Dextrins	36.7	6.9	19	—

Abbreviations, see preceding paper.
Reductometrical determination. Potassium copper carbonate method [7].

obtained a dry product in a purity of 84 % and a yield of 45 g from 450 g potato starch. But crystallisation failed. By means of measurements before and after fermentation with *S. cerevisiae* it was possible to calculate the following constants of the trisaccharides $[\alpha]_D^{20} = 163°$, mol. wt. (freezing point) 500 (theory: 522), equiv. wt. (iodine) 265 (theory 261), RM = 71. The determinations were controlled by parallel experiments with glucose and maltose, specially prepared. The constants show that *the unknown carbohydrates must be aldotrisaccharides*, and since starch only contains glucoserests they must be glucotrisaccharides. The trisaccharides are hydrolyzed by enzymes from pancreas and yeast, not or only very slowly by β-amylase from barley. The first step of hydrolysis of glucotrisaccharides must yield glucose and disaccharides. The formation of glucose starts at a starch-degradation of about 65 % TMR (see preceding paper). By comparison of the described starch-degradation interrupted at 63.8 % TMR and a starch-

Table 2. Formation of glucose from potato starch by pancreatic amylase. Starch paste 3 % pH = 6.9, [Cl⁻] = 0.005.

	20°	33°
Degree of hydrolysis	63.8 % TMR	91.8 % TMR
Glucose	0 » TMG	11.3 » TMG
Disaccharides	32.8 » »	51.5 » »
Trisaccharides	30.5 » »	12.5 » »
Dextrins	36.7 » »	24.7 » »

Abbreviations, see preceding paper.
Reductometrical determination. Potassium copper carbonate method [7].

degradation stopped at 91.8 % TMR (table 2) it is found that in this step about 11 % glucose and about 19 % disaccharides were formed. The trisaccharides decreased simultaneously about 18 % and the dextrins about 12 %. These facts are best explained by assuming the formation of trisaccharides from dextrins and a partial hydrolysis of trisaccharides into glucose and disaccharides. The hydrolysis of the trisaccharides is most certainly a property of the amylase itself, experiments to eliminate this property by partial heat-inactivation of the pancreatic enzymes being without result.

The isolation of trisaccharides with similar $[\alpha]_D = 150—170°$ from starch or glycogen has been denied almost as often as it has been reported. We only want to cite a few examples. Barbour [2] stated that he had isolated a trisaccharide from glycogen, $[\alpha]_D = 154°$. The result could, however not be confirmed [3]. Ling and Nanji [4] reported that they had isolated a trisaccharide from starch with the constants RM = 66; $[\alpha]_D = 165°$. Later investigations by Ling [5] showed however, that the trisaccharide formerly prepared was a mixture. Smits van Waesberghe [1] isolated small amounts of a similar trisaccharide as ours $[\alpha]_D = 154°$. Using bacteriaamylase, which easily splits the trisaccharides, the yield was very poor.

The trisaccharide, isolated by Myrbäck and Ahlborg [6] from starch, $[\alpha]_D = 124°$, must be of a different constitution.

SUMMARY

The work of the preceding paper was continued in 1944—45. The degradation products of starch, hydrolyzed to about 64 %, contain 1/3 disaccharides, 1/3 trisaccharides and 1/3 dextrins. The glucotrisaccharides, $[\alpha]_D^{20} = 163°$, RM = 71, mol. wt. 500 (theory 522), equiv. wt. 265 (theory 261), are hydrolyzed by pancreatic amylase under formation of disaccharides and glucose.

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An Intermediate Compound in the Catalase-hydrogen peroxide Reaction

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A review of the recent developments in the study of catalase has been made by Theorell [1]. The bulk of the past literature contains the following types of studies on the mechanism of catalase action:

1. Determination of the overall catalatic activity in very dilute enzyme-solutions by extrapolation to the activity at $t = 0$ (Euler and Josephson [2]).

2. Comparisons of the activity of inorganic catalysts with catalase in order to determine the mechanism by analogy (for a summary see Stern [3]).

3. The study of the catalytic activity model systems such as catalase-ethylhydroperoxide (Stern [4]) and azide-catalase-peroxide (Keilin [5]) leading to various conclusions concerning the activity of the enzyme in the presence of hydrogen peroxide.

4. The study of the »coupled» or peroxidatic reaction of catalase, alcohols, and enzyme systems which produce hydrogen peroxide continuously (Keilin[6]).

In no case has the activity of this enzyme been studied by means of the characteristics of a spectroscopically-defined intermediate compound of enzyme and substrate as in the case of peroxidase [7]. In fact the papers of Haldane [8] and Keilin [5] tend to discourage the search for such a compound, to quote from Keilin [5], »the reaction with H_2O_2, which consists in a violent, almost explosive, decomposition of the latter, during which it is impossible to observe any changes in colour and absorption spectrum of the enzyme».

These studies [9] were initiated in 1938 in a serious effort to find an intermediate compound of catalase and hydrogen peroxide. Due to a large number of technical difficulties success has only been achieved in the last few months and this paper gives preliminary spectral, kinetic, and equilibrum data. This intermediate compound differs markedly from the one postulated by the

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Michaelis theory and stated by Euler and Josephson [2] to have a very low affinity for H_2O_2. This compound has a high affinity for hydrogen peroxide, exists at a constant concentration during catalatic activity, and decomposes slowly in the absence of alcohols and rapidly in their presence. The compound appears to be a ferric-iron hydrogen-peroxide complex, which has many properties in common with those of peroxidase. However, this complex has both peroxidatic and catalatic properties.

METHOD

The bulk of the data presented in this paper was obtained by what may be called »Spectrophotometry with Time Resolution». The cuvettes containing solvent and solution usually employed in the spectrophotometry of liquids are replaced by a single capillary »cuvette» into which the *mixed* but *unreacted* components of the solution are rapidly injected. Modifications of the mixing chambers of Roughton and Millikan [10] are incorporated in the syringe unit of fig. 1. Time resolution requires a small bore capillary (1 mm) which can be rapidly filled with unreacted solutions. Second order reactions require dilute solutions ($\sim 1 \times 10^{-6}$ *M*) in order to reduce their rate of reaction. The effective

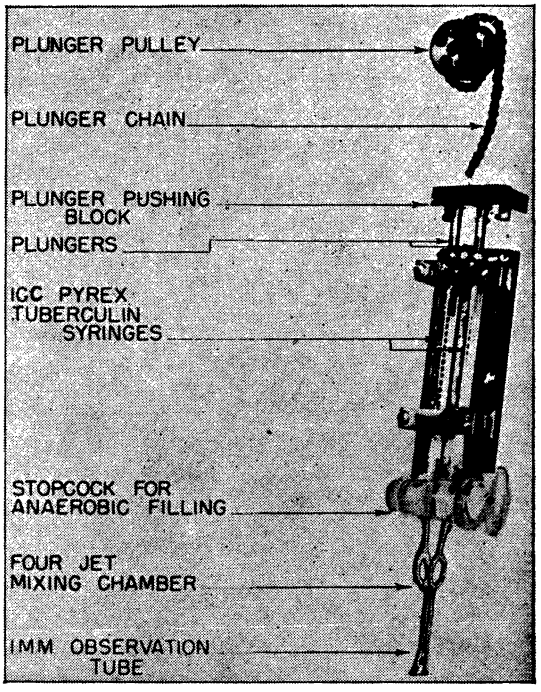

Fig. 1. The syringe unit.

depth of the capillary, as illuminated by the exit slit of the spectrophotometer, is only 0.47 mm. The density of strong enzyme solutions is, therefore, very small ($\log \frac{Io}{I} \leq 4 \times 10^{-2}$), and the density changes due to some intermediate compounds much smaller ($\log \frac{Io}{I} \approx 3 \times 10^{-3}$).

The measurement of these small density increments with an accuracy of 3 % ($\log \frac{Io}{I} \approx 1 \times 10^{-4}$), requires an extremely stable light source and photoelectric recording system. The technical details of this apparatus will soon appear elsewhere [11] and only an outline of the function is given here. As fig. 2 shows, the voltage for the tungsten lamp is stabilized by an electronic controller which reduces fluctuations by 3×10^4. The drift of the lamp voltage is less than 10^{-5} parts per minute. A double monochromator (Coleman Electric Company) covering a range from 340 to 1000 mμ and having a spectral

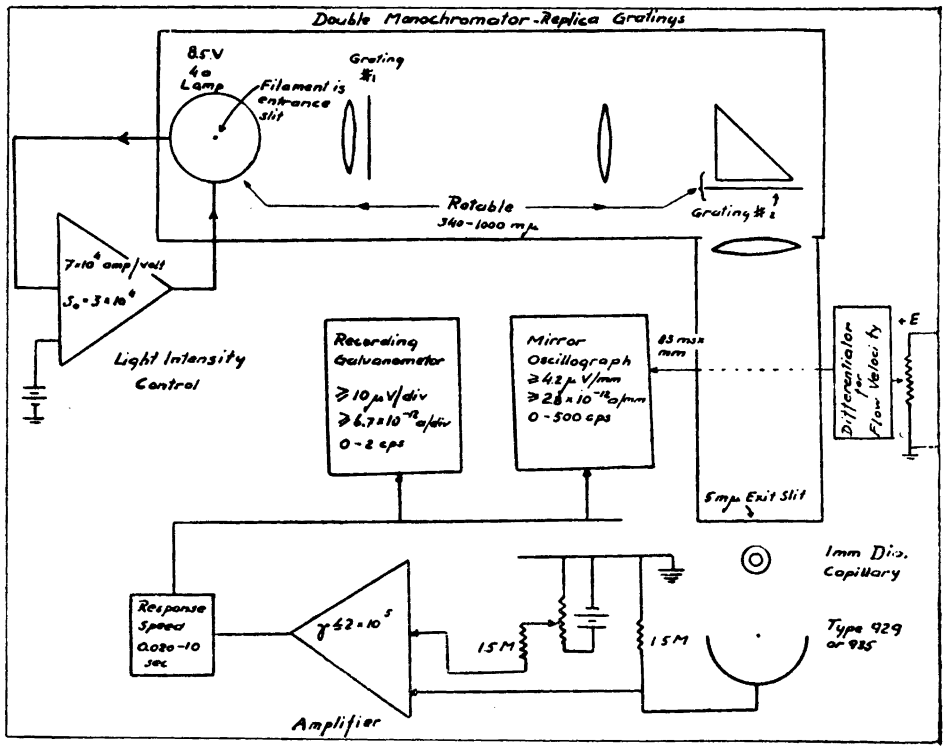

Fig. 2. Functional diagram of the method for obtaining spectroscopic data on unstable intermediate compounds.

interval of 5 mμ illuminates the photocell with light transmitted through the capillary. The photocurrent is increased in a stable amplifier of adjustable response speed and finally operates two recorders (1) an ink-writing recorder having a response to 2 cps. and (2) a photographic recorder using a galvanometer oscillograph responding up to 500 cps.

The method of operation consists simply of filling the syringes with enzyme and substrate, setting the gain, response speed, wavelength, and zero point appropriately, and pushing upon the bakelite block holding the syringe plungers together (fig. 1). The capillary is then filled with the mixed but unreacted components of the solution and, after the flow has stopped, the reaction is recorded directly in ink or upon photographic paper. This is called the »stopped flow method» (fig. 3).

If the reaction to be studied occurs in less than 0.05 s the flow method of Hartridge and Roughton [10] is employed — but no modification of the apparatus is required except, (1) the recording is done at an appropriately higher speed of photographic paper and amplifier response and (2) flow velocity is simultaneously recorded photographically. Each value of flow velocity corresponds to a certain time interval between mixing and observation and to a certain value of optical density or concentration of intermediate compound (fig. 5).

PREPARATIONS

All the catalase solutions used in these experiments were prepared and crystallized by Bonnichsen [12, 13] and the tests of a large number of different catalases (table 1) would have been impossible without his assistance. The concentration of the horse liver catalases was determined straightforwardly from $\varepsilon_{405\,(c\,=\,1\,mM)} = 340$ (Agner [14]). For the other catalases, the concentration was determined on a hematin-iron basis and, in the

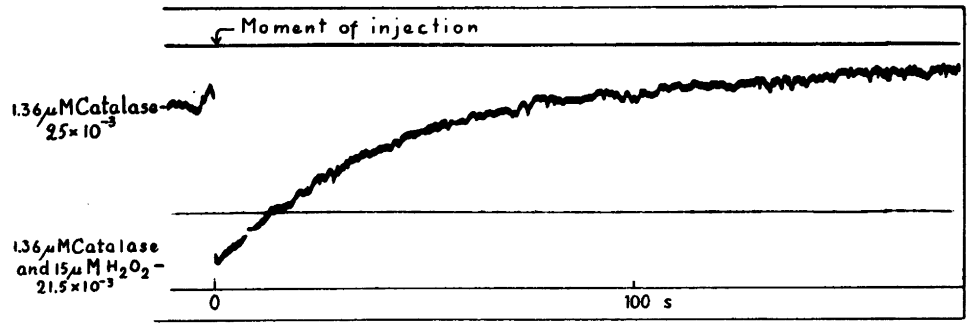

Fig. 3. The kinetics of the intermediate compound in the catalasehydrogen-peroxide reaction at 405 mμ.

case of the blood and kidney catalases where there are four hemin groups, the extinction per catalase molecule was taken as $\varepsilon_{405\,(c\,=\,1\,mM)} = 380$ (Bonnichsen [12]).

THE SPECTRUM OF THE INTERMEDIATE COMPOUND

Many, many experiments with an apparatus using glass color filters [7] and with this apparatus were fruitless because the assumption was made that the spectrum of the intermediate compound would resemble that of azide-catalase-peroxide. The first apparatus balanced 385 mμ against 420 mμ while in the second, a wavelength of 430—435 mμ was used. Also these fruitless experiments were made under optimum conditions dictated by the Michaelis theory; as dilute enzyme as feasible.

At a wavelength of 405 mμ and with a catalase concentration in excess of 1 μM and a several fold excess of H_2O_2, the transient intermediate compound of catalase and hydrogen peroxide is found and a typical record of its formation and disappearance is shown in fig. 3. At the moment of injecting catalase and hydrogen peroxide into the capillary a very small ($\log \frac{Io}{I} = 4 \times 10^{-3}$), but very rapid decrease of density occurs. The compound is momentarily stable

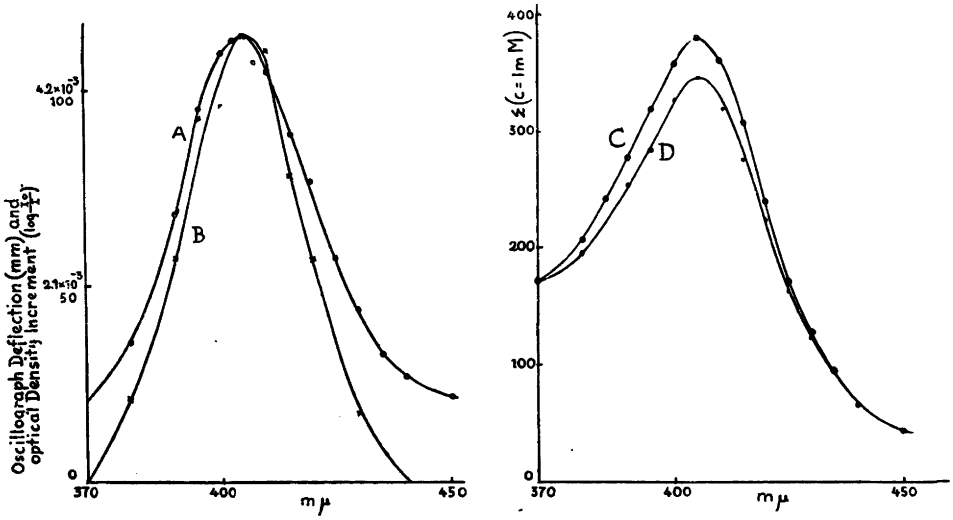

Fig. 4. The spectrum of the intermediate compound in the region of the Soret band. The left figure compares the differences between catalase and water (A) with those between catalase and the intermediate compound (B). The right figure shows an absolute spectrum computed from the difference spectrum. Expt. 51 b + d. 3.4 μM horse blood catalase, 7.5 μM H_2O_2, $S = 5$ for curve A, $S = 20$ for curve B (an eleven-fold change), pH = 6.7, 0.01 M phosphate. C is the catalase spectrum and D the catalase-hydrogen peroxide spectrum.

and then decomposes slowly liberating catalase in a reaction which requires 40 s for half completion in this experiment.

Fig. 4 (left) shows the variation of the oscillograph deflection (crosses) with wavelength. This is the »difference spectrum» between catalase and the intermediate compound over the range where the deflection exceeds the experimental error. For comparison the »difference spectrum» of the same catalase solution and water, but with the sensitivity reduced 11 times, is plotted with circles.

The difference spectrum of the intermediate compound can be converted into an absolute spectrum by applying these differences, with appropriate corrections, to a catalase spectrum determined in the ordinary manner. The following procedure is used:

1. The spectrum of a catalase solution is taken in the usual manner (Beckman spectrophotometer) and converted into units of $\varepsilon_{c=1\,mM}$.

2. This solution is used to calibrate the capillary by measuring the oscillograph deflection with respect to water — usually at 405 mμ where slight errors of wavelength have less effect.

3. At the same wavelength the oscillograph deflection corresponding to the difference between catalase and the maximum concentration of the intermediate compound is converted to absolute units by the formula

$$\Delta\,\varepsilon_I = \varepsilon_c \frac{D_k}{D_s} \cdot \frac{S_k}{S_s} \cdot \frac{C_s}{C_k}$$

where $\Delta\,\varepsilon_I$ = the difference of extinction between catalase and the intermediate at λ_1,
ε_c = the extinction of catalase at λ_1,
D_k = the maximum deflection of the oscillograph in the kinetic experiment at λ_1,
D_s = the deflection of the oscillograph in the static calibration at λ_1,
S_k = the sensitivity in the kinetic experiment (μvolts/mm),
S_s = » » » » static calibration (» / »),
C_s = » concentration » » » » (μM)
C_k = » » » » kinetic experiment (μM).

4. The value of $\Delta\,\varepsilon_I$ at $\lambda_1 \ldots n$ is obtained from the values of $(D_k)\,\lambda_1 \ldots n$ and the value of the photocurrent at the particular λ. The absorption of the catalase solution used was so small ($\log \frac{Io}{I} = 4 \times 10^{-2}$) that no corrections were made for this factor.

$$(\Delta \varepsilon_I)_{\lambda_n} = (\Delta \varepsilon)_{\lambda_1} \cdot \frac{E_{\lambda_1}}{E_{\lambda_n}} \cdot \frac{(D_k)_{\lambda_n}}{(D_k)_{\lambda_1}} \cdot \frac{(S_k)_{\lambda_n}}{(S_k)_{\lambda_1}}$$

where $E_{\lambda_1 \ldots n}$ is proportional to photocurrent (units are millivolts).

5. The values $(\Delta \varepsilon_I)_{\lambda_1 \ldots n}$ are then applied to the curve 1 above with the appropriate sign; $\varepsilon_I = \varepsilon_c - \Delta \varepsilon_I$.

The result is shown in the right-hand portion of fig. 4. The features of these data are

1. The Soret band of the intermediate compound is sharper than that of catalase.

2. It is shifted a few millimicrons to the visible (see left figure).

3.* The extinction coefficient is only slightly less than that of catalase ($\sim$ 90 %).

4.** The spectrum does not resemble those of the catalase-cyanide or azide-peroxide compounds.

Using even stronger catalase (7.5 μM) some deflections are obtained between 500 and 550 mμ ($(\Delta \varepsilon_I)_{\lambda = 530} \approx 5$) and have values approximately equal to the minimum detectable at the Soret band (at 425 mμ for example). The absence of spectral data in the intervals 430—490 mμ and 560—650 mμ is, therefore, no convincing proof that such data do not exist. It is hoped that a refinement of the method will give the isobestic points and the complete spectrum; at present, the data are apparently only obtainable when the difference between the absorption of catalase and the intermediate compound is large.

Table 1. The extent of the spectral shift at 405 mμ for various catalase preparations.

Type	Relative catalase activity	$\frac{D_{405}}{D_{280}}$	Conc. (μM)	$\Delta \varepsilon_{405}$	$\left(\frac{\Delta \varepsilon}{\varepsilon}\right)_{405}$
Horse blood	1***	1.2	1.9	42	0.11
Horse blood	—	0.96	4.3	32	0.08
Horse kidney	0.65	1.0	1.6	39	0.10
Horse liver	—	1.0	3.3	36	0.11
Horse liver	0.52	0.38	1.9	29	0.09
Horse liver	0.28	0.46	2.3	16	0.05
Human liver	0.31	0.7	3.4	19	0.06

* It is interesting to note that this is nearly equal to the difference between the extinction of three and four hemin catalases [12].

** A graphic illustration of the difference between the spectrum of the intermediate compound and that of the cyanide compound is given in fig. 22.

*** Cat. F. $\approx$ 60,000.

A large number of different catalase preparations gave similar values for $(\Delta \varepsilon_I)_{\lambda = 405}$ and the characteristics of the preparations and the spectral data are tabulated in table 1. In the last two preparations a somewhat smaller concentration of the compound was formed. This may possibly be correlated with the relative catalatic activity.

THE FORMATION OF THE INTERMEDIATE COMPOUND

Fig. 5 shows a 100-fold time expansion of the initial decrease of density of fig. 4. Here the records clearly show that the compound does not completely form until the flow stops — a time delay of nearly 30 milliseconds. In fact the compound is roughly half formed in about 8 milliseconds. Other records taken with larger concentrations of H_2O_2 show the intermediate compound to be completely formed at the earliest time after mixing (fig. 18 and 23).

The three experiments shown in fig. 5 were taken under widely different conditions, in A the compound is stable for about a second after its formation;

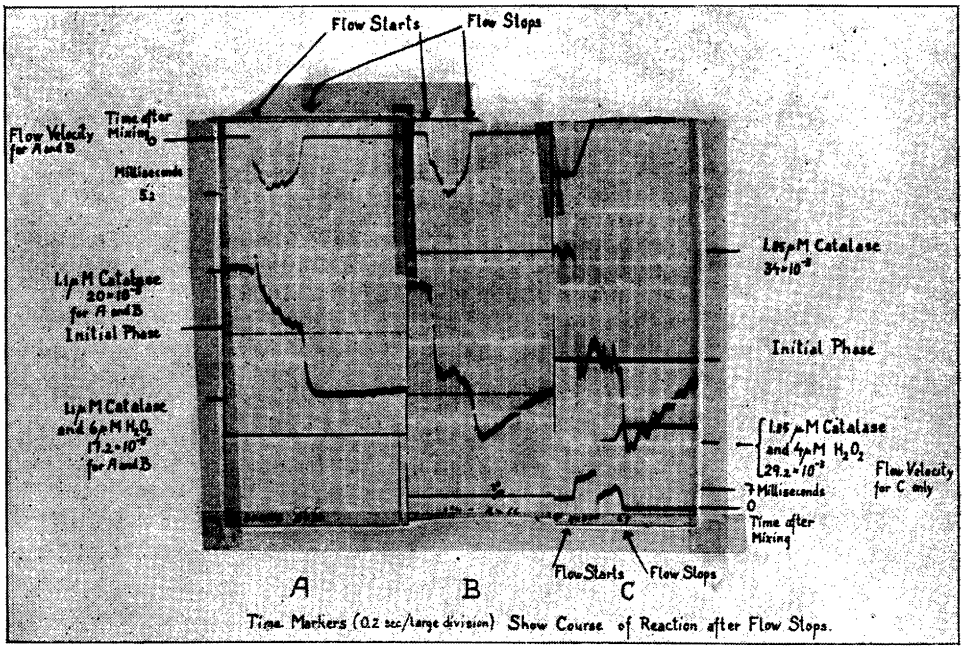

Fig. 5. The kinetics of the formation of the intermediate compound. Curves A and B were obtained with the apparatus of fig. 2 while C was obtained with an apparatus using a Jena UG — 3 filter. Ethanol was added in B and C (400 and 500 μM respectively). Time markers (0.2 s/large division) show course of reaction after flow stops.

in B and C it decomposes more rapidly due to added alcohol. This effect will be discussed later. Curves A and B were obtained using a monochromator while C was obtained in a different apparatus using a Jena-UG 3 filter and a more rapidly responding amplifier.

A second order velocity constant* was obtained from curves similar to C and the values are listed in table 2.

Table 2. Experiments 69 and 75.

H_2O_2 — μM	2	4	8	10	20
$k_1 \times 10^{-7}$ L $\times$ $M^{-1} \times s^{-1}$	7.0	2.4	3.1	3.8	1.4
e	1.85 μM horse blood catalase				
a	1000 μM ethyl alcohol				

The deviations of the values for $H_2O_2 = 2$ and 20 do not permit the statement that the reaction strictly obeys a second order equation over the entire range although the data for $H_2O_2 = 4$, 8, and 10 strongly favor this viewpoint and in fact are much more reliable for the following reasons:

1. At 20 μM the reaction is 85 % complete at the earliest time measured.
2. At 2 μM the amount of intermediate formed is 1/3 the saturation value and the errors are increased proportionally.

For the middle concentrations the value of 3×10^7 may be compared with that for peroxidase, myoglobin, and the calculated value for catalase, 1.2 (Chance [7]), 1.9 (Millikan [15]), 0.76 (Haldane [8]) $\times 10^7$ respectively and it is seen to be somewhat larger, especially with respect to the calculated value. This is an extremely important comparison since it reveals that *the rate of formation of this compound is sufficient to carry the entire catalatic activity of this enzyme* providing a mechanism for its rapid decomposition is afforded. The exact value of the velocity constant and the determination of the range over which a second order equation holds awaits further experiment.

THE DECOMPOSITION OF THE INTERMEDIATE COMPOUND

The experimental data, however, show that the decomposition of this intermediate compound is an extremely slow first-order reaction as is illustra-

* In this calculation it is assumed that the intermediate compound consists of one molecule of H_2O_2 per catalase molecule. On the basis of four H_2O_2 per catalase molecule the velocity constant would be somewhat larger.

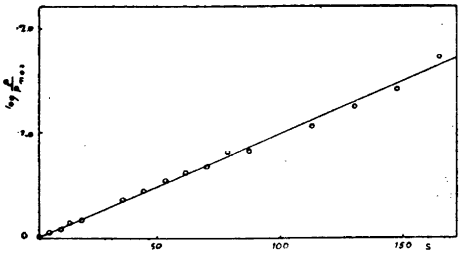

Fig. 6. The order of the reaction for the decomposition of the intermediate compound. The curve of fig. 3 plotted according to the first order equation, $k_3 = 0.02\ S^{-1}$. Expt. 59, 1.36 µM horse blood catalase, 12 µM H_2O_2, $\lambda = 405$, $S = 30$, $t = 24°\ C$, $pH = 6.7$, 0.01 M phosphate.

ted by fig. 6 where the data of fig. 3 are analysed. The reaction velocity constant is 0.02 S^{-1}. This decay time is much too long to account for the high catalatic activity of the enzyme. Furthermore the decay time and duration of the »cycle» do not in any way vary in the manner found experimentally and theoretically for the enzyme-substrate compound of peroxidase [13].

$$t_{1/_2\ \mathrm{off}} = \frac{x_0}{p_{\max} k_3}$$

Further support to this discrepancy is afforded by the data of fig. 7 where the time from the maximum to half the concentration of the intermediate is shown to be relatively *independant* of the peroxide concentration — certainly not proportional to it as the equation above requires.

These data also show another characteristic of the decay of the compound — considerable variation from preparation to preparation. In no case has the compound been completely stable and in cases where the compound decayed rapidly, a 24 hour dialysis removed the alcohol in the preparation and the usual rate was obtained — on the average about 40 s half time. In a particular case a preparation was repeatedly dialysed and then dried in order to remove all traces of alcohol. The usual rate was, however, obtained. The rate of decomposition is temperature sensitive and some of the systematic

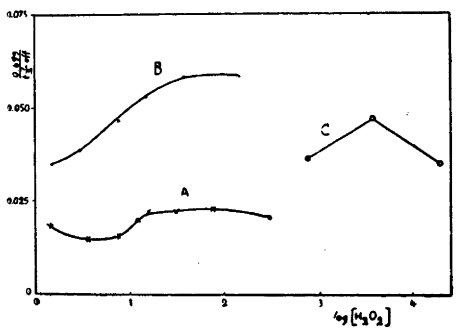

Fig. 7. The effect of initial hydrogen peroxide concentration upon the rate of decomposition of the intermediate compound.

× *Expt. 50 b, 0.68 µM horse blood catalase.*
• *Expt. 51 a, 3.4 µM » » »*
○ *Expt. 57, 1.36 µM » » »*
△ *Expt. 54, 55, 56, and 59.*

variation shown in the top curve is due to uncontrolled variation during the experiment.

Simple calculations indicate that the bulk of the catalatic activity can occur in the few moments while the concentration of the intermediate compound is constant — in fig. 3 e $= 1.36 \times 10^{-6}$ μM catalase, $x_0 = 15 \times 10^{-6}$ M H_2O_2. Since so little H_2O_2 is present the turnover of substrate will be governed entirely by the rate of combination of enzyme and substrate in the catalatic activity. Using the value just determined (3×10^7), the peroxide concentration can fall to a value roughly equal to the enzyme concentration in

$$\frac{2.3 \log \frac{15}{1.36}}{3 \times 10^7 \times 1.36 \times 10^{-6}} = 0.06 \text{ s}$$

— approximately the time interval that the concentration of the intermediate compound of fig. 3 remains constant. Therefore, this compound *does not begin to decompose until the bulk of the catalatic activity is complete* and the hydrogen peroxide concentration has fallen to a very low value (see »Controls»).

If, however, the substrate concentration approaches the saturation value for the catalatic activity (0.025 M^2), the »saturated» interval for this intermediate compound may be expected to increase and this is shown in fig. 8 where the hydrogen peroxide concentration is 4 mM. It is interesting that

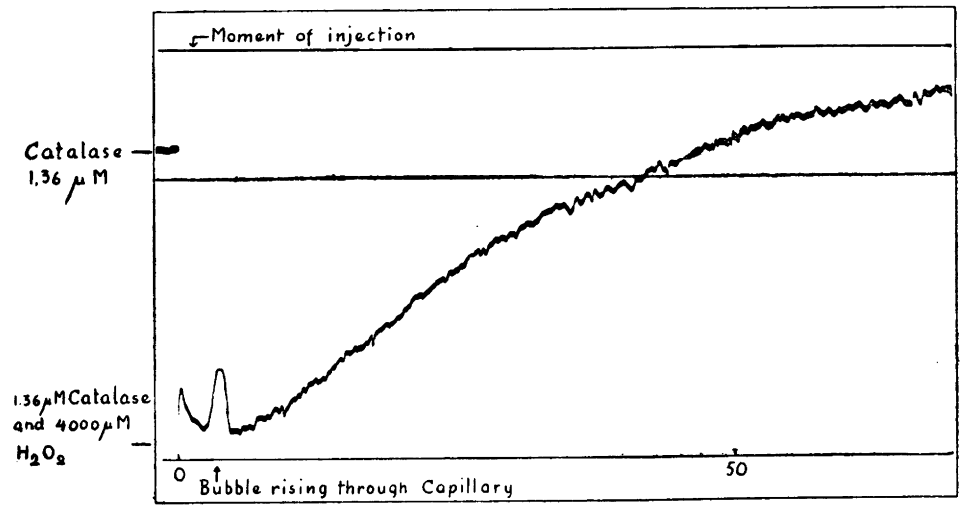

Fig. 8. The kinetics of the intermediate compound in the presence of a large initial excess (4 mM) of hydrogen peroxide. The conditions are otherwise as in fig. 3 except that the solutions were bubbled with H_2 to increase the solubility of O_2.

such a large increase of initial substrate concentration has such a small effect upon the kinetics of the intermediate compound.

Since catalase is approximately one-seventh saturated at this substrate concentration, a careful search of the spectrum was made to determine whether an addition intermediate compound could be found which had a life-time of a few hundredths of a second. So far no evidence has been obtained. It should be pointed out that these experiments are very difficult to carry out successfully since the initial portions of the curves are usually obliterated by oxygen bubbles and an inert gas was used to minimize this effect. An example of this appears in fig. 8.

THE EQUILIBRIUM CONSTANT FOR THE INTERMEDIATE COMPOUND

The relation between initial hydrogen peroxide concentration and density change is shown in fig. 9 (left), and in fig. 9 (right) the data are plotted as if a simple equilibrium existed (two opposing reactions) and on the assumption that only one of the four catalase hematins is involved in the intermediate compound. The latter assumption was required by the former since concentrations of the intermediate compound in excess of the initial peroxide concentration were obtained on the basis of more than one hematin participating in the reaction.

The value found by this method (1.6×10^{-6} M) greatly exceeds the ratio

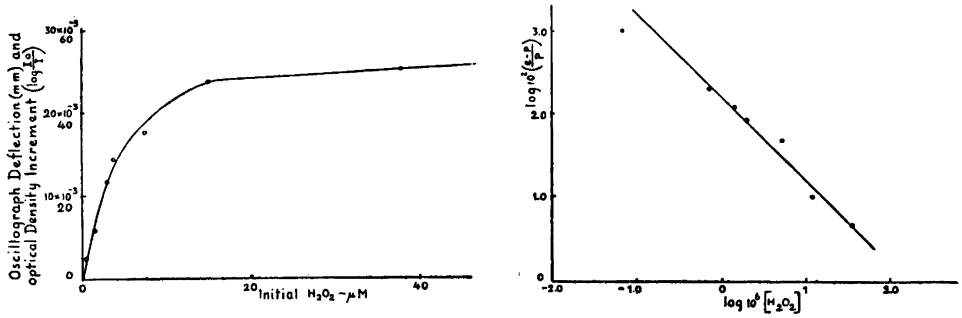

Fig. 9. The equilibrium of catalase and hydrogen peroxide. The left figure shows the variation of oscillograph deflection with initial hydrogen peroxide concentration and the right figure shows the data plotted assuming a simple equilibrium obtained and that the compound consists of one molecule of peroxide per catalase molecule. e = initial catalase, x = free H_2O_2, p = the concentration of intermediate compound.

Expt. 51 a, 3.4 μM horse blood catalase, λ = 410, s = 10, t = 25° C, pH = 6.7, 0.01 M phosphate, $t_{1/2\ off} = 14$ *s,* $k_3 = 0.05\ S^{-1}$.

of the rates of decomposition (fig. 6) and formation (table 2) of the intermediate compound (1×10^{-9} M). Therefore the equilibrium may be assumed to be composed of at least three reactions. The third is due to the high catalatic activity during the initial phases of the kinetics of the intermediate compound. (A high catalatic activity during the decay of the intermediate compound has already been shown to be unlikely because of the relation between $t_{1/2\,\mathrm{off}}$ and hydrogen peroxide concentration (fig. 7)). A first-order constant (k_7) for the catalatic activity is obtained, $K_c = \frac{k_3 + k_7}{k_1}$, $k_7 = 1.6 \times 3 \times 10^{-6+7} - 0.02 = 48\ \mathrm{s}^{-1}$, a value quite large compared with k_3. Since the catalatic activity is likely to occur at substrate concentrations slightly in excess of that of the enzyme (again since the intermediate compound decays slowly), a second-order constant is calculated; $\frac{48}{4 \times 10^{-6}} = 1.2 \times 10^7\ L \times M^{-1} \times s^{-1}$.

This value has a striking resemblance to the rate of combination of substrate with enzyme (3×10^7) and immediately suggests that the catalatic activity may be due to consecutive reactions of enzyme and substrate. Since a relatively stable compound (the intermediate compound) is seen by kinetic data (fig. 3) to exist throughout the catalatic activity, it is concluded that the catalatic reaction is due to a combination of more substrate molecules with the intermediate compound to yield a very unstable compound.

This sort of mechanism has been repeatedly proposed for hemin and for catalase activity[16, 17, 18]. In neither of these cases was any mechanism indicated whereby a peroxidatic activity and a very *high* catalatic activity could be obtained. In fact the experimental data for hemin[16], table 2) show just the opposite, catalatic/peroxidatic = 1×10^{-3}. The figure is about 4×10^4 for catalase, a net discrepancy of 4×10^7.

Unfortunately, the possibility of consecutive reactions vitiates the calculation of the composition of the intermediate compound on the basis of a simple equilibrium. The saturation value for the intermediate compound will vary with the ratio of k_1/k_7 and unless this ratio is known the calculation of the equilibrium constant cannot be made with accuracy. The calculation for k_7 given above must also be in error for the same reason.

To summarize, the catalatic activity during the initial phases of the kinetics of the intermediate compound results in the decomposition of an appreciable portion of the initial hydrogen peroxide in a reaction which has not yet been studied. The calculation of the equilibrium constant and the composition of the intermediate compound require such data. Until the composition of the intermediate compound is definitely known, the mechanism for the catalatic and peroxidatic action cannot be given with finality.

CONTROLS

A large number of control experiments were carried out in addition to those contributing to our understanding of the enzyme mechanism. Perhaps the most valid control is that of the next section which shows that this compound has sufficient activity to explain Keilins »coupled oxidation» of alcohols by catalase and hydrogen peroxide. However, an enumeration follows:

1. Does the apparatus show artifacts on mixing catalase and buffer? None in excess of 2 % of the maximum deflection and those are due to a weak mechanical mounting of the syringe unit. There is negligible drift of the baseline.

2. Is this a photochemical reaction excited by light at the peak of the Soret band? Certainly not under the conditions of 1. In the presence of hydrogen peroxide there is no difference irradiating the capillary first through a monochromator or directly from the tungsten lamp, followed by a filter (see fig. 5 and »Discussion»).

3. Is all the catalase liberated at the conclusion of the cycle? This is tested by immediately following a kinetic curve, for example that of fig. 3, by a mixture of the same catalase solution and buffer; the hydrogen peroxide is omitted. The baseline thus obtained corresponds with that obtained at the end of the cycle within the experimental error (5 %).

4. Was these an impurity in the H_2O_2 or buffer solutions? For the buffer solutions see 1. For the H_2O_2, the affinity data must indicate that the H_2O_2, if impure, is nearly completely impure and titres satisfactory with $KMnO_4$.

5. Were there impurities in the catalase preparations? In addition to the data of table 1, a particular catalase preparation was followed from start to finish and in all stages after the first, the compound was satisfactorily demonstrated. Also the same reasoning as 4 applies.

6. Since the compound is stable for a few moments can the measurement be made in the ordinary apparatus? Rapid manipulation of the Beckman spectrophotometer gives a measurable spectral shift at 405 mμ on addition of H_2O_2 to alcohol free catalase. The average of three experiments with liver catalase gave $(\Delta\varepsilon_1)\lambda = 405 = 38$; in good agreement with the data of table 1. This procedure is, however, not recommended for accurate determinations.

7. Was H_2O_2 present during the decay of the intermediate compound? A number of attempts were made to demonstrate the presence of free hydrogen peroxide during the decompostion of the intermediate compound. The following methods were tried:

1. Ceric sulfate.
2. Peroxidase and ascorbic acid.
3. Azide catalase.

In each case the peroxide-sensitive indicator was mixed with catalase (2 μM) and a large excess of H_2O_2 ($\approx$ 0.5 mM) within 15 seconds after mixing the latter two components. The sensitivity of the last two methods was surely adequate to detect 1 μM H_2O_2. The last tests will be explained in detail.

Catalase (2 μM) and H_2O_2 (0.4 mM) were mixed in a test tube and rapidly transferred to one syringe of the rapid reaction apparatus (10 s). This mixture was immediately mixed with 40 μM azide in the capillary and the azide-catalase H_2O_2 compound was sought at 430 mμ. The procedure was similar in 2 but azide was replaced by peroxidase and ascorbic acid.

In no case was there any evidence of H_2O_2 in excess of the experimental error. The conclusion is that the catalatic activity removed nearly all the H_2O_2 in a short time while the high affinity of the intermediate compound held the remainder from the indicators. In the case of 3 it is believed that a small amount of H_2O_2 could be demonstrated provided the test were carried out more rapidly.

THE PEROXIDATIC PROPERTIES OF THE INTERMEDIATE COMPOUND

Since this compound appeared to have may properties in common with peroxidase-H_2O_2, a number of acceptors were added to determine their effect upon the stability of the compound.

Ascorbic acid

The addition of a millimole of ascorbic acid to the intermediate compound formed in the usual way caused a definite acceleration of its decomposition. Since catalase has a very high affinity for hydrogen peroxide, it was possible to form the intermediate compound directly from the hydrogen peroxide present in ascorbic acid solution. The data of fig. 10 show the increase in the amount of intermediate compound and the increase in the rate of its decomposition as a function of initial ascorbic acid concentration. The acceleration of the rate is more than two fold for 80 μM ascorbic acid and a velocity constant of 360 $L \times M^{-1} \times s^{-1}$ is obtained by deducting the »blank» rate. This is quite low compared with the activity of horse-radish peroxidase which has a constant of 1.8×10^5 $L \times M^{-1} \times s^{-1}$. It appears that the influence of the protein component of catalase has resulted in a different acceptor specifi-

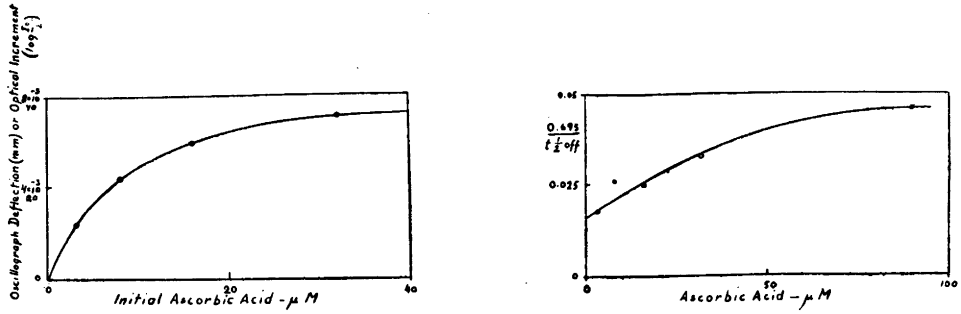

Fig. 10. The formation of the intermediate compound in the presence of ascorbic acid (left). The acceleration of its decomposition by ascorbic acid (right) ($k_4 = 360$ $L \times M^{-1} \times s^{-1}$). Expt. 55, 1.36 μM horse blood catalase, $\lambda = 405$, $s = 30$, $t = 23°$ C, pH = 6.7, 0.01 M phosphate.

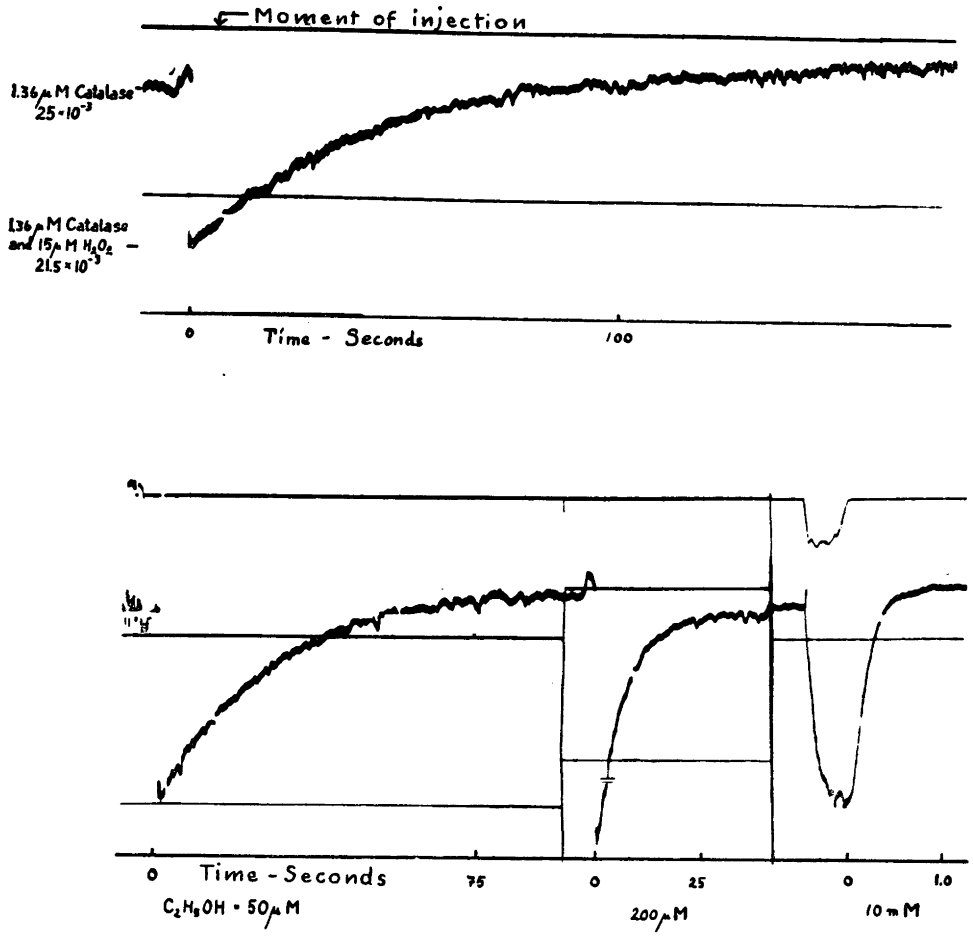

Fig. 11. The kinetics of the intermediate compound in varous alcohol concentrations. The alcohol concentration is zero in the top record.

city and that other types of acceptors must be employed in order to obtain optimal acitivity, for example, the alcohols used in Keilins »coupled oxidations»[6].

The effect of ethanol

The rather dramatic effect of ethanol upon the stability of the intermediate compound is shown in the series of kinetic curves of fig. 11. The half-life of the compound is decreased from 30 to 0.1 s by 10 *mM* ethanol. Fig. 12 shows that the effect of ethanol is to increase in a regular fashion the first-order

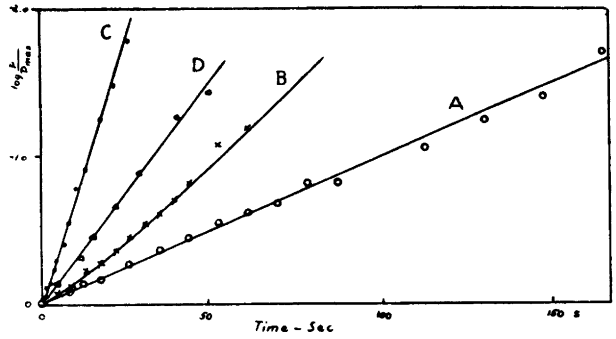

Fig. 12. The order of the reaction. The data of fig. 11 are plotted according to a first order equation. Note that abscissa is divided by 180 for the 10 mM curve (D). A = 0, B = 50, C = 200, D = 10,000 μM ethanol.
Expt. 59, 1.36 μM horse blood catalase, 12 μM H_2O_2, λ = 405, S = 30, t = 24° C, pH = 6.7, 0.01 M phosphate.

velocity constant for the decomposition. This suggests that there is a second-order reaction of ethanol and the intermediate compound and the data of fig. 13 verify this. Since the rate of decomposition is not zero with zero alcohol concentration, the original data appear as the two curves (circles and crosses) approaching a straight line asymptotically. Subtraction of the »blank» rate

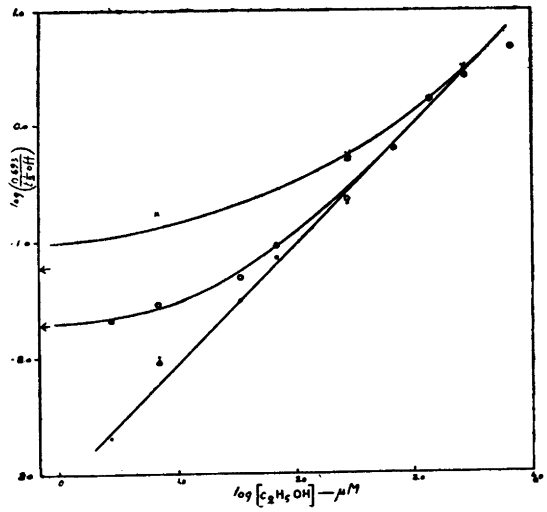

Fig. 13. The second-order velocity constant k_4 = 1000 L × M^{-1} × s^{-1} for the reaction of the intermediate compound and ethanol. The curves are uncorrected for the blank reaction.
o, · Expt. 59, 1.36 μM horse blood catalase, 15 μM H_2O_2.
×, Δ Expt. 62, 1.94 μM horse liver catalase, 6 μM H_2O_2.
λ = 405, S = 30, t = 20° C, pH = 6.7, 0.01 M phosphate.

from the original data gives the remainder of the points on the straight line. At the highest concentration of alcohol there is some evidence of a »saturation» effect and experiments with still higher alcohol concentrations verify this; 0.085 *M* ethanol gives a constant of only 100 L × M^{-1} × s^{-1}. The breakdown constant for such an alcohol-peroxide-catalase complex can be estimated from the value $k_4 = 100$ above since $100 \times 0.085 = 8.5$ s^{-1} or roughly 10 s^{-1}. assuming that a further increase of alcohol brings about a slight further increase of rate. Thus a »Michaelis Constant» would be roughly 10^{-2} *M*.

This effect is very similar to that of ascorbic acid upon the intermediate compound of peroxidase and hydrogen peroxide and may be termed a peroxidatic activity of catalase. Apparently the extreme linearity of the effect of alcohol concentration is only limited by the rate of breakdown of a catalase-peroxide-alcohol complex as is also the case in the reaction of peroxidase and ascorbic acid or leucomalachite green [7]. The peroxidatic activity of catalase for ethanol is, however, less than that of peroxidase for ascorbic acid, 1×10^3 compared with 1.8×10^5, although the maximum turnover ($k_5 \approx 10$ s^{-1}) does not greatly differ.

As in the case of peroxidase and ascorbic acid, the rate of decomposition is not accelerated by mixing the alcohol with the enzyme before mixing with the hydrogen peroxide compared with the results obtained on mixing the enzyme with substrate and acceptor sumultaneously (at least up to 4 *mM* ethanol).

It is unfortunately much more difficult to correlate the kinetics of the intermediate compound with the turnover of ethanol than in the case of peroxidase where dyes of intense absorption are produced (malachite green, etc.). Also in these studies the maximum amount of ethanol consumed would be related to the *enzyme concentration* and not to the peroxide concentration since the excess peroxide is removed by the catalatic pathway. However, Keilins' [6] experiments were all carried out with a continuous supply of H_2O_2 from either an enzyme system or a mechanical device, and the production of aldehyde from alcohol was demonstrated by the Schiff test. It is possible that the decomposition of the intermediate compound could cause the oxidation of an equivalent amount of alcohol and its activity may be compared with that found in Keilins' manometric tests.

From Keilins' fig. 1, the rate of oxygen uptake during the initial phase of the reaction is 145 μl in 20 min. or 5.4×10^{-3} μM/s in terms of O_2, hydrogen peroxide, ethanol, or acetaldehyde. The amount of catalase present is 8.7×10^{-3} μM and hence the turnover is 0.7 s^{-1}.

In these experiments, k_4 has fallen to 100 L × M^{-1} × s^{-1} at this high alcohol concentration and the rate of breakdown of the intermediate compound is 100 × 0.07 *M*

$= 7\ s^{-1}$, a turnover ten times as great as in Keilins' experiments if the assumption is made that the decomposition of the intermediate compound in the presence of alcohol gives an equivalent amount of aldehyde.

In this case it is clear that *the intermediate compound can easily be responsible for the »coupled oxidation» in Keilins' experiments.*

The smaller turnover is probably due to the partial saturation of catalase with H_2O_2 furnished by the enzyme system. This will also explain the data of Keilins' table 4 where the first ten-fold reduction of the enzyme concentration caused less effect than subsequent ten-fold reductions. As the catalase concentration is decreased, first the saturation will increase without much loss of turnover. However, when catalase is saturated, the excess of hydrogen peroxide will be decomposed catalatically resulting in the decrease of oxygen uptake shown in Keilins' table 4. The ratio of catalatic to peroxidatic activity is a function of the relative concentrations of catalase and hydrogen peroxide.

The effect of alcohol upon the intermediate compound may then be formulated in general terms. The bulk of the data indicate that n = 1.

$$\underbrace{\mathrm{Cat} + \mathrm{n(H_2O_2)}}_{\substack{\text{Very rapid combination} \\ k_1 \approx 3 \times 10^7\ \mathrm{L} \times \mathrm{M^{-1}} \times \mathrm{s^{-1}}}} \xrightarrow{k_1} \underbrace{\mathrm{Cat(H_2O_2)_n} + \mathrm{nC_2H_5OH}}_{\substack{\text{Rapid second order reaction} \\ k_4 = 100\ \mathrm{L} \times \mathrm{M^{-1}} \times \mathrm{s^{-1}}}} \xrightarrow{k_4} \underbrace{\mathrm{Cat(H_2O_2)_n(C_2H_5OH)_n}}_{\substack{\text{Relatively unstable compound} \\ k_5 \text{ estimated } 10\ \mathrm{s^{-1}}}} \xrightarrow{k_5} \mathrm{Cat} + 2\mathrm{nH_2O} + \mathrm{nCH_3CHO}$$

Spectrum

The spectrum of the intermediate compound in the presence of alcohol was measured for these reasons. 1) The measurements can be carried out in more dilute solutions since the »cycle» is more rapid and slow fluctuations of the light intensity have less effect. 2) It is of interest to determine whether an intermediate compound of catalase, peroxide, and alcohol can be detected.

Using a 1 *mM* ethanol concentration, the spectrum of fig. 14 was obtained by the same calculations as used for fig. 4. The shape of the curve and the values of extinction are equal to those of fig. 4 to within the experimental error. Therefore neither a change of enzyme concentration (or source) nor the presence of alcohol alter the spectrum appreciably. Thus there is only kinetic evidence for the catalase-hydrogen peroxide-alcoholcomplex.

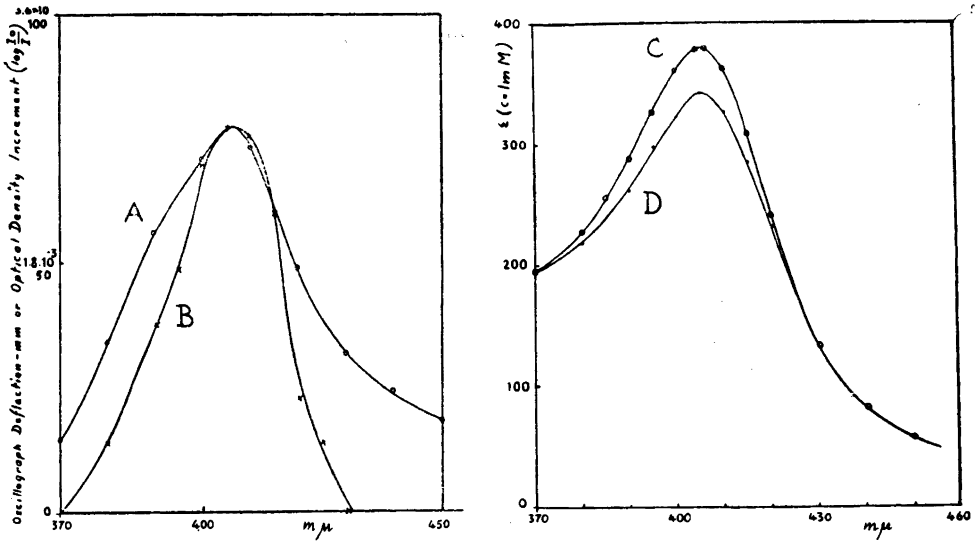

Fig. 14. The spectrum of the intermediate compound in the presence of ethanol. Other features as in fig. 4. A = catalase compared with water, B = catalase compared with the intermediate compound, C = catalase, D = catalase-hydrogen peroxide spectrum.
Expt. 66, × = 1.63 M horse kidney catalase, 12 M H_2O_2 1 mM C_2H_5OH, 0 = 0.16 μM horse kidney catalase, S = 30, t = 23° C, pH = 6.7, 0.01 M phosphate.

The equilibrium constant in the presence of alcohol

Fig. 15 shows the increase of the concentration of the intermediate compound with increasing hydrogen-peroxide concentration. Similar data are plotted in fig. 16 using the same assumptions as fig. 9 and with approximately

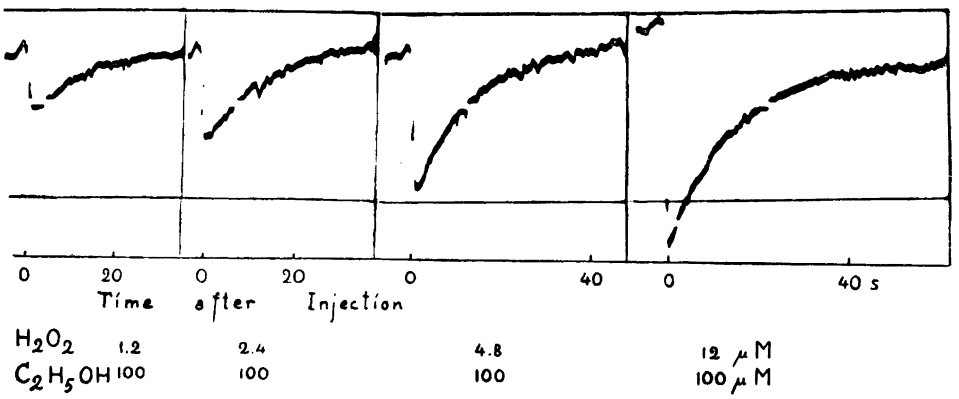

Fig. 15. The effect of hydrogen peroxide upon the kinetics of the intermediate compound in the presence of 100 μM ethanol.

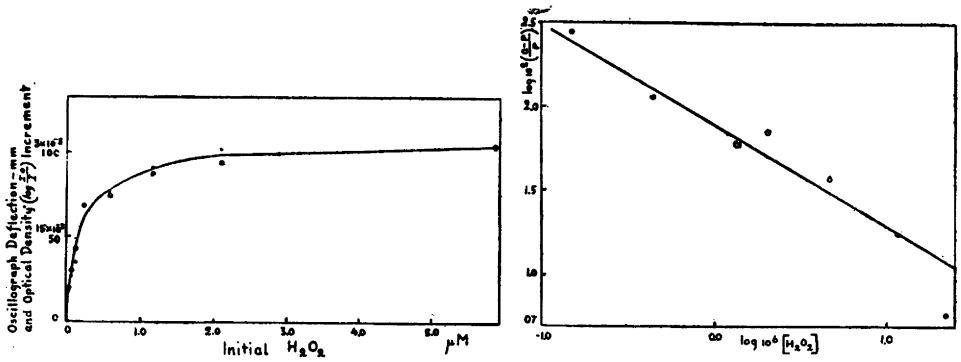

Fig. 16. The equilibrium of catalase and hydrogen peroxide in the presence of ethanol. assumptions were the same as in fig. 9. $K_M = 0.8\ 10^{-6}\ M$, $a = 0.58$, *Expt. 66 d, 1.6 μM horse kidney catalase, 1 mM ethanol,* $\lambda = 405$, $S = 50$, $t = 26°\ C$, $pH = 6.7$, *0.01 M phosphate.* $t_{1/2\ off} = 0.7\ s$, $k_4 = 1000\ L \times M^{-1} \times s^{-1}$

the same result except that a line of slope = 0.58 was drawn. No explanation of this difference is proposed.

The increase of alcohol concentration should lead to an increase in the amount of hydrogen peroxide required to saturate the intermediate compound.

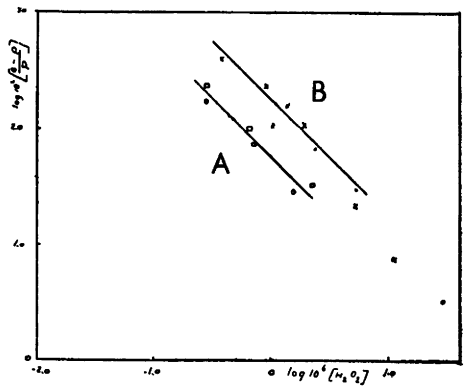

Fig. 17. The effect of ethanol upon the equilibrium constant. The assumptions were the same as in fig. 9.

For curve A, $K_M = 0.56\ 10^{-6}\ M$, $a = 1$,
For curve B, $K_M = 1.7\ 10^{-6}\ M$, $a = 1$.
Expts. 60 a + 61, 1.1 M horse blood catalase,
$\square = 0$, $O = 100$, $\times = 400$, $\cdot = 4000\ \mu M\ C_2H_5OH$,
$\lambda = 405$, $S = 30$, $t = 25°\ C$, $pH = 6.7$, *0.01 M phosphate.*

This effect is qualitatively illustrated by fig. 17 were there is a shift of the »constant» from 0.56 to 1.7 $\times$ 10^{-6} M from the lower to the higher concentrations. As in the case of the fig. 9, the effect of the catalatic disappearance of H_2O_2 must not be forgotten and not only is the equilibrium constant much larger than the ratio of the measured rates of decomposition and formation of the intermediate compound but it must be also relatively insensitive to a change in the rate of decomposition as indeed it is. In fact a rate constant for the catalatic activity can be now estimated for both values of the equilibrium constant: $\frac{0.03 + k_7}{3 \times 10^7} = 0.56 \times 10^{-6}$ and $\frac{4.0 + k_7}{3 \times 10^7} = 1.7 \times 10^{-6}$ giving 18 and 49 s^{-1} respectively or in second order units, between 2 and 5 $\times$ 10^7. Apparently an accurate value for the catalatic activity cannot be found on the basis of such simple assumptions.

The effect of excess hydrogen peroxide

As already illustrated in fig. 7 for the absence of alcohol, the rate of decomposition of the intermediate is not greatly affected by excess hydrogen peroxide, presumably because the catalatic activity rapidly removes this excess. This presumption is strongly supported by the data of fig. 18 and, since the decay times are shorter, the data are more reliable than those of fig. 7.

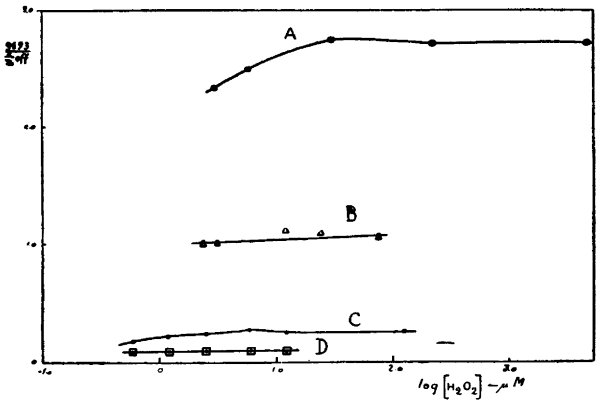

Fig. 18. The effect of initial hydrogen peroxide concentration upon the rate of decomposition of the intermediate compound in the presence of varying amounts of alcohol.

Expts. 60 a + b, 61, and 66.
A = 4000 = B 1000, C = 400, D = 100 μM C_2H_5OH.

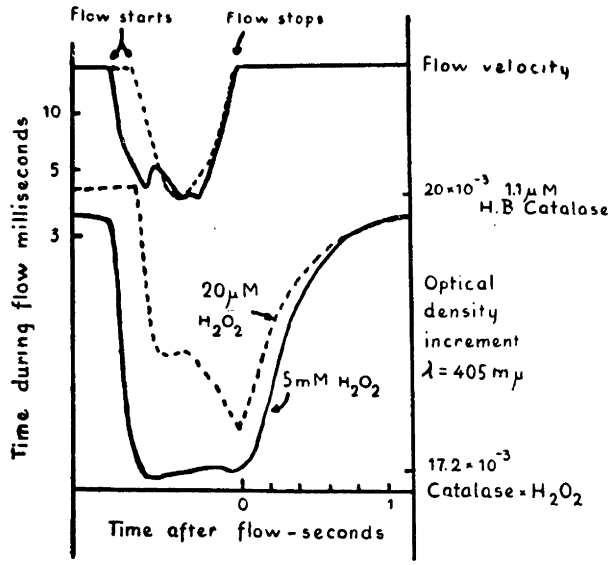

Fig. 19. The competition between hydrogen peroxide and ethanol for the intermediate compound. Excess initial hydrogen peroxide causes a delay of about 0.1 s. Expt. 67b. Other conditions as in fig. 8 except no H_2 was used.

The competition between ethanol and hydrogen peroxide for the intermediate compound

In the absence of alcohol, fig. 8 showed a considerable delay in the decomposition of the intermediate compound in the presence of excess hydrogen peroxide. When alcohol is present it and the last traces of hydrogen peroxide would be expected to compete for the intermediate compound and thereby to cause a considerable reduction of the delay. This is demonstrated by fig. 19 where the tracings of two kinetic curves with 20 μM and 5 mM H_2O_2 are shown. The delay in strong peroxide and alcohol is about 0.1 s. This again indicates that the intermediate compound does not begin to decompose until the catalatic activity is complete.

The effect of a variation of enzyme concentration

Since the rapid kinetics can be recorded more reliably, an illustration of the effect of a five-fold decrease of enzyme concentration is shown in fig. 20. The activity of the enzyme is the same and the greater instability of the apparatus in the more dilute solution causes only minor differences between the two curves.

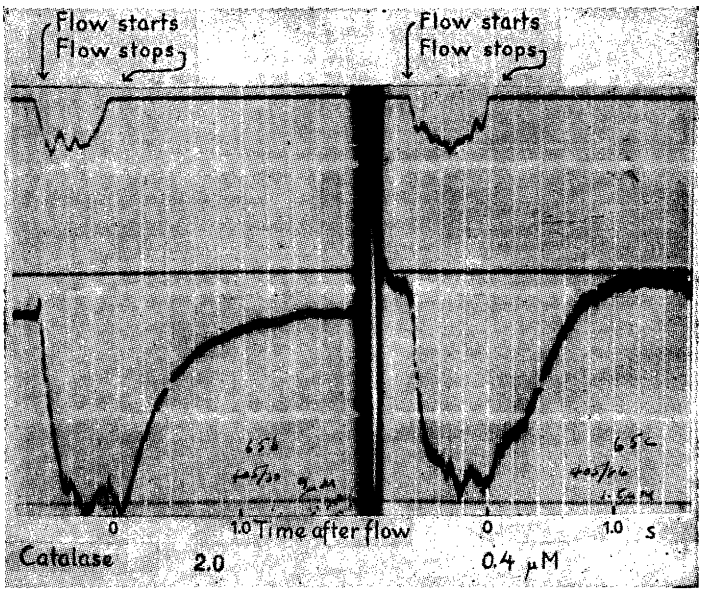

Fig. 20. The effect of a five-fold variation of enzyme concentration upon the kinetics of the intermediate compound in the presence of 4 mM C_2H_5OH and 12 μM H_2O_2.

The effect of other alcohols

Methanol. Fig. 21 shows that the variation of the corrected rate is linear over about the same range as with ethanol and that a similar saturation effect occurs. Within the experimental error the velocity constants are identical although the volatility of methanol might cause the values to be somewhat low.

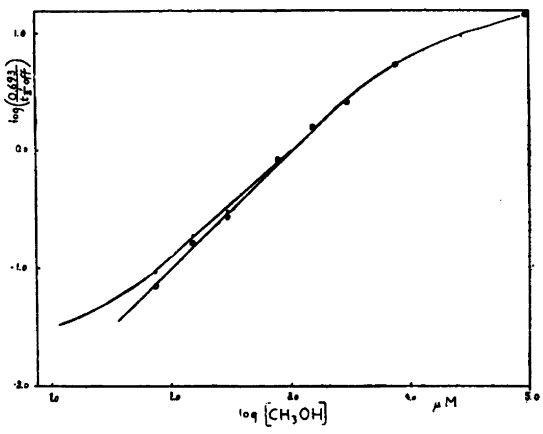

Fig. 21. The second-order velocity constant $k_4 = 100\ L \times M^{-1} \times s^{-1}$ for the reaction of the intermediate compound with methanol. The left-hand curve is uncorrected for the blank rate.
Expt. 70, 1.85 μM horse blood catalase, 30 μM H_2O_2 λ = 405, S = 30, t = 22° C, pH = 6.7, 0.01 M phosphate.

Agner* has recently determined the kinetics of the disappearance of methanol from the blood of rabbits [19]. The data of fig. 3 fit a first order equation having a rate constant of 1.8×10^{-5} s^{-1}. It has been shown that methanol is oxidized to formaldehyde in the liver [20]. The liver catalase concentration calculated on the basis of the liquid phase (since the alcohol is uniformly distributed in this phase) is 0.17 μM.

For those experiments, the saturation of catalase is assumed to be constant and the alcohol to disappear according to the following equation:

$$\underset{a}{CH_3OH} + \underset{p}{Cat \cdot H_2O_2} \xrightarrow[k_4]{} HCHO + 2H_2O + Cat$$

$$\frac{da}{dt} = k_4 ap \text{ (at } t = 0,\ a = a_o); \log \frac{a_o}{a} = k_4\ p\ t.$$

Since Agner's figure is k_4p, p was assumed equal to e,

$$\log \frac{a_o}{a} = k_4\ e\ t$$

$$k_4 e = 1.8 \times 10^{-5}\ s^{-1}$$

$$k_4 = \frac{1.8 \times 10^{-5}}{0.17 \times 10^{-6}} = 100\ L \times M^{-1} \times s^{-1}$$

But in these experiments with the intermediate compound the kinetics of p, and not a, were measured; $\log \frac{e}{p} = k_4\ a_o t$, and k_4 is found to be 1000 L $\times$ M^{-1} $\times$ s^{-1} for low methanol concentrations and roughly 500 L $\times$ M^{-1} $\times$ s^{-1} over the range of alcohol concentrations used in Agner's experiments five times that required by his data.

It is seen that more than enough catalase is present in the rabbit liver to account for Agner's kinetic data. Furthermore, if it is assumed that all the liver catalase is equally exposed to methanol in this reaction, the saturation of the enzyme would appear to be about 20 % and the peroxide concentration (supplied by other enzyme systems) in the neighborhood of 1 μM in this liver.

Therefore the knowledge of the kinetics of methanol uptake of an organ, its catalase content, and the velocity constant for the intermediate compound and methanol (1000 L $\times$ M^{-1} $\times$ s^{-1}) permits a determination of the hydrogen peroxide turnover in that organ, and very small concentrations of hydrogen peroxide may readily be determined. It is suggested that this technique may be a useful tool in physiology.

It is also of interest that the concentration of H_2O_2 found in rabbit liver is so low — one wonders whether catalase ever acts catalatically *in vivo*. On the other hand it would be difficult to imagine a substance that could be better suited to permitting a small concentration of H_2O_2 to exist for metabolic processes and also for rapidly decomposing a concentration of H_2O_2 in *excess* of the amount of catalase. Certainly the H_2O_2 and catalase concentrations found in this experiment are too nearly equal to permit a clear cut decision.

There is still no evidence of the relation between alcohol dehydrogenase and catalase and, although these data clearly indicate that catalase is present in rabbit liver in sufficient concentration to account for Agner's data, no proof is yet afforded that catalase and

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* These calculations were made in collaboration with Docent Agner.

not alcohol dehydrogenase actually carried out the oxidation. Also the inhibition of methanol oxidation by ethanol as observed *in vivo*[19] and *in vitro* with alcohol dehydrogenase[21] is unlikely in the case of catalase unless ethanol inhibits those enzyme systems which, *in vivo*, supply catalase with hydrogen peroxide.

A comparison of five alcohols. Fig. 22 shows the relative rates of combination of various alcohols with the intermediate compound. While ethyl and methyl alcohol have quite similar rates, *n*-propanol and *n*-butanol are respectively only 1/60 and 1/500 as effective. The rate with the highest concentration of iso-amyl alcohol is so nearly equal to the blank rate that the difference is subject to considerable error. The data also indicate that saturation may occur at a lower concentration with the higher alcohols. It is quite possible that steric factors are responsible for the slower rates of combination of the higher alcohols.

These data may be compared with Keilins'[6] figs. 3 and 4 with the following reservations.

1. Since the catalase is not completely saturated with H_2O_2, Keilins' system may be non-linear for small changes of the rate of combination of alcohol and intermediate compound.

2. It is essentially the rate of breakdown of the alcohol-peroxide-catalase compound that is measured in Keilins' experiments since his alcohol concentrations approach the saturation values.

3. Apparently his results vary with the enzyme system and/or the turnover number. In fig. 3 the effect of ethyl to propyl was equivalent to a 10 fold reduction of catalase concentration (table 4) while in fig. 4 the effect is equivalent to in excess 100 fold reduction of catalase (table 4). A greater sensitivity with a higher turnover would be expected.

4. The small but consistent difference between ethanol and methanol shown in Keilins' data may be attributed to 2.

Although there are differences in the conditions under which the relative activities were measured by the two methods, there are certainly no differences concerning the decrease of activity with the higher alcohols.

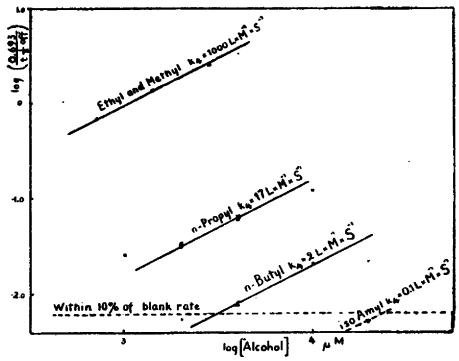

Fig. 22. A comparison of the reaction velocity of the intermediate compund with five different alcohols. Expt. 71, 1.85 μM horse blood catalase, 20 μM H_2O_2 λ = 405, S = 30, t = 23° C, pH = 6.7, 0.01 M phosphate.

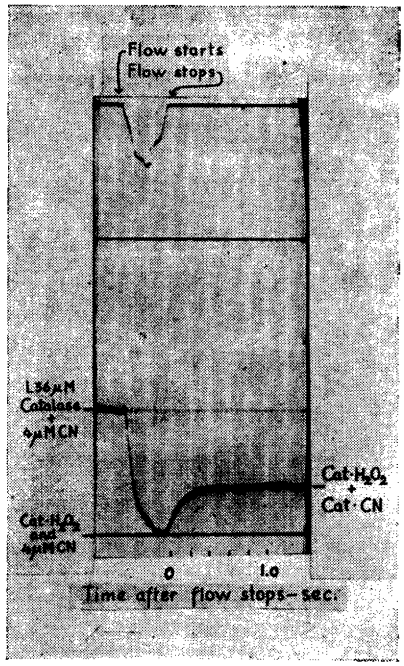

Fig. 23. The kinetics of the intermediate compound in the presence of cyanide — initial phase only.

The effect of inhibitors

Cyanide. The competition between hydrogen peroxide and cyanide is shown in fig. 23. At this wavelength (420 mμ) the density change due to the catalase-cyanide compound (+) has the opposite sign to that of the intermediate compound (—). The abrupt drop in density at the moment of injection is due of to the replacement of catalase-cyanide compound remaining in the capillary from the preceeding injection by a mixture of the intermediate compound, catalase, and cyanide. The cyanide then rapidly combines with a portion of the catalase as indicated by the increase of density after the flow stops. The intermediate compounds is momentarily stable but decays as shown in the first of the series of curves of fig. 24 which are recorded on a much slower time scale.

This series of curves shows the regular increase of the concentration of the cyanide compound and a decrease of the concentration of the intermediate compound as the cyanide concentration is increased. In fact in the last two records very little intermediate is formed.

Azide. The effect of azide is not so straightforward because: 1) more than one azide-catalase-peroxide compound can form and 2) the azide-catalase-

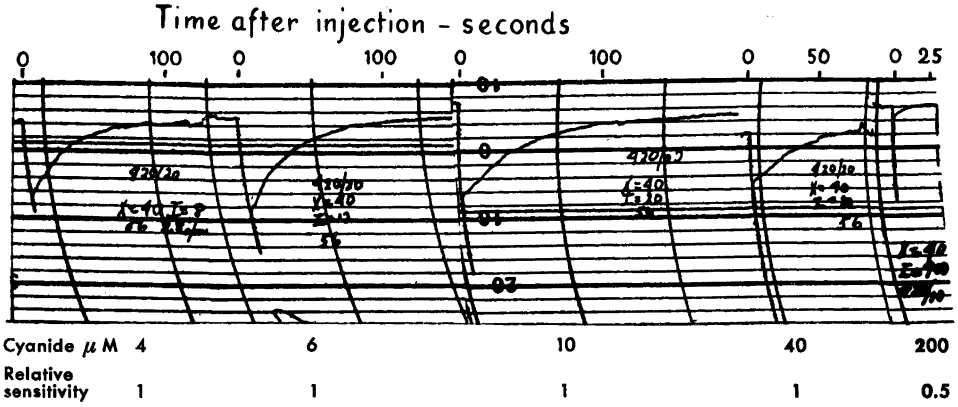

Fig. 24. The kinetics of the intermediate compound in the presence of varying amounts of cyanide. Note that the sensitivity was reduced in the last figure.

peroxide compound itself decomposes in a few minutes. Fig. 25 shows at 410 mμ that the rapid formation of the intermediate compound is followed by the slow formation of the azide-catalase-peroxide compound when dilute azide is used (10 μM). With more azide (100 μM) the distinctive spectrum and kinetics of this compound are shown in fig. 26 on a slower time scale. In these records, however, the initial phase appears to consist of the primary formation of the intermediate compound followed by the formation of at least two different types of azide-catalase-peroxide compounds during the course of its decomposition. A wide variation of experimental conditions does not appear to alter the rate of formation of the azide-catalase-peroxide compound and, therefore, it is concluded that *the extremely high affinity of this compound,* to quote Keilin[5] »Azide-catalase is certainly one of the most sensitive reagents for the detection of H_2O_2 . . . . . .», *is due entirely to the high affinity of the intermediate compound,* and not azide-catalase, *for* H_2O_2. This finding does not conflict with previous data, it merely explains them.

The nature and significance of the complex kinetics of the azide-catalase-peroxide compounds shown in fig. 26 is indeed beyond the scope of this paper. A significant factor is, however, that the life-time of this compound is quite independent of hydrogen peroxide concentration over a very wide range (in fact — as large as that of the intermediate compound). Under these experimental conditions, the same explanation may apply; the catalatic pathway first reduces the peroxide concentration to a very low value and then the azide-catalase-peroxide compound forms and goes through its complicated course of decomposition.

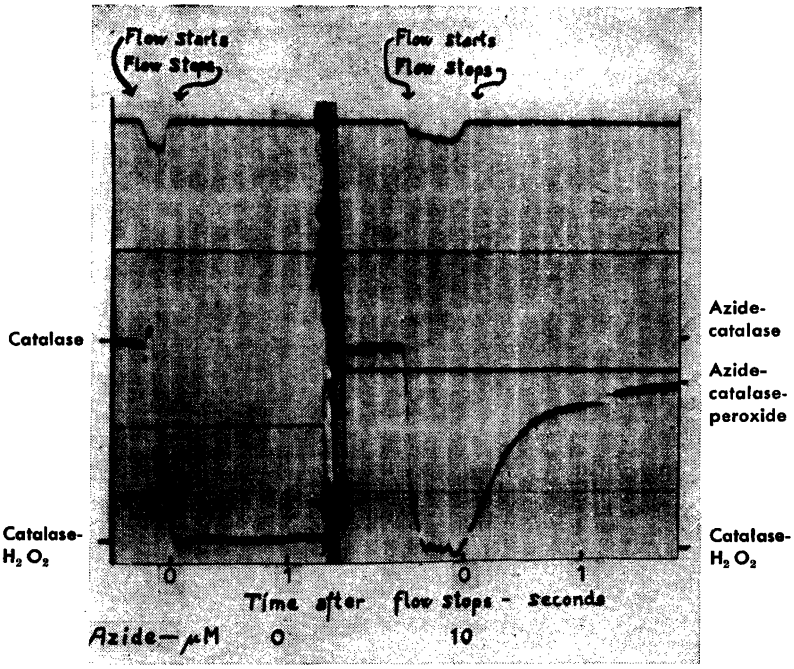

Fig. 25. The effect of 10 μM NaN_3 upon the kinetics of the intermediate compound 400 μM H_2O_2.

Since azide-catalase-peroxide decomposes at a constant rate, can this be accelerated by alcohol? Evidently the peroxidatic activity is completely inhibited by 100 *μM* azide since 1 *mM* ethanol has no effect upon the kinetics of the azide-catalase-peroxide compound.

Ethyl hydroperoxide. With strong ethyl hydroperoxide (0.1 *M*) as used by Stern [4] the intermediate is rapidly converted into the ethyl hydroperoxide compound.

Carbon Monoxide. There is no inhibition of the effect of 4 *mM* ethanol by 500 *μM* CO. Also there are no measurable spectral changes under aerobic or anaerobic concitions.

Anaerobic conditions. The rate of decomposition of the intermediate compound in the presence (4 *mM*) or absence of ethanol was not greatly affected by anaerobic conditions. (Solutions were bubbled for 20 min. with H_2 passed over heated platinized asbestos.)

The data above strong support the view that the intermediate compound of peroxide attached to hematin and that no detectable amount of a reduced

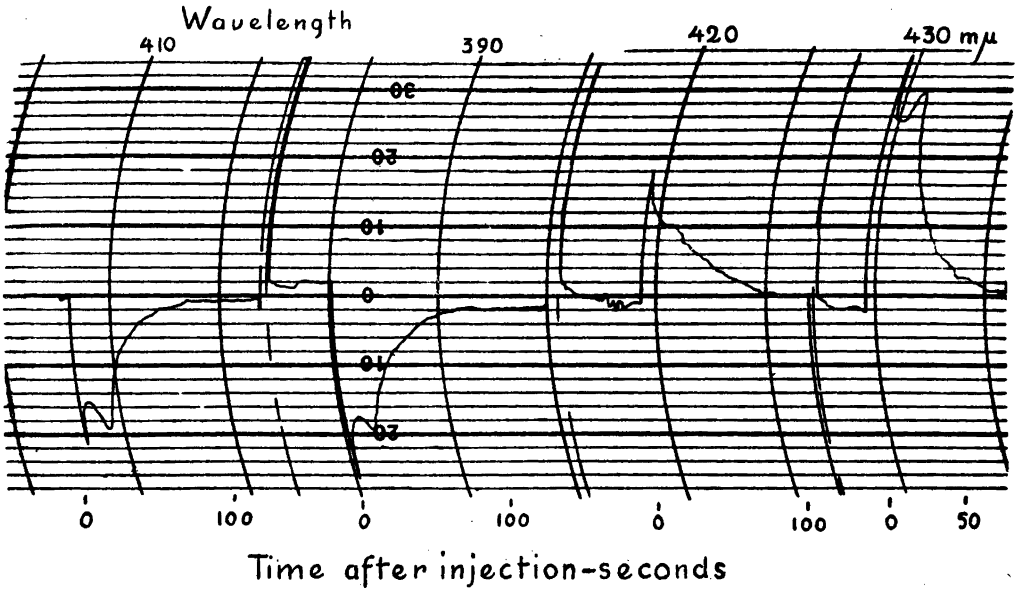

Fig. 26. The kinetics of azide-catalase-peroxide at three wavelengths — 100 μM NaN_3, 400 μM H_2O_2.

compound is formed during the kinetics of the intermediate compound. This further increases the similarity to peroxidase — H_2O_2.

SUMMARY

A complete discussion of these and other data will appear in a series of papers[22]; at present the results are only summarized as follows:

1. Catalase forms an intermediate compound in the presence of hydrogen peroxide.
2. This compound reacts with acceptors and inhibitors much in the same way as peroxidase — H_2O_2 and is believed to be a similar peroxide complex with catalase.
3. The spectrum of the intermediate compound has been measured from 380 to 430 mμ and is slightly shifted towards the visible with respect to catalase. $\varepsilon_{405\ (c\ =\ 1\ mM)} = 340$ for blood catalase. The spectrum is unlike that of cyanide-catalase or azide-catalase-peroxide.
5. The rate of formation of the complex is very high and exceeds the value required for the catalatic activity of the enzyme as well as those for peroxidase and myoglobin with hydrogen peroxide and oxygen respectively. A preliminary value for the constant is 3×10^7 L × M^{-1} × s^{-1},

6) With pure crystalline catalases the intermediate compound decomposes very slowly and with slight variability from preparation to preparation as in the case of peroxidase-hydrogen peroxide. An average value for the breakdown constant is 0.02 s^{-1}.

7. The intermediate compound has a very high affinity for hydrogen peroxide. Since the calculation of the data requires the postulation of a comprehensive enzyme mechanism, no correct figure can be given as yet. The simple equilibrium equations give $1 \times 10^{-6} M$, the kinetic data give $1 \times 10^{-9} M$ indicating a large catalatic activity during the formation of the intermediate compound ($k_3 = 30\ s^{-1}$).

8. The intermediate compound reacts slowly with ascorbic acid ($k_4 = 340\ L \times M^{-1} \times s^{-1}$), more rapidly with ethyl and methyl alcohols ($k_4 = 1000\ L \times M^{-1} \times s^{-1}$) and less rapidly with the higher alcohols, *n*-propanol ($k_4 = 17\ L \times M^{-1} \times s^{-1}$), *n*-butanol ($k_1 = 2\ L \times M^{-1} \times s^{-1}$), and very slowly with iso-amyl alcohol ($k_4 = 0.1\ L \times M^{-1} \times s^{-1}$).

9. Saturation effects with excess ethanol and methanol lead to the conclusion that a further intermediate compound is formed in these reactions as in the case of peroxidase. The velocity constant for the break-down of such a complex is 10 s^{-1}, similar to the value found for peroxidase.

10. The peroxidatic activity of the intermediate compound is more than adequate to account for Keilin's experiments on »coupled oxidations» and Agner's experiments on the kinetics of methanol disappearance in vivo.

11. Where catalase is solely responsible for methanol oxidation the velocity constants in 8 permit determinations of the hydrogen peroxide concentration or turnover in vivo by simply following the kinetics of methyl alcohol oxidation and determining the catalase content.

Many thanks are due to Dr. Roger K. Bonnichsen, who prepared all the pure catalase preparations used but one, and thanks are due to Prof. B. Sumner for that excellent preparation. Miss Pero Kara rendered helpful service in standardization, etc. Many helpful suggestions were due to Drs. K. Agner, R. K. Bonnichsen and R. Abrams and are gratefully acknowledged.

But the real credit for this work is due to Prof. Hugo Theorell, who has constantly advised, supervised, and planned for its successful conclusion and has been of immeasurable assistance in shaping the logic of this paper.

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ACTA CHEMICA SCANDINAVICA 1 (1947) 268

Über die Bromierung des Tetraäthylsilans

Vorläufige Mitteilung

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Friedel und Crafts [1] fanden, dass Tetraäthylsilan und Brom in geschlossenem Gefäss bei 140° bromsubstituierte Tetraäthylsilane ergaben. Aus dem Reaktionsgemisch wurden zwei Produkte mit den Siedepunkten 230—240° (1 atm.) und 120—140° (»Vakuum») isoliert, die unreine Monobrom- und Dibromtetraäthylsilane waren.

Wir haben nun Tetraäthylsilan bei seinem Siedepunkte bromiert und dabei ein Monobromtetraäthylsilan vom Sdp. 87—88° (15 mm) und ein Dibromtetraäthylsilan vom Sdp. 112—114° (15 mm) erhalten. Beide Verbindungen spalten das Brom langsam ab, wenn sie mit 1 *N* alkoholischer Kalilauge bei Zimmertemperatur behandelt werden. Auf siedendem Wasserbade gehen die Spaltungen leichter. Dabei gibt die Monobromverbindung Triäthylvinylsilan. Die Dibromverbindung gibt ausser Triäthylsilanol noch Vinylbromid und/oder Acetylen. Hieraus können wir schliessen, dass die Dibromverbindung ein Triäthyl-dibromäthyl-silan $(C_2H_5)_3(C_2H_3Br_2)Si$ sein muss.

Wir sind jetzt damit beschäftigt, die Strukturen der beiden Bromverbindungen festzustellen und ihre Verwendbarkeit zur Darstellung organischer Siliziumverbindungen zu untersuchen.

BESCHREIBUNG DER VERSUCHE

200 g Tetraäthylsilan wurden während 1—2 Stunden unter gelindem Sieden tropfenweise mit 36 ml Brom versetzt. Bei der Fraktionierung des Reaktionsgemisches unter vermindertem Druck wurden etwa 100 g Tetraäthylsilan, 30—35 g Monobromverbindung vom Sdp. 87—88° (15 mm) und 50—55 g Dibromverbindung vom Sdp. 112—114° (15 mm) erhalten.

$C_8H_{19}BrSi$ (223,2)	Ber.	C 43,1	H 8,6	Br 35,8	Si 12,6
	Gef.	» 42,9	» 8,5	» 35,4	» 12,7
$C_8H_{18}Br_2Si$ (302,1)	Ber.	C 31,8	H 6,0	Br 52,9	Si 9,3
	Gef.	» 31,6	» 5,9	» 52,5	» 9,3

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Investigations on Malt Amylase

I. On the Viscosimetrical Determination of α-Amylase

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Staudinger and co-workers [1] established, for polymeric homologous substances in collodial solution, the following relation between viscosity, concentration and molecular weight:

$$\eta_{sp} = K_m c_{gm} M$$

where

η_{sp} = the specific viscosity,
K_m = the viscosity-molecular weight constant,
c_{gm} = the concentration in basic moles per litre, and
M = the molecular weight.

This relation applies, among other substances, to dilute solutions of starch and partially degraded starch [2] up to a maximum concentration depending on the molecular weight.

Myrbäck and Sillén [3, 4] showed that α-amylase would break all linkages between the basic molecules of starch at the same rate, except certain linkages at the ends of the molecules and near the points of ramification. Since there are comparatively few linkages of the latter kind, we will disregard them in this work.

The enzymic decomposition of starch by α-amylase will hence follow the equation established by the present author [5]:

* For this investigation the author received financial su pport from *Statens Tekniska Forskningsråd.* The viscosity measurements were performed by miss Anna Hörnfeldt, and the translation into english was carried out by Mr Georg Horovitz, to whom I wish to express my cordial thanks.

$$A^{t^\circ}_{\alpha/s} = \mathfrak{c}^2 \cdot \frac{d\frac{1}{\eta_{sp}}}{dt}$$

where

$A^{t^\circ}_{\alpha/s}$ = enzymic activity in units per gram of solution at t° C,
t° = temperature,
α/s = abbreviation for α-amylase and starch,
$\mathfrak{c}_s$ = concentration of starch in grams per gram of solution, and
t = time in seconds.

PREVIOUS INVESTIGATIONS

According to Davison [6], the activity of an enzyme is to be defined as a function of the time necessary to effect a certain change in a substrate, rather than a function of the change effected in a certain time. He expressed the enzyme concentration as the reciprocal of the time in hours required for the reduction of the viscosity of a Lintner [7] starch solution, the original viscosity of which was about 2 ½ times that of water, by 20 %. Davison attempted to obtain the required initial viscosity by choosing a suitable concentration for the starch solution. The effect of the concentration may not, however, be neglected when the results of series of experiments with different starch solutions are compared.

This was also the opinion of Thompson, Johnson and Hussey [8], who used a 7 % solution of the s a m e batch of Baker's soluble starch in all their experiments, which made the initial viscosities almost identical in all experiments. The enzyme concentration proved to be inversely proportional to the time necessary to reduce the viscosity by 20 %.

Broeze [9] employed the specific viscosity as an expression for the internal state of a sol. He refered to Einstein's [10] formula, which was calculated for a suspension of particles:

$$\eta = \eta_0 (1 + \varkappa\varphi)$$

where

η = viscosity of the »solution»,
η_0 = viscosity of the »solvent»,
$\varkappa$ = a constant depending on the shape of the particles, and
φ = ratio between the total volume of the dispersed substance and the volume of the »solution».

Broeze used a solution of Merck's soluble starch of constant concentration (2 %). In relative enzyme determinations he compared the reaction curves obtained by plotting the specific viscosity at the time t, expressed in per cent of the specific viscosity at the beginning of the experiment, against the time t. Broeze claimed that the times required for equal decomposition are comparable, although he did not read the absolute enzyme concentration from the reaction curves.

To obtain the time for which a certain viscosity measurement was valid, Broeze added half the outflow time of the liquid to the time at which the measurement was commenced. This is mathematically correct, provided that the fluidity is a linear function of the time.

Instead of solutions of Lintner starch, enzymically degraded starch etc., Józsa and Gore [11] used potato-starch solutions, with which they obtained fairly reproducible viscosities. However, the concentration employed was so high (4.211 %; diluted in the experiments with 1/10 enzyme solution), that Staudinger's formula, $\eta_{sp} = K_m c_{gm} M$, was no longer valid in the beginning of the experiment, since the volume of the hydrated starch must be low in relation to the volume of the free solvent.

In amylase determination, Józsa and Gore calculated a function **D** of the viscosity of a starch solution submitted to enzymic degradation. For mixtures of undegraded and completely degraded starch, they empirically determined a curve showing the relation between **D** and the percentage of starch decomposed.

Józsa and Gore used this standard curve to calculate, from viscosity measurements, the percentage of starch decomposed per hour. They defined the activity (the »liquefying power», **LP**) of the enzyme preparation as the number of grams of starch decomposed by 1 g enzyme preparation per hour at 21° C.

To give this definition real significance, we must suppose that the enzyme preparation will decompose as many 1,4 linkages per hour as occur in the above quantity of starch (or half that number, if we prefer to regard maltose as the elementary component, or perhaps some other fraction). When employing their standard curve, Józsa and Gore tacitly assumed that the viscosity of a starch solution changes as though every starch molecule were completely degraded before the next one is attacked.

This supposition is unpermissable, as has been shown experimentally by Fletcher and Westwood [12]. These authors, however, found a proportionality within a certain range, which allows the application of the method, provided that almost equal and adequate quantities of amylase are used in the experiments. Fletcher and Westwood also pointed out that the viscosity of Józsa and Gore's starch solution varies appreciably with the time of stirring.

Willaman, Clark and Hager [13] attempted to improve Józsa and Gore's method by using a 2 % starch solution and introducing a formula for the calculation of the enzymic activity. According to Józsa and Gore, the function **D** (»decline in outflow time») is

$$\mathbf{D} = 100 \cdot \frac{\tau_0 - \tau_t}{\tau_0 - \tau_\infty}$$

where τ_t is the outflow time of the solution measured in a viscosimeter at the time t after the start of the enzymic degradation etc. Under the above experimental conditions, the percentage **L** of starch decomposed per hour is, according to Willaman, Clark and Hager,

$$\mathbf{L} = \frac{\mathbf{m}}{\frac{\mathbf{a}}{\mathbf{D}} - 1}$$

where **m** and **a** are constants. In the calculation of **LP** (grams of starch decomposed per gram of enzyme preparation) it is said to be necessary to increase **L** to the empirically found constant 1.6.

An examination of Willaman, Clark and Hager's expression **L** suggests that the expression for the enzymic activity

$$A^{t^\circ}_{\alpha/s} = \mathbf{c}_s^2 \cdot \frac{d\frac{1}{\eta_{sp}}}{dt}$$

may be transformed in the following manner:

$$A^{t^\circ}_{\alpha/s} = \mathbf{c}_s^2 \cdot \frac{\frac{1}{\eta} - \frac{1}{\eta_0}}{t} = \mathbf{c}_s^2 \cdot \frac{\eta_0 - \eta_t}{\eta_0 \cdot \eta_t \cdot t} = \mathbf{c}_s^2 \cdot \frac{\eta_0 - \eta_t}{\eta_0 [\eta_0 - (\eta_0 - \eta_t)] t} =$$

$$= \mathbf{c}_s^2 \cdot \frac{1}{\eta_0 \left(\frac{\eta_0}{\eta_0 - \eta_t} - 1\right) t} = \mathbf{c}_s^2 \cdot \frac{1}{\eta_0 \left(\frac{\eta_0 \cdot 100}{(\eta_0 - \eta_\infty)\,\mathbf{D}} - 1\right) t}$$

if **D** is rewritten as $\mathbf{D} = 100 \cdot \frac{\eta_0 - \eta_t}{\eta_0 - \eta_\infty}$, where η denotes the specific viscosity.

The expression thus obtained can easily be identified with Willaman, Clark and Hager's term for **L**, whereby

$$\mathbf{m} = \frac{\mathbf{c}_s^2}{\eta_0 \cdot t} \text{ and } \mathbf{a} = \frac{100 \cdot \eta_0}{\eta_0 - \eta_\infty}$$

Johnston and Jozsa [14, 15] finally corrected Józsa and Gore's method by graphical extrapolation of a series of measurements to the time of the start of the experiment. This constituted a clear advance in comparison with the method of Willaman, Clark and Hager, which required several empirical constants for the calculations.

Blom and Bak [16] took up, and applied in practice, the idea of Davison [6] and Broeze [9] that the quantity of enzyme is directly proportional to the time required for a certain degree of degradation of the substrate. These authors prepared a homogenous potato-starch solution by stirring a starch suspension at room temperature under the addition of caustic soda [17], after which they adjusted the pH with acetic or phosphoric acid. They eliminated the errors originating from the circumstances that starch solutions of the same concentration may have different initial viscosities and that the mixing of the enzyme and starch solutions requires a certain time, by timing the enzymic activity, not, as hitherto, from the moment of the addition of the enzyme to the starch solution, but from a time at which degradation has proceeded to an arbitrary, but welldefined, stage. As the termination of the enzymic activity, they take the time at which the starch solution has reached another stage of degradation.

These two degradation stages were taken as having been attained when the viscosity of the mixture was twice and equal to the viscosity of a 45 % cane-sugar solution respectively. These times were interpolated from outflow times obtained at different intervals after the beginning of the experiment.

The expression for the enzymic activity

$$A^{t^\circ}_{\alpha/s} = \mathbf{c}_s^2 \cdot \frac{d\frac{1}{\eta_{sp}}}{dt}$$

may be written:

$$A^{\circ}_{\alpha/s} = \mathbf{c}^2 \cdot \frac{\frac{\eta_w}{\eta_{t_2} - \eta_w} - \frac{\eta_w}{\eta_{t_1} - \eta_w}}{t_2 - t_1}$$

where η_t denotes the viscosity of the mixture at the time t and η_w that of water. If $\mathbf{c}_s$, η_{t_2} and η_{t_1} are fixed and another unit chosen, this expression may,

according to Blom and Bak, be written as the enzymic activity per gram of enzyme preparation

$$= \frac{1000}{a(t_2 - t_1)}$$

where a is the weight of enzyme preparation in grams, which, although this is not expressly stated by the author, when in 5 ml of aqueous solution, is added to 50 ml of a 3 % starch solution, will reduce the viscosity in the above-mentioned measure during the time t_2—t_1 at the temperature + 20° C.

EXPERIMENTAL

Enzyme solution

Malt meal was stirred with 3 parts of water and after one hour the mixture was centrifuged. β-amylase in the liquid was inactivated by heating according to Ohlsson [18]. After filtration, the amylase was purified according to Weidenhagen [19] by precipitation with tannin, washing with acetone, dissolution in water and filtration.

Starch solution

An approximately calculated quantity of starch was weighed accurately into a round-bottomed flask of 250 ml with a wide neck. 200 ml of water was added. The contents were stirred with a motor-driven propeller. After the starch had been completely suspended, 20 ml of 2 N sodium hydroxide was added at room temperature. The mixture became viscous, but the stirring was made effective by short-circuiting some series resistances of the motor. After 15 minutes, a quantity of 4 N acetic acid (standardized against the sodium hydroxide) equivalent to 25 ml of the sodium hydroxide was added, followed by enough water to give the starch solution the required concentration. The final weight of the mixture was about 250 g. After stirring for another 15 minutes, the solution was poured into a 300 ml conical flask which was placed in a thermostat at 30°.

A layer of starch paste adhing to the interior of the round-bottomed flask indicated that stirring had not been effective enough to make the solution homogenous. On such occasions the solution was rejected, since its concentration was unknown.

Viscosimeter

An Oswald viscosimeter was used in the experiments. If

η = viscosity in CGS units,
τ = time of flow,
D = specific gravity, and
k_1 and k_2 = apparatus constants,

the viscosity is expressed by the equation:

$$\eta = D\tau\left(k_1 - \frac{k_2}{\tau^2}\right)$$

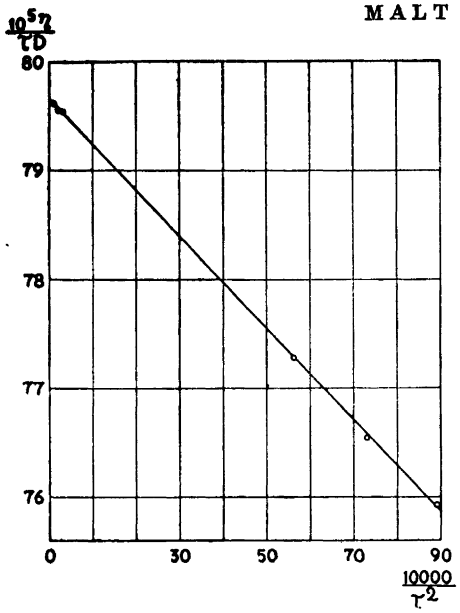

Fig. 1. Calibration of the viscosimeter.

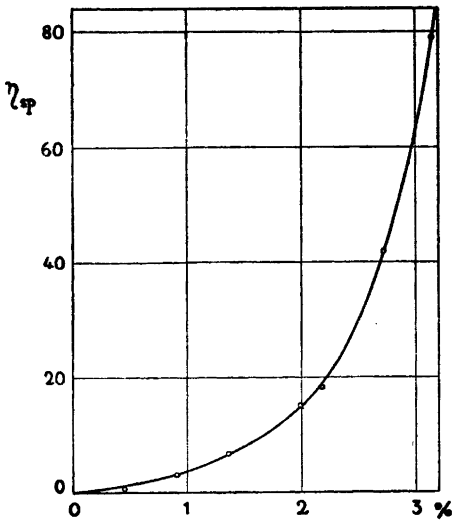

Fig. 2. Specific viscosities of the potato-starch solutions.

The apparatus constants were determined empirically by measurements of the times of flow for water and known cane-sugar solutions. The specific gravity of water has been determined by Thiesen, Scheel and Diesselhorst[20], and the specific gravities of sugar solutions have been calculated from the determinations of Plato[21]. Bingham and Jackson[22] determined the viscosities of water and of cane-sugar solutions at, among other temperatures, 30°. From these values the viscosities of the sugar solutions used to determine the apparatus constants were calculated. In these calculations the following interpolation formula was employed:

$$\frac{1}{3 + \log \eta} = 0.72654 - 0.012035\ (C-30) + 0.00002743\ (C-30)^2 + \\ + 0.000000202\ (C-30)^3$$

where C is the percentage concentration of the sugar solution.

The viscosimeter was cleaned with bichromate-sulphuric acid, rinsed with water and finally with alcohol. It was then placed in a thermostat at 30.00 $\pm$ 0.01° C, and dried by suction for about 10 minutes. 3 ml fluid was then introduced. The values obtained in the calibration of the viscosimeter are shown in fig. 1, and the apparatus constants are $k_1 = 0.0007966$ and $k_2 = 0.00421$.

Viscosity of starch solutions

The method employed for measuring the viscosity of starch solutions correspond to the method of determining enzymic activity described below. The results of viscosity measurements on some of my potato-starch solutions of different concentrations are shown in fig. 2.

Measurement of enzymic activity

3 ml enzyme solution was transferred to a 50 ml conical flask which was placed in a thermostat at 30°. The water reached the neck of the flask. A motor-driven stirring propeller was inserted into the flask, the neck of which was packed with cotton. The temperature became constant after few minutes, whereupon the starch solution was added with a 30 ml pipette. A stopwatch and the stirring apparatus were started. The pipette, containing residues of the starch solution, was weighed. The pipette was also weighed both dry and filled with the starch solution. In the latter case, wighing is rendered easier by allowing the bulk of the starch solution to flow into a tared flask. These weighings made possible an exact calculation of the quantity of starch solution introduced and thus of the final concentration. With a pipette, brought to the correct temperature by standing in the thermostat in a glass tube with a fused bottom, 3 ml of the mixture was transferred to the viscosimeter in the termostat. The outflow time for the mixture was determined an adequate number of times, the moment at which the outflow began being read on the stopwatch and noted down.

Calculation of the results

Calculations of series of specific viscosities are best made in the following manner (the index w indicates that the value is for water):

$$\eta = \tau D \left(k_1 - \frac{k_2}{\tau^2}\right) = \tau D k_\tau$$

$$\eta_w = \tau_w D_w \left(k_1 - \frac{k_2}{\tau_w^2}\right) = \tau_w D_w k_w$$

$$\frac{1}{\eta_{sp}} = \frac{\eta_w}{D \cdot k_\tau} \cdot \frac{1}{\tau - \frac{D_w k_w}{D \cdot k_\tau} \tau_w} = \frac{\eta_w}{D \cdot k_\tau} \cdot \frac{1}{\tau - \tau_0}$$

The value τ_0 is tabulated against τ; example: Table 1. Furthermore, $\frac{\eta_w}{Dk_\tau}$ is tabulated against τ; example: Table 2.

To obtain the time for which a measurement is valid, we add half the outflow time to the time of starting[9].

$\frac{1}{\eta_{sp}}$ is plotted graphically against time. The points are joined by a straight

Table 1. τ *and* τ_0.

τ	τ_0
	10.0
40	
	10.1
20	
	10.2
15.1	
	10.3
12.6	
	10.4
11.2	
	10.5

Table 2. τ *and* $\frac{\eta_w}{Dk_\tau}$.

2.5 % stock starch solution.

τ	$\frac{\eta_w}{Dk_\tau}$
	9.93
84	
	9.94
57	
	9.95
45	
	9.96
38	
	9.97
34	
	9.98
30.5	
	9.99
28.0	
	10.00
26.0	
	10.01
24.7	

line, the slope of which, $\Delta \frac{1}{\eta_{sp}} / \Delta t$, is determined. The activity of the enzyme *in the mixture with starch* is

$$A^{t^\circ}_{\alpha/s} = \mathbf{c}_s^2 \cdot \frac{\Delta \frac{1}{\eta_{sp}}}{\Delta t}$$

If **a** g enzyme solution (preparation) has been mixed with **b** g starch solution, the activity of the original enzyme solution (preparation) will be

$$A^{t^\circ}_{\alpha/s} = \frac{\mathbf{a} + \mathbf{b}}{\mathbf{a}} \mathbf{c}_s^2 \frac{\Delta \frac{1}{\eta_{sp}}}{\Delta t}$$

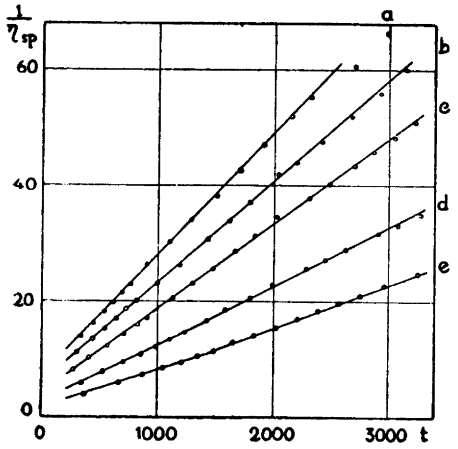

Fig. 3. Enzymic degradation of starch solutions of various concentrations with the same α-amylase solution.

a) $c_s = 0.01995$.
b) $c_s = 0.02179$.
c) $c_s = 0.02402$.
d) $c_s = 0.02719$.
e) $c_s = 0.03173$.

EXPERIMENTS

The results of specific gravity determinations on starch solutions will be found in table 3.

Table 3. Specific gravity of starch solutions with acetate buffer.

Starch %	D_4^{30}
0.000	1.0029
1.995	1.0113
2.179	1.0118
2.402	1.0130
2.722	1.0142
3.173	1.0159

Table 4. Enzymic activity at different substrate concentrations.

c_s	$\frac{1}{\eta_{sp}}$	$A^{30°}_{\alpha/s} \cdot 10^8$
0.01995	0.14—0.66	90.3
0.02179	0.11—0.60	90.8
0.02402	0.08—0.51	91.1
0.02719	0.06—0.15	80
	0.15—0.25	86
0.03173	0.05—0.12	75
	0.13—0.21	85

An enzyme solution, which had been kept in a refrigerator, was measured against several starch solutions of different concentrations. The values obtained in the different series are given in fig. 3 and table 4.

Potato starch was treated with 1.5 *N* hydrochloric acid at room temperature for 10 hours, washed and dried. The activity of an enzyme solution was measured in comparative experiments with solutions of this starch and of potato starch. The results are given in tables 5 and 6.

Table 5. 3.00 g enzyme solution + 29.63 g of a 2.495 % stock potato-starch solution.

$t + \frac{\tau}{2}$	τ	$\frac{1}{\eta_{sp}}$
344	201.2	0.0520
578	173.2	0.0609
801	156.8	0.0677
991	145.4	0.0734
1178	135.1	0.0795
1398	125.4	0.0862
1574	118.6	0.0916

$d\frac{1}{\eta_{sp}}/dt = 0.0310 \cdot 10^{-3}$. $c_s = 0.02214$.
$A^{30°}_{a/s} = 16.5 \cdot 10^{-8}$.

Table 6. 3.00 g enzyme solution + 29.96 g of a 2.322 % stock solution of potato-starch treated with hydrochloric acid.

$t + \frac{\tau}{2}$	τ	$\frac{1}{\eta_{sp}}$
224	48.7	0.258
300	48.3	0.260
607	46.8	0.271
781	45.9	0.278
990	45.2	0.283
1372	43.7	0.296
1612	42.8	0.304

$d\frac{1}{\eta_{sp}}/dt = 0.0335 \cdot 10^{-3}$. $c_s = 0.02211$.
$A^{30°}_{a/s} = 16.4 \cdot 10^{-8}$.

To ascertain to what extent the method of preparing a starch solution affects the enzymic decomposition of the latter, the activity of an enzyme solution was measured with several starch solutions prepared in different ways. The results are recorded in tables 7—9.

Table 7. 3.00 g enzyme solution + 29.60 g of a 1.998 % stock starch solution prepared by the method described in the text.

$t + \frac{\tau}{2}$	τ	$\frac{1}{\eta_{sp}}$
303	119.3	0.0908
473	112.8	0.0966
698	106.2	0.1031
995	99.0	0.1107
1373	91.0	0.1228
1764	84.6	0.1330
2005	80.9	0.1402
2304	78.1	0.1459

$d\frac{1}{\eta_{sp}}/dt = 0.0282 \cdot 10^{-3}$. $c_s = 0.01814$.
$A^{30°}_{a/s} = 10.0 \cdot 10^{-8}$.

Table 8. 3.00 g enzyme solution + 29.65 g of a 1.998 % stock starch solution prepared by the method described in the text, but subsequently heated to 95° for 15 minutes.

$t + \frac{\tau}{2}$	τ	$\frac{1}{\eta_{sp}}$
260	105.5	0.1040
469	100.3	0.1100
614	97.2	0.1139
858	92.1	0.1209
1151	88.2	0.1270
1476	82.5	0.1370
1773	79.3	0.1432
2069	75.2	0.1522

$d\frac{1}{\eta_{sp}}/dt = 0.0260 \cdot 10^{-3}$. $c_s = 0.01814$.
$A^{30°}_{a/s} = 9.3 \cdot 10^{-8}$.

Table 9. 3.00 g enzyme solution + 29.59 g of a 2.000 % stock starch solution prepared from an aquous suspension of starch by boiling and violent stirring for 15 minutes. Acetate buffer was added on cooling.

$t + \frac{\tau}{2}$	τ	$\frac{1}{\eta_{sp}}$
617	198.8	0.0526
850	183.9	0.0571
1216	154.5	0.0687
1569	137.7	0.0778
1979	122.6	0.0882
2403	111.1	0.0981
2892	98.9	0.1119

$$d\frac{1}{\eta_{sp}}/dt = 0.0260 \cdot 10^{-3}. \quad c_s = 0.01816 \cdot A^{30^C}_{a/s} = 9.4 \cdot 10^{-8}.$$

DISCUSSION OF THE EXPERIMENTAL RESULTS

Table 4 and fig. 3 show that, when 3.5 and 3 % stock starch solutions are used, deviations from Staudinger's[1] formula are so serious in the beginning of the experiment, that the points of the graph no longer lie on a straight line. These concentrations are hence too high. However 2.65, 2.40 and 2.20 % stock starch solutions give very satisfactory results. A comparison with fig. 2 shows that, at these low concentrations, the deviations from Staudinger's formula begin to assume moderate proportions for undegraded starch. The deviations may be expected to decrease rapidly as the starch is degraded. The measurements cannot, in point of fact, begin before the starch has been appreciably degraded.

Fig. 3 also shows that starch with a high initial degree of degradation cannot be a suitable substrate for the viscosimetric determination of α-amylase, since $d\frac{1}{\eta_{sp}}/dt$ is independent of the time t only before degradation has proceeded to a certain stage. Lintner's[7] starch is therefore not a suitable substrate in this case.

When very low enzyme concentrations are to be determined, it is important to degrade the starch, before the beginning of the viscosity measurement, sufficiently to permit the application of Staudinger's formula with respect to the concentration of the starch solution. We gather from tables 5 and 6 that the same result is obtained in enzyme determinations with starch mildly degraded by hydrochloric acid, and with potato starch previously untreated. In the preparation of a starch solution by working the material with an alkali,

the starch is also subjected to degradation. There was good reason to expect the same result in these cases, since the degradation of starch by α-amylase and that by acids and alkalis must be supposed to take place similarly, *i. e.* all 1,4 linkages are exposed to the same probability of rupture (there are, however, certain exceptions [3, 4]). For the determination of low enzyme concentrations, it is therefore advantageous to use starch that has been very mildly degraded with hydrochloric acid.

It appears from tables 7—9 that a starch solution, made up by dissolving starch in alkali and adjusting the acidity with acetic acid, when afterwards heated to 100° for some time, will be digested at the same rate as a solution made by dissolving starch in boiling water with subsequent addition of the buffer solution.

Howevers, a starch solution that has not been heated, will have its viscosity diminished more rapidly by the influence of amylase than a heated solution, just as if its concentration were less, *i. e.* as if all starch particles had not been completly dissolved. Thus, a starch solution for viscosimetric amylase determination, which has been made up by dissolving starch in alkali and adjusting the pH with acid, should be heated to almost 100° C for some minutes before immersed in the thermostat.

SUMMARY

A relation — recently deduced theoretically by the present author — connecting the enzymic activity, the substrate concentration, the specific viscosity and the time — has been applied in the examination of the reliability of the viscosimetrical methods used earlier in calculating the activity of α-amylase solutions and preparations.

It has been demonstrated experimentally that when using the formula the degradation of starch solutions of various concentrations with the same α-amylase solution results in the same values of enzymic activity.

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The Structure of 1,2-Epoxy-cyclohexane

An Electron Diffraction Investigation Based on the Rotating Sector Method

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CHEMICAL PREPARATION

1,2-Epoxy-cyclohexane, $C_6H_{10}O$, was prepared according to the method of Brunel [1] by treating 2-iodo-cyclohexanol-(1), $C_6H_{10}OH \cdot I$ with KOH in ether. The epoxide was purified by distillation.

ELECTRON DIFFRACTION-DIAGRAMS

A number of electron diffraction diagrams of 1,2-epoxy-cyclohexane were taken, using the rotating sector method. The microphotometer records of the different plates showed intensity curves in satisfactory agreement. The Fourier analysis was therefore based on a mean intensity curve. The evaluated $\sigma(r)/r$ curve is shown in fig. 1 a. With regard to the theoretical deduction of the $\sigma(r)/r$ function attention is drawn to a survey of the method developed at this institute. Reference to the original papers, published in the years 1940—46, will be found in this survey [2].

DETERMINATION OF THE STRUCTURE OF THE EPOXIDE FROM THE $\sigma(r)/r$ CURVES

From chemical evidence we may draw the conclusion that the epoxide consists of one six-membered ring of C-atoms and one three-membered ring containing two C-atoms and one O-atom. These two rings have one edge in common. To simplify the discussion of the different models of the molecule, the C-atoms are given numbers from 1 to 6, as shown in fig. 2.

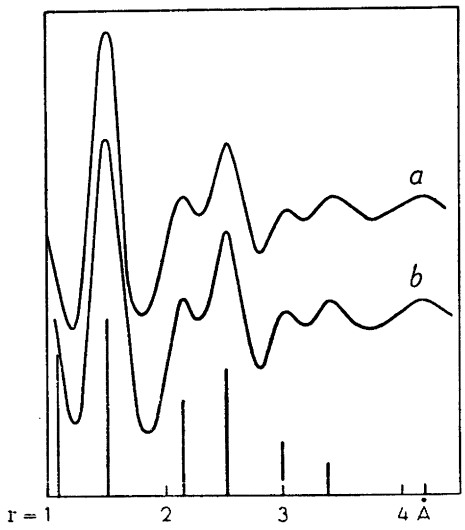

Fig. 1. a Experimental σ(r)/r curve. b Theoretical σ(r)/r curve. The vertical lines indicate height and r-value of the maxima of the seven normal curves, the sum of which is the theoretical σ(r)/r curve.

From a previous investigation of the molecular structure of cylohexane [3], we know that the cyclohexane ring has the »stair case» form, but an electron diffraction examination of C_6H_{12} does not give conclusive evidence as to whether or not small amounts of the »cradle»-shaped molecules are present. The repulsion between the H-atoms in this form, however, should render this form energetically unfavourable and under normal conditions the tilted ring is more likely than the »cradle»-shaped. All experimental facts seem to support this view. In general the H-atoms of two neighbouring CH_2-groups of a cyclic polymethylene weill tend to occupy »trans» positions, similar to the stable form of ethane or the symmetrical form of the cyclohexane molecule. In three- or four-membered rings the »cis» positions cannot be avoided. In these cases the H-C-H-angle of the methylene group is not necessarily the tetrahedral angle (109.5°). In cyclopropane, for example, an angle of 118.2 ± 2° is found [4].

Starting with a normal cyclohexane ring, it is easy to see that the O-atom may be attached to two neighbouring C-atoms in different ways:

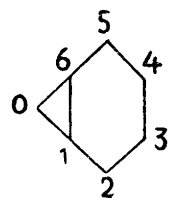

Fig. 2. Numeration of the atoms in 1,2-epoxy-cyclohexane.

1. We may engage two $\varkappa$-bonds in the formation of the three-membered ring, bringing the two C-O-bonds into the same plane. This will necessarily result in a deformation of the six-membered ring, the C-C-C angles becoming smaller than the tetrahedral angle of the undeformed cyclohexane-ring. It is seen from fig. 3 a that the resulting model will be assymmetrical and should exist in two optically active forms, the one being the mirror image of the other. A transformation of one of these forms into the other or into any other form, cannot be performed without breaking chemical bonds.

2. By using one $\varkappa$-and one ε-bond of the cyclohexane ring and bringing the two bonds into the same plane, two different situations may arise.

In the first place the tilted six-membered ring may be transformed into a »cradle»-shaped ring. The resulting model will be either that of fig. 3 b or that of fig. 3 c. The two models may be transposed with each other without breaking chemical bonds. If the stresses present in the three-membered ring cause deformations of the »cradle»-shaped six-membered ring, this will produce an increase of the C-C-C angles above the value 109.5°.

In the second place the choice of one $\varkappa$- and one ε-bond may also lead to a deformed six-membered ring of the type shown in fig. 3 d. Here the atoms C_1, C_2, C_5, C_6, are coplanar and one of the atoms C_3 and C_4 is situated above and one below this plane. Of all the models this involves the least deformation of the »staircase»-shaped cyclohexane-ring.

3. Two ε-bonds point in opposite directions and any formation of an epoxide based on an ε, ε-model is impossible, unless the six-membered ring is transposed; thereby transforming all ε-bonds into $\varkappa$-bonds.

In fig. 3 the four types of models are shown in projection. The double rings indicate two C-atoms placed one above the other.

In order to decide which model corresponds to the real structure of the epoxide, we may construct theoretical $\sigma(r)/r$ curves for the different models and compare them with the experimental $\sigma(r)/r$ curve.

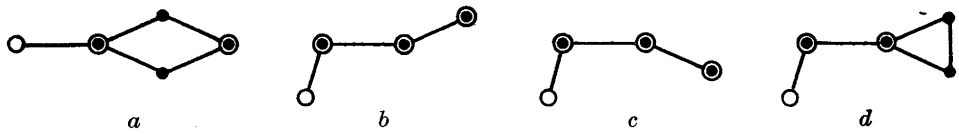

Fig. 3. Different types of models of the 1,2-epoxy-cyclohexane molecule shown in projection. The open ring indicates the O-atom. The double rings indicate two C-atoms, one placed above the other. Model a is produced by engaging two $\varkappa$-bonds, and the others by engaging one $\varkappa$-bond and one ε-bond in the formation of the three-membered ring.

These $\sigma(r)/r$ curves may, however, be interpreted as the sum of two sets of normal curves [7]. The first set is derived from the distances between all C- and H-atoms, the second from the distances between the O-atom on the one hand and all the C- and H-atoms on the other. In all the proposed models the distances of the first set must lie within comparatively narrow limits. This is a consequence of the fact that the distance between neighbouring C-atoms is 1.54 Å and the valency angles in the six-membered ring does not differ very much from 109.5°. From this it is evident that it will be difficult to differentiate between the various models on the basis of the distances in the six-membered ring.

In order to attain any result we must use the distances of the second set, where the C-O-distances dominate. For each proposed model a $\sigma(r)/r$ curve for the first set was constructed by correcting an experimental $\sigma(r)/r$ curve for cyclohexane, by means of normal curves for the single distances. The resulting curve was subtracted from the experimental $\sigma(r)/r$ curve for the epoxide. The difference curve represents the sum of the normal curves for the second set of distances. From this difference curve we can deduce the O-C-distances, and if we have chosen the correct model for the six-membered ring, these experimentally determined O-C-distances have to agree with the model.

$\sigma(r)/r$ curves for the first set of distances and corresponding difference curves were then constructed for models of all the proposed types (fig. 3), the models being deformed in varying degree. The O-C-distances which emerged were largely independent of the particular form of the six-membered ring. All reasonable models, that is to say those with a angle C-C-C- between 100° and 120°, give four different O-C-distances, all lying within the limits below:

$O—C_1 = O—C_6$	= 1.42 Å
$O—C_2 = O—C_5$	= 2.49—2.53 Å
$O—C_3$	= 2.98—3.10 Å
$O—C_4$	= 3.25—3.40 Å

This obliges us to reject model 3 a. The O-C-distances cannot agree with this model, unless all the C-C-C-angles are deformed so as to make the six $C_1—C_3$-distances approximately 1.9 Å. Such a short $C_1—C_3$-distance is very improbable, and moreover the experimental $\sigma(r)/r$ curve plainly shows that this distance is very nearly 2.50 Å. In addition to this, the model cannot give different $O-C_3$ and $O-C_4$ distances.

Further investigations prove that the models 3 b and 3 c may be adjusted to agree with the O-C-distances, if all the C-C-C-angles are deformed to approximately 118° and the two forms exist in equilibrium. So large a deformation

of all the angles seems improbable, however, it is certainly reasonable to assume that the angles C_1-C_6-C_5 and C_6-C_1-C_2 are about 118°. In cyclopropane [4] the corresponding angle is found to be 116.4 ± 2°. A simple quantum mechanical calculation of the effect produced on the valency angles at the C-atom, when one of the valency angles is decreased, supports this assumption [5].

It should be pointed out however that a deformation of the other four C-C-C-angles in the six-membered ring to approximately 118°, is energetically improbable. On the contrary there is every reason to believe that these angles lie much closer to the value of the tetrahedral angle. Assuming the angles C_1-C_6-C_5 and C_6-C_1-C_2 to be approximately 118°, then models of the type, shown in fig. 3 d, are produced by building up the rest of the six-membered ring, leaving the other C-C-C-angles as close to the tetrahedral value (109.5°) as possible. We may presume that particularly the angles C_3-C_4-C_5 and C_2-C_3-C_4 deviate little from the tetrahedral value. Models of this type will also cause as many as possible of the H-atoms connected to neighbouring C-atoms to come into trans position. When testing different models of this type, the angles C_3-C_4-C_5 and C_2-C_3-C_4 were assumed to be tetrahedral. Different values for the angles C_6-C_1-C_2 and C_1-C_6-C_5 were then systematically tried. The last two angles are determined, when the six-membered ring is assumed to have a symmetry axis through the middle points of the C_1-C_6 and C_3-C_4 bonds.

In fig. 4 theoretical $\sigma(r)/r$ curves for the second set of distances (the O-C- and O-H-distances) are shown together with the corresponding difference curves derived by subtracting the theoretical $\sigma(r)/r$ curve for the first set of distances from the experimental $\sigma(r)/r$ curve for the epoxide. A model of type 3 d with the angle C_6-C_1-C_2 = C_1-C_6-C_5 = 118.5° apparently gives the best agreement between the two corresponding curves.

From fig. 3 d it is easily seen that the model may correspond to two optically active isomers. These can be transposed with each other without any chemical bond being broken, and it will scarcely be possible to separate them. But this transposition of the six-membered ring may also involve the occurrence of models of the type 3 b and 3 c as unstable forms during the transposition.

CONSTRUCTION OF THE THEORETICAL $\sigma(r)/r$ CURVE FOR THE EPOXIDE

If we now try to build up a theoretical $\sigma(r)/r$ curve for the epoxide corresponding to the model of type 3 d with an angle C_1-C_6-C_5=118.5°, this may be done in a simple way by putting all the interatomic distances of any significance in the model into seven groups. Then the difference between the distances within each group will not be more than 0.15 Å. We may now build up the

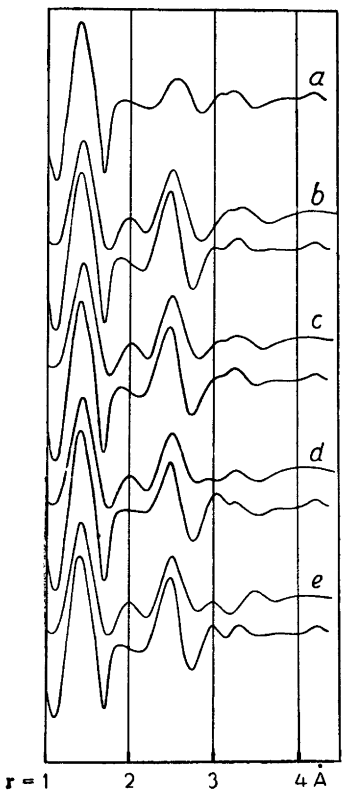

Fig. 4. Theoretical $\sigma(r)/r$ curves for the second set of distances (the O-C- and O-H-distances) shown together with the corresponding difference $\sigma(r)/r$ curves: a Difference between experimental $\sigma(r)/r$ curves of 1,2-epoxycyclohexane and cyclohexane. b Theoretical $\sigma(r)/r$ curve and below this the corresponding experimental difference $\sigma(r)/r$ curve for the model of the epoxide molecule shown in fig. 3 d with the angle $C_6—C_1—C_2 = 117°$, c the same for the angle $C_6—C_1—C_2 = 118.5°$, d the same for the angle $C_6—C_1—C_2 = 120°$, e the same for an equilibrium between equal parts of the molecular models shown in fig. 3 b and fig. 3 c, both with all the angles $C—C—C = 118°$.

$\sigma(r)/r$ curve from seven normal curves, one for each group. Each of these will have a maximum of a certain height at a given r-value, which was evaluated by adding the maxima corresponding to the single distances within the group. The resulting theoretical $\sigma(r)/r$ curve fig. 1 b is in complete agreement with the experimental curve fig. 1 a.

This confirms the result of the last chapter, but it also plainly shows the limits of the method. It is evident from fig. 1 that, when keeping the six middle distances constant, we have a certain although limited choice of single distances in the molecule, without coming into conflict with the experimental facts. Especially is this the case if the middle distance is built up of many single distances.

A closer examination of the middle distances, however, reveals that the single distances which are of special importance for the choice of molecular structure type, are not liable to any variation of consequence. It is thus not possible to get away from:

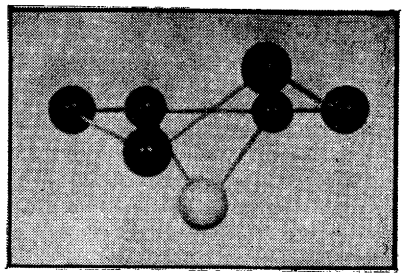

Fig. 5. Model of the 1,2-epoxy-cyclohexane molecule.

$O—C_1 = 1.42$ Å, $O—C_3 = 3.01$ Å and $O—C_4 = 3.35$ Å.

The distances which show the greatest variability, are those embraced in the middle distance of 2.53. This uncertainty is not sufficient to make our choice of model (fig. 3 d) unreliable, but it prevents an exact determination of the angles O-C-C- and C-C-C. These angles however, cannot differ by more than a few degrees from the values given for the model fig. 3 d with an angle C_6-C_1-$C_2 = 118.5°$.

SUMMARY

The molecular structure of 1,2-epoxy-cyclohexane, $C_6H_{10}O$, has been determined by an electron diffraction investigation of the vapour, using the rotating sector method. The resulting structure of the six-membered ring is a sort of deformed »staircase» form (fig. 5). The four C-atoms nearest to the O-atom are coplanar. The two remaining are placed one above and one below this plane. The interatomic distances and angles in this model are:

$C_1—C_2 =$	1.54 Å
$O—C_1 = O—C_6 =$	1.42 »
$O—C_2 = O—C_5 =$	2.51 »
$O—C_3 =$	3.01 »
$O—C_4 =$	3.35 »
C_6-C_1-$C_2 = C_1$-C_6-$C_5 =$	118.5°
C_1-C_2-$C_3 = C_6$-C_5-$C_4 =$	116.0°
O -C_1-C_2 = O -C_6-C_5 =	115.0°

The two remaining C-C-C angles are 109.5°. The investigation leaves no doubt about the structure type, but a variation of about $\pm$ 2° in the angles mentioned above cannot be detected if the middle value of all the corresponding distances is kept constant at 2.53 Å.

The resulting structure of the six-membered ring, has a great resemblance to the six-membered ring we may expect to find in cyclohexene, C_6H_{10}, where the distance C_1-C_6 must be approximately 1.34 Å and the four atoms C_1, C_6, C_5 and C_6 are necessarely coplanar.

Finally I wish to express my gratitude to Dr. O. Hassel, professor at the University of Oslo, for giving me the opportunity to carry out the foregoing work in his laboratory and for his helpful criticism and advice.

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Some Measurements of Double Refraction of Flow on Carbohydrates

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In connection with other investigations of high polymers being carried out at the Institute of Physical Chemistry in Upsala, the double refraction of flow of certain carbohydrates has been studied.

A modification of the so-called Kundt apparatus for the measurement of double refraction of flow was used in these investigations. The construction and use of the apparatus will be described in another connection. The apparatus consists of a fixed outer cylinder and a rotating inner one. The space between the cylinders is filled with the liquid under investigation.

If the rate of rotation of the inner cylinder is lower than a certain limit a definite laminar movement occurs. A definite gradient of velocity is therefore found in the liquid. We can fix three directions: — the direction of flow x_1, the direction of the gradient x_2 and the direction of the light rays x_3 which is perpendicular to x_1x_2. The components of the velocity are $U_1 = Gx_2$; $U_2 = 0$; $U_3 = 0$.

Under the influence of flow the liquid behaves as a doubly refractive crystal with one axis in the direction x_3 and the other two in the plane x_1x_2.

We will now go into the attempts which have been made to interpret this phenomenon with special reference to the highly polymeric carbohydrates.

To begin with we will treat the particles as long rigid ellipsoids or cylinders. Under the influence of the flow a certain anisotropic distribution of the particles occurs. It is thus found that particles arrange themselves in such a way that their arrangement is different along the path of the beam of light ($z = x_3$) and in a plane at right angles to this. The axes of distribution x and y, in the plane x_1x_2 are perpendicular to one another, and the x axis lies at an angle (χ) to the x_1 direction.

The distribution function is as follows:

$$\varrho_x = 1 + \frac{\sigma}{2}; \varrho_y = 1 - \frac{\sigma}{2}; \varrho_z = 1 + \frac{\sigma^2}{60}; \sigma = \frac{pG}{2D}; p = \frac{a^2-b^2}{a^2-b^2}; \qquad (1)$$

where ϱ_x, ϱ_y, and ϱ_z are the functions of the distribution in the directions x, y, z and a and b the length of the axes of the ellipsoids. G is the gradient of the velocity and D the constant of rotational diffusion.

This distribution function is valid even for long particles. The forces of inertia however impose a limit and these begin to exert an effect when the particles are of the order of sizes of 50,000 Å.

From the optical theory for cases where the field is quasi-stationary a formula can be calculated for the angle between the x-axis and the direction of flow, and, when the orientation is not large, we get the following formula for long cylinders in such dilute solutions that no interaction between them occurs.

$$\lambda = 45° - \chi = \frac{\pi \eta_0 G l^3}{36 k T (\ln {}^{l}/_{d} - 0.11)} \qquad (2)$$

In this formula η_0 is the viscosity of the solvent, l is the length of the particles, d is the diameter of the particles, T is the absolute temperature and k is Boltzmann's constant.

As is seen from the formula, the angle of orientation is not determined by the optical constants (provided that there is no optical activity). It has therefore frequently been assumed without any further thought that this formula had the same field of application as the hydrodynamic theory. In the following pages we shall see that this seems empirically to be the case. However, from a theoretical point of view matters stand rather differently.

According to Snellman and Björnståhl [1] the following formula* is valid for the calculation of χ

$$\begin{aligned} \operatorname{tg} 2\chi &= \frac{2a_{12}}{a_{11} - a_{22}} \\ a_{12} &= \frac{1}{8\pi} [H - H^1] \iint \sin^3 \Theta \sin 2\varphi \, d\Theta \, d\varphi \\ a_{11} - a_{22} &= \frac{1}{4\pi} [H - H^1] \iint (-1) \sin^3 \Theta \cos 2\varphi \, d\Theta \, d\varphi \end{aligned} \qquad (3)$$

In deriving this expression the assumption has been made that the electrical moment $f_i = H_i E_i$ where E_i is the light vector in the direction i. This is the

* For definitions of terms see the paper referred to.

first term in a power series in which the subsequent terms can be ignored as we are assuming the field to be quasi-stationary, *i. e.* the size of the particles $\lesssim 1500$ Å. The subsequent terms may be expected to play a part for larger particles so the optical quantities, will also enter into formula (3). The various parts of the particle do not excite the same phase of the light-wave and such a calculation leads to very complicated functions.

It is not possible to give a general solution to the problem owing to the extremely complicated expression which results when the dimensions of the particle are greater than the wave-length of light in all directions. There is a possibility for particles which have only one dimension longer than the wave-length of light. We have discussed this in another connection [2]. It is found here also that equation (2) should be valid. The magnitude of the double refraction is thus a function of the cylinderradius of the particles.

THE KUHN-HERMANS THEORY

Kuhn [3] and later Hermans [4] have proposed a statistical theory for high-polymeric compounds which takes into account their greater or less degree of coiling. The theory contains many crude approximations and can only be considered as a first attempt to tackle the special conditions which may be supposed to prevail among high polymers. In this connection Kuhn has only treated a two-dimensional case.

The actual statistical treatment for the coiled molecule has been principally developed by Kuhn. The first assumption, that the molecule is not straight but more or less curled up owing to the greater or less degree of free rotation, appears to be confirmed by the investigations which have been carried out concerning the rotation of the groups in simpler molecules.

In order to avoid the question of interference with this free rotation Kuhn has divided the thread-like molecules into sections (fibre-elements). The direction of each fibre-element is chosen so that it can be treated as being independent of all others fibre-elements. A statistical calculation is made as to the probability of there being a given distance between the end-points of the whole molecular chain. In the calculations it has been found desirable to treat this distribution of end-points as a diffusion equilibrium.

It cannot be denied that there are certain difficulties inherent in the actual treatment of the orientation which only takes account of the distribution of the end-points of the molecules. The impulse to execute Brownian movement, and thus to sway in the direction of the gradient of flow must be attributed to the whole fibre-molecule. All the fibre-elements must be affected and be connected with one another in this orientational effect.

In this connection it is strange that in double refraction of flow the molecules are acted on as units while in magnetic or electrical double refraction this appears not to be so. From the experiments which the present author did with the latter types of double refraction it appears that, in very dilute solutions such double refraction as can be attributed with certainty to free molecules is very slight and below the limit of observation. Those phenomena of double refraction which sometimes appear among highly polymeric compounds can usually be attributable to aggregated molecules where, as a result of this stabilisation of the long threads, such double refraction appears.

An interpretation of these conditions would certainly be possible on the assumption that, during orientation in a magnetic or electrical field, the individual elements try to move independently of one another. The forces by which a fibre-element is linked to other elements which do not respond oppose the orientation of that element. These forces are so strong that little or no orientation occurs. The kinematic orientation of double refraction of flow, on the other hand, is determined by movement of the neighbourhood.

A certain difficulty exists in arriving at a satisfactory treatment of the optical side of the problem in accordance with the theories developed by Kuhn and Hermans. The whole molecule should not be regarded as contributing to the polarisation of the individual residue but it may be tentatively supposed that the immediate environment, with its axial character, contributes to this polarisation. On account of the dependence of double refraction on the properties of the solvent it cannot either be assumed that the individual elements are optically free particles. As a first approximation it may perhaps be assumed that the element which is polarised by the light wave coincides in length with a fibre-element, and treat this as an elongated cylindrical resonator. If this cylinder has a sufficiently great length the end effects can be ignored. Such an assumption is perhaps the best that can be made at present. In the future, when much more experimental material is available it will beccome apparent what corrections must be made.

Calculations based on these premises can only be made when the molecule has, on the average, rotational symmetry about the line joining its end-points. It must also be postulated that the molecules are coiled to so slight an extent that the various fibre-elements do not interact optically as this makes the calculations extremely complicated. The assumptions which have been made here should be valid for nitrocellulose where the residues are comparatively large and the chain is loosely coiled. The fibre-element must not be so large that the field cannot be treated as quasi-stationary.

We also make the assumption that, in such an element, the residues affect one another and that the fibre-element therefore consists of several coupled

resonators. In the limiting case, for a large number of coupled resonators the fibre-element may be treated as a material having the optical principal dielectric constants ε and ε'.

It is now possible to calculate the relative principal polarisabilities h and h' for the general case where the light vector is not parallel with the optical axes. We further assume that the material is anisotropic and that the optical and geometrical axes of the element coincide. We can then write:

$$h = \frac{(\mu_r^2 - 1)}{4\pi + (\mu_r^2 - 1)P} \; ; h' = \frac{(\mu'^2_r - 1)}{4\pi + (\mu'^2_r - 1)P'} \; ; \tag{4}$$

where μ_r and μ_r' are the complex relative optical principal dielectric constants and P and P' are functions of the dimensions of the element.

The use of Hermans' methods of calculation leads to the final formula given below.

For small deviations of the extinction angle from 45° to the direction of flow the amount of the deviation is given by the expression:

$$\lambda = \frac{\eta_0 G l_g^3 Z^2}{3kT} \frac{s^3}{p^2} \tag{5}$$

where η_0 is the viscosity of the solvent
G is the gradient of velocity
T is the absolute temperature
k is Boltzmann's constant
l_g is the length of the component residue
Z is the degree of polymerisation
s is a factor dependent om the relation between the mean length of the element and the length of the residue
p is the number of residues in an element.

For the difference in the refractive index between the axes which lie in the plane normal to the axis of symmetry of the cylinder we get the following expression:

$$n_x - n_y = \frac{8\pi}{15} G \eta_0 \frac{v\,(h - h')^R}{n_0 k T} l_g^3 \frac{s^3}{p^2} Z^2 c' \tag{6}$$

c' is the number of chain-molecules dissolved per unit volume
v is the volume of the fibre-element
n_0 is the refractive index of the solvent

or when $c' = \frac{Nc}{Z10^3}$

where N is Avogadro's number and c is the number of component residues per litre

$$n_x - n_y = \frac{8\pi}{15} G \eta_0 \frac{Nv (h - h')^R}{n_0 k T} l_g^3 \frac{s^3}{p^2} Zc \tag{7}$$

If the lengths of the elements are several times as great as their diameters $[h - h']$ is independent of the length and therefore it is a constant for a homologous series of polymers in one and the same solvent. For an anisotropic non-absorbing polymer one may therefore write:

$$[h' - h']^R = \frac{{n_0}^2}{4\pi} \cdot \frac{m^2 - 3\ m'^2 + \frac{m^2\ m'^2}{{n_0}^2} + n_0^2}{{n_0}^2 + m'^2} \tag{8}$$

where m and m' are the refractive indices of the fibre-element in different directions.

If we now vary the refractive index of the solvent we find that for $m<m'$ the value of $[h - h']^R$ is always positive while for $m<m'$ the value of $[h - h']^R$ is negative between

$${m_0}^2 = \frac{m^2 - 3\ m'^2}{2} \pm {}^1/_2 \sqrt{(m^2 - 3\ m'^2) - 4\ m'^2\ m^2}.$$

POLYDISPERSITY

All the calculations which have been made are applicable to monodisperse solutions. Polydispersity upsets the results of the measurements very considerably. Calculations of length from double refraction of flow using formula (2) gives a mean value for length in which the longer molecules have the greatest weight. We have taken weighted means as the values for the degree of polymerisation and the length.

In such investigations one may generally expect to get higher values for length from experiments with double refraction of flow than from data obtained with the ultracentrifuge so long as other factors do not come in. This is due to polydispersity.

The Kuhn-Hermans theory also gives a mean value which is strongly influenced by polydispersity. Our experimentally derived data as well as the theoretical deduction are based on the assumption that the solution is monodisperse.

Hermans has shown that for degrees of polymerisation from equ. 5 one gets $\frac{\overline{Z}^4}{\overline{Z^2}}$ where as from the actual double refraction one gets thus $\frac{\overline{Z}^2}{\overline{Z}}$ in the latter case there is less dependence on polydispersity.

VISCOSE

Two different preparations of viscose in strong sodium hydroxide (2 *N*) were investigated. The solutions were very dilute. In these investigations it was observed that the solutions only consisted of free particles at the point where their viscosities were minimal. Both before and after this point aggregated particles appeared in the solution. Investigation of the magnetic double refraction reveated the same phenomenon. Before and after the point of minimum viscosity there was strong magnetic double refraction which immediately became saturated. It was also possible to observe long relaxation times when the field had been removed. On the other hand we found no measurable double refraction at the point of minimal viscosity. The time during which it was possible to carry out measurements of double refraction of flow without being interfered with by aggregated particles in the solution was about one day. In table 1 the degrees of polymerisation are calculated both from measurements of double refraction of flow and from molecular weight determinations carried out by Gralén.

Table 1. Viscose. The degree of polymerisation from double refraction of flow and from the ultracentrifuge.

l	Degree of polymerisation	Degree of polymerisation determined ultracentrifugally
2200 Å	113000	113000
1200 Å	62000	62000

This result appears to indicate that the molecules are very loosely coiled. This may perhaps be expected to be the case when the molecules are highly charged.

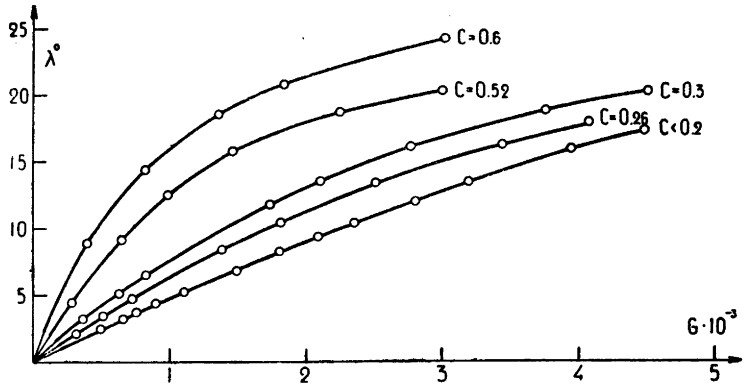

Fig. 1. The dependence of the angle of extinction on the gradient at different concentrations (g/100 ml) for a highly polymeric nitrocellulose (solvent butyl acetate).

NITROCELLULOSE

The preparation of nitrocellulose was dried over silica gel before weighing. The solutions were alloved to stand for 24 hours before the measurement were made so that the nitrocellulose might become evenly dispersed. Berl and Lange [6] have found that a solution of nitrocellulose in acetone shows anomalies of viscosity at first. These are caused by incompletely dissolved nitrocellulose. Most of the measurements reported here were carried out on solutions of nitrocellulose in butyl acetate at 20° C.

Gralén [5] carried out molecular weight determinations and viscosity measurements on some of the preparations and Jullander [7] carried them out on the others. These are described in their papers.

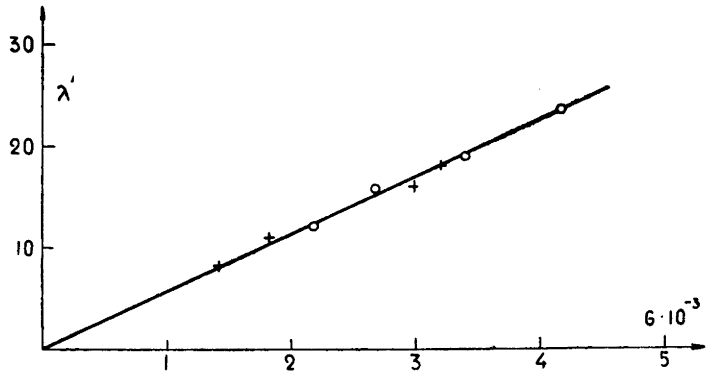

Fig. 2. The dependence of the angel of extinction on the gradient at two different concentrations for a less highly polymeric nitrocellulose (solvent cyclohexanone).

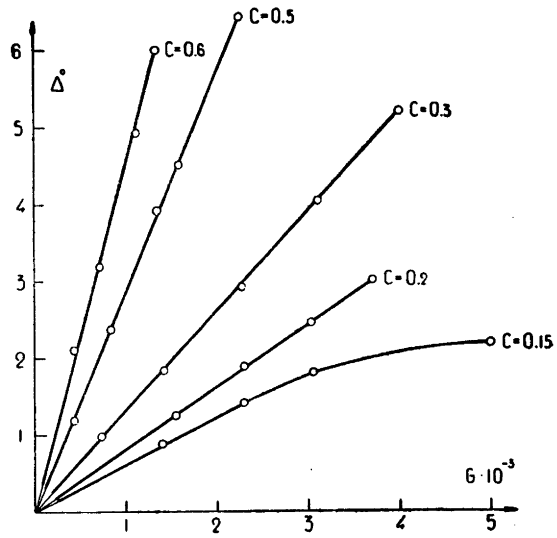

Fig. 3. The dependence of the phase-difference (Δ) on the gradient for a highly polymeric nitrocellulose (solvent butyl acetate).

Figures 1, 2 and 3 are given as examples of the dependence of the angle of extinction (λ) and phase-difference (Δ) on the gradient *(G)* at different concentrations.

At the lower concentrations the angle of extinction varies linearly with the gradient, at least to begin with. This agrees with the theories of double-refraction of flow. When there is a high degree of polydispersity other conditions

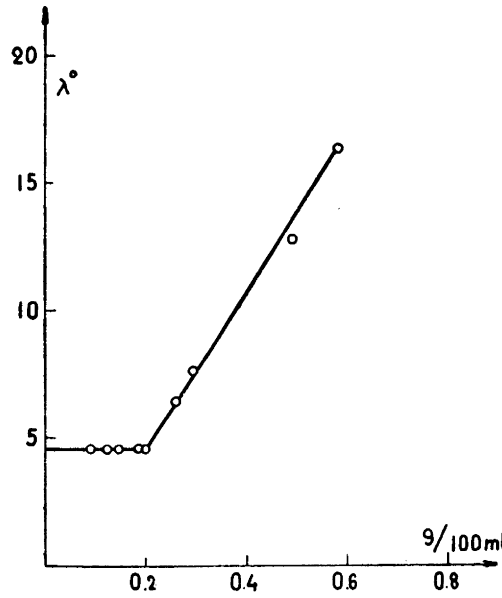

Fig. 4. The dependence of the angle of extinction on the concentration when the gradient is constant.

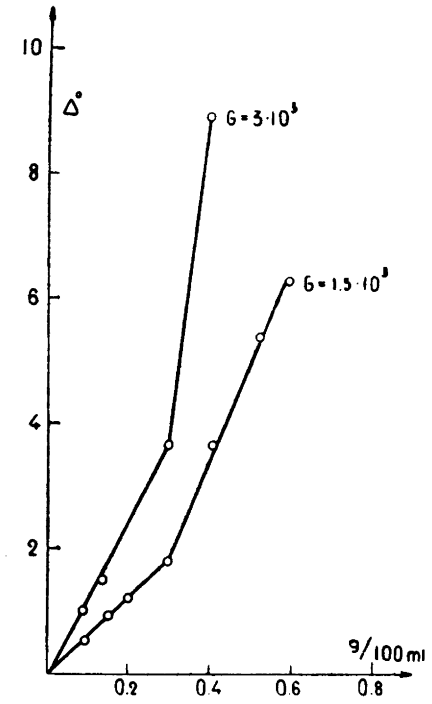

Fig. 5. The dependence of the phase-difference on the concentration when the gradient is constant.

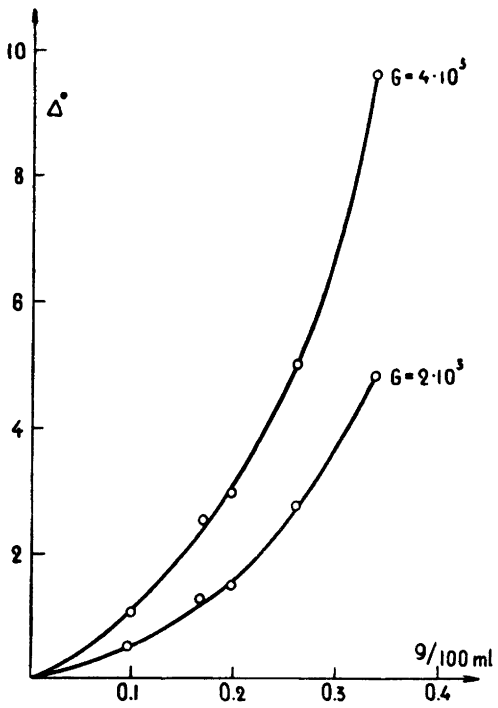

Fig. 6. The dependence of tye phase-difference on the concentration when the gradient is constant (great polydispersity).

may be expected to obtain. The double refraction should also stand in a linear relation to the concentration.

The dependence of the angle of extinction and the double refraction on the concentration at various gradients are shown for two preparations (figs. 4 and 5). These may serve as typical examples. In almost all cases the relationship to the concentration changes markedly at a certain concentration. Figure 6 shows that, in certain exceptional cases, another appearence is found. This is probably caused by greater polydispersity.

In table 2 data are given for the degree of polymerisation, calculated from data obtained by means of the ultracentrifuge, and for the lengths of the molecules determined both by the dissymmetry factor (f/f_0) and by the measurements of double refraction of flow and viscosity. The length is calculated from the measurements of double refraction of flow by means of formula (2).

The length determined by the double refraction method is usually greater than that derived from f/f_0. If the length is calculated directly from the

Table 2. Nitrocellulose. The lengths of the molecules at different degrees of polymerisation.

Degree of polymerisation (ultracentrifuge)	Length in Å (from f/f_0)	Length in Å (double refract. of flow)
2000	5300	6100
1700	6300	5700
1200	4600	4000
1010	4100	4700
1000	3700	4500
1000	2800	3800
1000	2800	3100
997	2900	3000
854	2600	2500
820	3700	3400
528	1700	2100
320	1200	1800
71	450	550

molecules weight the result obtained is always greater than that obtained by the other methods except in the case of very low molecular weights where the results of all methos are the same.

The applicability of the Kuhn-Hermans theory has been tested in respect of some preparations of nitrocellulose. The data are given in tables 3 and 4. For this purpose s^3/p^2 has been calculated from previously known data by the use of formula (5) in order to see whether this value remained constant. The same calculation was made in respect of formula $p^3/s^2\ (h - h')\ v$ which should also remain constant for the same solvent. There is no obvious alteration in the values though there is a certain scatter, probably due to polydispersity.

Table 3. Nitrocellulose. The value of the factor s^3/p^2 in the theory of flexible molecules.

Degree of polymerisation	s^3/p^2
1700	26.0
1200	20.3
1010	30.2
1000	35.5
1000	20.3
1000	22.9
820	25.5
560	21.7
512	25.6
350	32.4
320	16.9

Table 4. Nitrocellulose. The value of the constant factor of the double refraction in the theory of flexible molecules.

Degree of polymerisation	$s^3/p^2\,(h - h')^R v$
1000	300×10^{-24}
854	338 »
512	273 »
350	286 »
320	240 »

POLYURONIC ACIDS

In collaboration with Säverborn [8] the author has investigated the double refraction of flow of some highly polymeric polyuronic acids. The determinations of molecular weight and other information concerning this work are described in Säverborn's paper [9]. The pectins themselves do not show any double refraction as may easily be understood on theoretical grounds. However hydrolysed or nitrated pectins give results which are in agreement. The degrees of polymerisation calculated in this way agree with those obtained from determinations of the molecular weights of pectins. With gum arabic direct determinations can be made. There are no anomalies here.

Table 5. Polyuronic acids. The lengths of the molecules from molecular weight and from double refraction of flow.

Molecular weight	Length in Å	Length in Å (double refract. of flow)
Apple pectin 99000	2850	2600
Citrus pectin 210000	7000	3800
Gum arabic 285000	945	1000

The descrepancy in the measurements on citrus pectin was probably due to some peculiarity in the preparation used. Later determinations of the molecular weight gave results of only about half this size, which agree with the results of the measurements of the double refraction of flow.

It is the side-chains of gum arabic which make its molecular weight so large. Its double refraction is but small which also indicates that this is the case. The theory dealing with rigid rods ought to be applicable here.

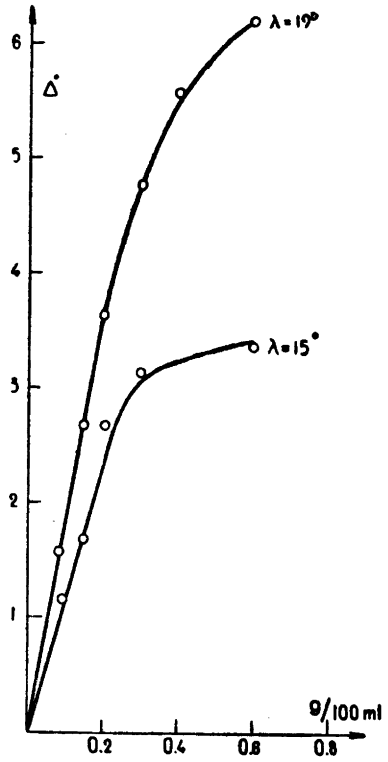

Fig. 7. The dependence of the phase-difference on the concentration when the angle of extinction is constant.

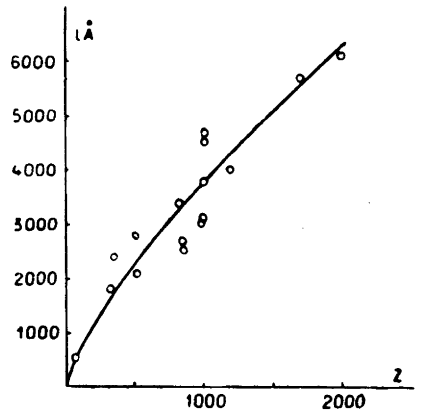

Fig. 8. The dependence of the length on the degree of polymerisation for the nitrocelluloses here investigated.

DISCUSSION OF THE RESULTS OF THE MEASUREMENTS

It is scarcely possible to enter on a discussion of details before the problem of the production of more or less monodisperse polymers has been satisfactority solved. Polydisperse solutions give shifting values which are hard to compare with those expected on theoretical grounds. External circumstances are responsible for the fact that this work has been carried out entirely with polydisperse solutions.

Table 2 gives an indication of the significance of polydispersity. Comparison at the lengths of substances having a degree of polymerisation of 1000 shows that they vary from 3000 to 4700 Å.

As is seen from the material, calculations of length from the double-refraction of flow by formula (2) gives lengths which, for nitrocellulose are

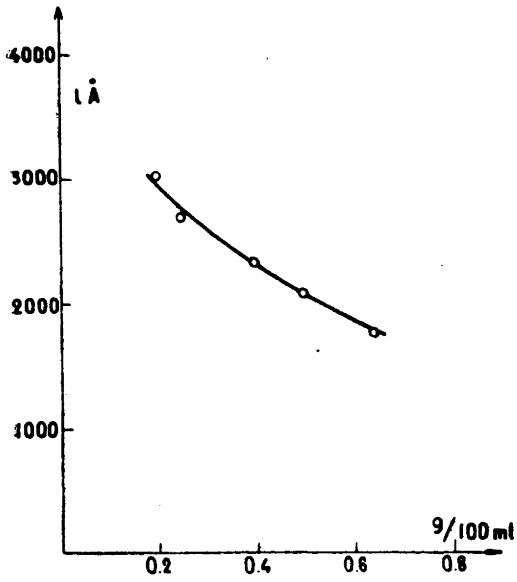

Fig. 9. The dependence of the critical concentration on the length.

somewhat larger than those calculated from f/f_0. Similarly, if the degree of polymerisation is calculated from the double-refraction of flow it is found to be less than that calculated from the date obtained with the ultracentrifuge at high degrees of polymerisation.

The critical concentration where λ and Δ become dependent on concentration has been determined. The relation between the degree of polymerisation and this point is seen in fig. 9. It appears empirically has though log c were proportional to l (fig. 10).

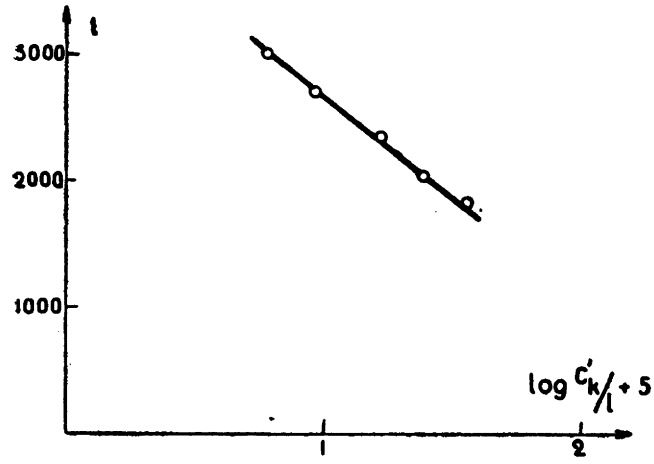

Fig. 10. The logarithmic relationship between the critical concentration and the length.

The results calculated from the Kuhn-Hermans theory show a relatively great degree of constancy. As we did not compare substances of the same degree of polydispersity, but substances which were derived from material treated in different ways, our results may be considered satisfactory. Our values for s^3/p^2 are higher than those calculated by Hermans on the basis of other measurements. However we used figures for the degree of polymerisation derived from data obtained with the ultracentrifuge while he did not.

The difficulty in comparing the different theories on the basis of the present data lies in the fact that here $Z^2 = K \frac{l^3}{ln\, l/d - 0.11}$ as a rough aproximation. It is possible that other classes of substances might give more decisive evidence as to the applicability of the Kuhn-Hermans theory.

We have also tried to check the theory by investigating the constancy of $\frac{\Delta}{c\lambda}$ which may be derived from the formulas (table 5). Other premises however lead to similar formulae. The viscosity is not included in this expression. It may therefore be expected to be independent of the viscosity over a long period. This appears from table 6 to be the case.

Table 6. The factor independent of the viscosity for a nitrocellulose at different velocity gradients.

G	$\Delta/c\lambda$		
	$C = 0.5510^{-2}$	$C = 0.2710^{-2}$	$C = 0.09310^{-2}$ g/ml
10^3	3.70	3.65	3.61
3.10^3	3.72	3.68	3.61
6.10^3	3.61	3.64	3.62

It must be pointed out that the theory is not valid for greatly coiled chain-molecules, and also that when the component residues have a small diameter in comparison with those of the solvent the optical quantity $h - h'$ has a different significance from that which it has in the theory used here.

Amongst other evidence we have Gralén's numerous observations in the determination of their molecular weights, to show that the derivatives of cellulose are very loosely coiled. The differences between the values for the lengths calculated on the basis of data from the ultra-centrifuge, directly from the molecular weight or from the dissymmetry factor (the differences between the last two are obvious) are thought by Gralén to be due to internal movements

in the chain (Brownian movement) which shorten it. This lends support to the Kuhn-Hermans theory. The fact that the nitrocelluloses are so slightly coiled is thought by Huggins [10] to be due to the existence of hydrogen bonds between the — O and — OH groups in adjacent glucose residues.

SUMMARY

Measurements of the double-refraction of flow have been carried out on several carbohydrates. The values obtained have been compared with those expected according to the different theories. Certain of the theoretical assumptions underlying these theories have been discussed. No experimental evidence could be established in favour of either of the theories on account of the polydispersity of the material under investigation.

The author wishes to express his sincere gratitude to Professor The Svedberg for enabling him to carry out this work. The investigation was supported financially by grants from *Nitroglycerin AB, AB Bofors Nobelkrut, AB Svenskt Konstsilke.*

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Kationoid Reactivity of Sulphur

Sulphenyl Compounds

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It was demonstrated in previous papers [1, 2] that various polythionic compounds in reactions with anionoid reagents behave as derivatives of divalent electropositive sulphur S^{++} and S_2^{++}. Thus the following compounds were found to react with piperidine to give the corresponding sulphur piperidides, as do the sulphur chlorides [3, 4] and thiocyanates [5, 6]: Sulphur di-O-methylmonothiophosphates, sulphur ethanethiosulphonates, sulphur *p*-toluenethiosulphonates, and sulphur thiosulphates (pentathionate and hexathionate).

The present paper deals with the chemistry of sulphenyl compounds, *viz.*, derivatives of alkylsulphur or arylsulphur RS^+. A rather comprehensive material concerning sulphenyl compounds is available in literature. A recent review on »The Sulfenic Acids and their Derivatives», mainly from an organic synthetic point of view, is by Kharasch, Potempa and Wehrmeister [7]. The authors, however, fail to recognize the so-called »esters of thiosulphonic acids» and »esters of thiosulphuric acid» as derivatives of sulphenic acids, as also the sulphenyl thiosulphonates of Brooker, Child and Smiles [8]. In most of the reactions with anionoid reagents the above compounds eliminate their sulphenyl group as a monovalent kation RS^+. It should be emphasized that this is the case within the majority of derivatives of sulphenic acids.

The structure of »esters of thiosulphonic acids» $RSSO_2R$ as *sulphenyl sulphinates* finds a clear proof in their reactions with cyanide ion and with mercaptide ions:

$$RSSO_2R' + CN^- = RSCN + R'SO_2^-$$
$$RSSO_2R' + R''S^- = RSSR'' + R'SO_2^-$$

Reactions of this type were demonstrated by Otto and Rössing [9], Smiles and Gibson [10], and Footner and Smiles [11]. Other examples are the reactions with

sodium derivatives of certain organic compounds [12] (*cf.* also Connor [13]), and displacement reactions [14, 15] of the type:

$$RSSO_2R' + R''SO_2^- = RSSO_2R'' + R'SO_2^-$$

The so-called »esters of thiosulphuric acid» $RSSO_3^-$ in reactions with anionoid reagents behave as *sulphenyl sulphites*, with fission in the sense (RS^+) (SO_3^{--}). Thus in reactions with cyanide ion [11, 16] and mercaptide ions [11]:

$$RSSO_3^- + CN^- = RSCN + SO_3^{--}$$
$$RSSO_3^- + R'S^- = RSSR' + SO_3^{--}$$

1-Anthraquinonesulphenyl sulphite on alkaline hydrolysis [17] yields stable 1-anthraquinonesulphenic acid:

$$RSSO_3^- + OH^- = RSOH + SO_3^{--}$$

Sulphenyl thiosulphonates were prepared by Brooker, Child and Smiles [8] by the action of the chlorides on aromatic potassium thiosulphonates:

$$RSCl + S_2O_2R^- = RSS_2O_2R + Cl^-$$

They react in two ways, *viz.*, as sulphenyl thiosulphonate, or as a mixed monosulphur mercaptide-sulphinate. Reaction products according to both modes of fission were encountered in reactions with sodium mercaptides and 2-naphtoxide. Fission exclusively as sulphenyl thiosulphonate takes place in the reactions of *o*-nitrobenzenesulphenyl *p*-toluenethiosulphonate with sulphinate ions [14]:

$$RSS_2O_2R' + R''SO_2^- = RSSO_2R'' + R'SO_2S^-$$

as also in displacement reactions demonstrated on pp. 315—321 of this paper. The pseudohalide nature of thiosulphonate ions, as well as other thio anions, has been discussed in a previous paper [18].

The present investigation was begun with the purpose of examining displacement reactions from RS^+, in order to obtain further evidence concerning base strength sequences of thio anions and anthio anions (see p. 321) found to hold in the case of displacement reactions from S^{++} and S_2^{++} [1, 2]. Some new types of sulphenyl compounds are described (chapter I). The base strength sequences and displacement reactions are considered in chapters II and III.

I. NEW TYPES OF SULPHENYL COMPOUNDS

The following new types of sulphenyl compounds are described in this chapter (Ar = 2-nitro-5-methylphenyl and, in some cases, *o*-nitrophenyl):

Sulphenyl di-O-alkylmonothiophosphates $ArSSPO(OR)_2$ (R = methyl, ethyl, *iso*-propyl).

Sulphenyl alkanethiothiosulphonates $ArSS_2O_2R$ (R = methyl, ethyl).

Solutions of sulphenyl thiosulphates $ArSS_2O_3^-$.

The only thio derivatives of RS^+ previously described are the sulphenyl thiocyanates of Lecher [19, 20] and the sulphenyl arylthiosulphonates of Brooker, Child and Smiles [8].

The sulphenyl di-O-alkylmonothiophosphates were prepared from the sulphenyl bromides and finely powdered sodium or potassium di-O-alkylmonothiophosphates (10—20 % excess) in ether or carbon disulphide suspension. The solid particles were treated with a glass rod for a few minutes, the suspension filtered, and the product isolated from the filtrate through evaporation or cooling.

The sodium and potassium di-O-alkylmonothiophosphates employed were prepared from the corresponding phosphites as described elsewhere [18].

The sulphenyl di-O-alkylmonothiophosphates form yellowish-green crystals, which are quite stable, and readily soluble in alcohols, benzene, and chloroform. The methyl compounds are slightly soluble in ether, more soluble in warm carbon disulphide. The ethyl and *iso*-propyl compounds are extensively soluble in both solvents. The compounds are insoluble in water, and are not affected by moisture. With alcoholic potassium hydroxide they slowly give a blue-violet colour, characteristic of alkaline solutions of sulphenic acids [7].

They react with secondary amines to give sulphenamides, as do sulphenyl halides and sulphenyl thiocyanates [7]:

$$RSSPO(OR)_2 + 2\ R_2NH = RSNR_2 + R_2NH_2^+ + SPO(OR)_2^-$$

Since in these reactions the amine undoubtedly is the anionoid reagent, the reactions prove the properties of the reacting compounds as derivatives of RS^+.

The sulphenyl alkanethiosulphonates were prepared from the sulphenyl bromide and sodium or potassium alkanethiosulphonates in carbon disulphide as described for the sulphenyl di-O-alkylmonothioposphates. They form stable, yellowish-green crystals, insoluble in water, practically insoluble in ether, soluble in ethanol, carbon disulphide, benzene, and chloroform.

2-Nitro-5-methylbenzenesulphenyl ethanethiosulphonate was found to react with piperidine:

$$RSS_2O_2C_2H_5 + 2\ C_5H_{10}NH = RSNC_5H_{10} + C_5H_{10}NH_2^+ + S_2O_2C_2H_5^-$$

Solutions of sulphenyl thiosulphates were obtained

(1) from 2-nitro-5-methylbenzenesulphenyl thiocyanate by reaction with thiosulphate ion:

$$RSSCN + S_2O_3^{--} = RSS_2O_3^- + SCN^-$$

(2) from sulphenamides by reaction with thiosulphuric acid:

$$RSNR_2 + S_2O_3^{--} + 2\ H^+ = RSS_2O_3^- + R_2NH_2^+$$

The reactions are rapid and quantitative, and may be employed for the iodometric analysis of sulphenyl thiocyanates and sulphenamides. The following procedures have proved their value:

(1) 0.0002 mole sulphenyl thiocyanate are dissolved by gentle heating in 2 ml ethylacetate, and 10 ml ethanol and 25 ml 0.01 *N* sodium thiosulphate are added. After standing for 5 minutes, 40 ml water, 10 ml 10 % acetic acid, and a few crystals of potassium iodide are added, and the excess of thiosulphate is back-titrated with 0.01 *N* iodine.

(2) 0.0002 mole sulphenamide are dissolved by gentle heating in 15 ml ethanol or *iso*-propanol, and 25 ml 0.01 *N* sodium thiosulphate (no turbidity should occur, or more alcohol must be added) and 5 ml 10 % acetic acid are added. After standing for 10 minutes, 40 ml water are added, and the excess of thiosulphate is backtitrated with 0.01 *N* iodine.

The sulphenyl thiosulphate $RSS_2O_3^-$ forms green solutions which seem to be quite stable. It is the structural analogon of monosulphur di-(thiosulphate) $S(S_2O_3)_2^{--}$ (pentathionate) and disulphur di-(thiosulphate) $S_2(S_2O_3)_2^{--}$ (hexathionate) [1, 2].

A method corresponding to (2) is available [2] for the iodometric analysis of sulphur piperidides, by use of the reactions (x = 1 or 2):

$$S_x(NC_5H_{10})_2 + 2\ S_2O_3^{--} + 4\ H^+ = S_x(S_2O_3)_2^{--} + 2\ C_5H_{10}NH_2^+$$

If sodium hydroxide be added to sulphenyl thiosulphate solutions, an intense blue colour is developed. The sulphenyl thiosulphate thus undergoes hydrolysis as follows:

$$RSS_2O_3^- + OH^- = RSOH + S_2O_3^{--}$$

The sulphenyl thiosulphates react rapidly with cyanide and sulphite ions:

$$RSS_2O_3^- + CN^- = RSCN + S_2O_3^{--}$$
$$RSS_2O_3^- + SO_3^{--} = RSSO_3^- + S_2O_3^{--}$$

The reactions may be employed for the iodometric analysis of sulphenyl thiosulphates (for procedures see p. 313 f.).

The reactions are polar displacements of thiosulphate ion by cyanide and sulphite ions, respectively, as are the cyanide and sulphite reactions of polythionates [1, 2].

Experimental

The melting points reported in this paper are in ° C (corr.).

Phosphorus analyses were made volumetrically by the method of Neumann (for literature references see Nylén [21]).

No compounds containing the 2-nitro-5-methylphenylsulphur group are hitherto described in literature. The sulphenyl bromide was obtained as follows:

3,4-Dinitrotoluene was converted into 2,2′-dinitro-5,5′-dimethyldiphenyldisulphide by the action of sodium disulphide. This method of preparing disulphides from aromatic dinitrohydrocarbons is due to Blanksma[22]. Displacement of the nitro group (as nitrite) by sulphide or other anionoid reagents takes place only when the nitro groups are in *ortho* or *para* positions [23]. In 3,4-dinitrotoluene (and 3,4-chlorobenzene) it is the 3-nitro group which undergoes displacement [24].

The structure of the disulphide was confirmed through convertion into 2-mercapto-6-methylbenzthiazol [25] by the method of Teppema and Sebrell [26] (see pp. 315—321).

The disulphide was treated with bromine in carbon tetrachloride to obtain the sulphenyl bromide.

2,2′-Dinitro-5,5′-dimethyldiphenyldisulphide. In ethanol at boiling temperature only poor yields were obtained. In methanol at room temperature, however, the yields are quantitative:

To 75 g 3,4-dinitrotoluene* dissolved in 500 ml methanol are added with stirring, during a period of one hour, 48 g sodium sulphide nonahydrate and 7 g sulphur dissolved in 25 ml water and 50 ml methanol. After one hour's standing the product is filtered off and washed with methanol and water. It may be recrystallized from glacial acetic acid or from benzene. Greenish-yellow crystals, m. p. 152°.

0.1447 g subst.: 0.2002 g $BaSO_4$.

$(O_2NC_7H_6S)_2$ (336,4) Calc. S 19.1 Found S 19.0

2-Nitro-5-methylbenzenesulphenyl bromide. 20 g disulphide (the crude product may be employed) in 100 ml carbon tetrachloride are heated with 5 ml bromine for 2 hours. The

* The author is indebted to Chem. Eng. Jørgine Stene Sørensen, *Institutt for Organisk Kjemi*, for a gift of 3,4-dinitrotoluene (isolated from a technical T. N. T. mother liquor).

excess bromine and the solvent are evaporated, and the product recrystallized from petroleum. Brownish-yellow crystals, m. p. 84°.

0.1347 g subst.: 0.1016 g AgBr.

$O_2NC_7H_6SBr$ (248.1) Calc. Br 32.2 Found Br 32.1

2-Nitro-5-methylbenzenesulphenamide and *piperidide* were prepared from the bromide, dissolved in ether, by the action of ammonia and piperidine, respectively. Recrystallized from methanol. Brownish-yellow crystals, m. p. 151° (amide) and 92° (piperidide).

0.1677 g subst.: 0.2130 g $BaSO_4$.

$O_2NC_7H_6SNH_2$ (184.2) Calc. S 17.4 Found S 17.5

0.1850 g subst.: 0.1705 g $BaSO_4$.

$O_2NC_7H_6SNC_5H_{10}$ (252.3) Calc. S 12.7 Found S 12.7

2-Nitro-5-methylbenzenesulphenyl di-O-methylmonothiophosphate. Coarse crystals, m. p. 93° (from carbon disulphide).

0.09344 g subst.: 0.1421 g $BaSO_4$. 0.09977 g subst.: 18.54 ml 0.4836 *N* NaOH.

$O_2NC_7H_6SSPO(OCH_3)_2$ (309.3) Calc. S 20.7 P 10.0

Found » 20.9 » 9.9

2-Nitro-5-methylbenzenesulphenyl di-O-ethylmonothiophosphate. Coarse crystals, m. p. 53° (from ether).

0.1169 g subst.: 0.1618 g $BaSO_4$. 0.1553 g subst.: 26.78 ml 0.4836 *N* NaOH.

$O_2NC_7H_6SSPO(OC_2H_5)_2$ (337.4) Calc. S 19.0 P 9.2

Found » 19.0 » 9.2

To 0.69 g dissolved in 20 ml ether were added 1 ml piperidine. The ether was evaporated off, and the crystalline residue treated with water. Yield 0.51 g (theor. 0.52 g 2-nitro-5-methylphenylsulphenpiperidide), m. p. (recrystallized from methanol) 92°, not depressed in mixture with a specimen obtained from the bromide (p. 311 f.).

*2-Nitro-5-methylbenzenesulphenyl di-O-*iso*-propylmonothiophosphate.* Coarse crystals, m. p. 44° (from ether or petroleum).

0.1398 g subst.: 0.1784 g $BaSO_4$. 0.1521 g subst.: 24.02 ml 0.4836 *N* NaOH.

$O_2NC_7H_6SSPO(OC_3H_7)_2$ (365.4) Calc. S 17.5 P 8.5

Found » 17.5 » 8.4

o-Nitrobenzenesulphenyl di-O-methylmonothiophosphate. Prepared from the bromide [27]. Prisms from carbon disulphide, m. p. 72°.

0.1083 g subst.: 0.1714 g $BaSO_4$. 0.1142 g subst.: 22.41 ml 0.4836 *N* NaOH.

$O_2NC_6H_4SSPO(OCH_3)_2$ (295.3) Calc. S 21.7 P 10.5

Found » 21.7 » 10.5

To 3 g dissolved in 20 ml methanol were added 5 ml 30 % aqueous dimethylamine. After standing for one hour the formed *o*-nitrobenzenesulphendimethylamide [27] was precipitated by means of water. Recrystallized from methanol, it had the correct melting point, *viz.*, 63°.

2-Nitro-5-methylbenzenesulphenyl methanethiosulphonate. Prisms, m. p. 98° (from carbon disulphide).

0.0527 g subst.: 0.1301 g $BaSO_4$.

$O_2NC_7H_6SS_2O_2CH_3$ (279.3) Calc. S 34.4 Found S 34.3

2-Nitro-5-methylbenzenesulphenyl ethanethiosulphonate. Small crystals, m. p. 91° (from carbon disulphide).

0.05776 g subst.: 0.1380 g $BaSO_4$.

$O_2NC_7H_6SS_2O_2C_2H_5$ (293.4) Calc. S 32.8 Found S 32.8

To 0.77 g dissolved in 25 ml chloroform were added 1 ml piperidine. After 10 minutes at room temperature the solvent and the excess of piperidine was evaporated off, and the crystalline residue treated with water. Yield 0.65 g (theor. 0.66 g 2-nitro-5-methylphenyl-sulphenpiperidide). Recrystallized from methanol, it melted at 92°, not depressed in mixture with a specimen obtained from the bromide (p. 311 f.).

2-Nitro-5-methylbenzenesulphenyl thiocyanate. Prepared from the chloride (obtained from the disulphide in carbon tetrachloride at 50—60° in presence of traces of iodine) by the method of Lecher [19]. When excess of potassium thiocyanate is employed (finely powdered, suspended in benzene) the reaction is complete after less than 5 minutes. Yellowish-green plates from benzene, m. p. 115°.

0.1027 g subst.: 0.2113 g $BaSO_4$.

$O_2NC_7H_6SSCN$ (226.3) Calc. S 28.3 Found S 28.3

Iodometric analysis of 2-nitro-5-methylbenzenesulphenyl thiocyanate (for procedure see p. 310).

Substance employed, g	Iodine consumed ml 0.01074 *N*	Substance found, g
None	22.78	
0.04439	4.54	0.04434
0.04421	4.62	0.04414
0.04503	4.22	0.04509

Iodometric analysis of sulphenamides (for procedure see p. 310). $2\text{-}O_2N\text{-}5\text{-}CH_3C_6H_3SNC_5H_{10}$ and $o\text{-}O_2NC_6H_4SN(CH_3)_2$ were dissolved in 15 ml *iso*-propanol, the more soluble $2\text{-}O_2N\text{-}5\text{-}CH_3C_6H_3SNH_2$ in 15 ml ethanol.

Compound	Substance employed, g	Iodine consumed ml 0.009993 *N*	Substance found, g
	None	23.96	
(1)	0.03683	4.00	0.03674
(1)	0.03617	4.33	0.03614
(1)	0.03704	3.92	0.03689
(2)	0.05183	3.49	0.05164
(3)	0.04946	4.36	0.04941
(4)	0.05126	3.65	0.05119
(5)	0.03872	5.12	0.03866

The employed compounds were the following:

(1) $2\text{-}O_2N\text{-}5\text{-}CH_3C_6H_3SNH_2$ from sulphenyl bromide

(2) $2\text{-}O_2N\text{-}5\text{-}CH_3C_6H_3SNC_5H_{10}$ from sulphenyl bromide

(3) —»— from sulphenyl di-O-ethylmonothiophosphate

(4) —»— from sulphenyl ethanethiosulphonate

(5) $o\text{-}O_2NC_6H_4SN(CH_3)_2$ from sulphenyl di-O-methylmonothiophosphate

Iodometric analysis of solutions of 2-nitro-5-methylbenzenesulphenyl thiosulphate.

1. 0.0008 mole 2-nitro-5-methylbenzenesulphenyl thiocyanate were dissolved by gentle heating in 10 ml ethylacetate in a 100 ml volumetric flask, and 20 ml ethanol and 10 ml 0.1 *N* sodium thiosulphate were added. After standing for 5 minutes the mixture was made up to 100 ml with water, and 3 times 25 ml were pipetted out. 40 ml water

were added to each sample. One of them (a) was titrated with 0.01 *N* iodine for excess thiosulphate (after addition of 10 ml 10 % acetic acid and a few crystals of potassium iodide). To the other two there were added (b) 5 ml 0.2 sodium sulphite, and (c) 3 ml 1 *M* potassium cyanide, respectively. After standing for 5 minutes there were added 1 ml 40 % formaldehyde, 10 ml 10 % acetic acid, and a few crystals of potassium iodide, and the solutions were titrated with 0.01 *N* iodine.

Substance employed, g	Iodine consumed ml 0.01074 *N*			Substance found calc. from (a), g
	(a)	(b)	(c)	
None	22.78			
0.1821	4.12	22.78	22.80	0.1814
0.1817	4.10	22.77	22.79	0.1816
0.1817	4.10	22.78	22.78	0.1816

2. 0.0008 mole 2-nitro-5-methylbenzenesulphenamide and piperidide were dissolved by gentle heating in 30 ml ethanol and 40 ml *iso*-propanol, respectively, in 100 ml volumetric flasks, and 10 ml 0.1 *N* sodium thiosulphate and then 10 ml 10 % acetic acid were added. After standing for 5 minutes the mixture was made up to 100 ml with water, and 4 times 20 ml were pipetted out. 40 ml water were added to each sample. One of them (a) was titrated with 0.01 *N* iodine for excess thiosulphate. To the other three were added: (b) Amide. 5 ml 0.2 *M* sodium sulphite and 5 ml 1 *N* sodium hydroxide. (c) Piperidide. 3 ml 1 *M* potassium cyanide and 5 ml 1 *N* sodium hydroxide. After standing, there were added 1 ml 40 % formaldehyde and 10 ml 10 % acetic acid (in the case of (c) also a few crystals of potassium iodide) and the solutions were titrated with 0.01 *N* iodine.

Compound	Substance employed, g	Time of standing (minutes)	Iodine consumed ml 0.009993 *N* (a)	(b)	(c)	Substance found calc. from (a), g
	None		17.88			
Amide	0.1465		2.03			0.1459
		5		17.88		
		10		17.88		
		15		17.89		
Piperidide	0.2036		1.74			0.2034
		5			17.89	
		7			17.90	
		11			17.84	

On addition of potassium cyanide to the 2-nitro-5-methylbenzenesulphenyl thiosulphate solutions the green colour rapidly faded, and crystals separated out. After the titrations the crystals were filtered off and identified as 2-nitro-5-methylphenyl thiocyanate, m. p. (recrystallized from glacial acetic acid) 135°, not depressed in mixture with a specimen obtained from the sulphenyl thiocyanate (p. 324).

II. OXIDABILITY AND BASE STRENGTH (ANIONOID REACTIVITY) OF THIO ANIONS. DISPLACEMENT REACTIONS

In previous papers [1, 2] polar (electron-sharing) displacement reactions from S^{++} and S_2^{++} were demonstrated, of the type:

$$S(SX')_2 + 2\ SX''^- = S(SX'')_2 + 2\ SX'^-$$
$$S_2(SX')_2 + 2\ SX''^- = S\ (SX'')_2 + 2\ SX'^-$$

The base strengths (anionoid reactivities) of the thio anions SX^- towards S^{++} and S_2^{++} were found to increase with decreasing redox potentials of the systems:

$$2\ SX^- \rightleftarrows (SX_2) + 2\ e$$

viz., in the order thiocyanate, di-O-methylmonothiophosphate, ethanethiosulphonate, *p*-toluenethiosulphonate, thiosulphate, and thiocarbonyl anions and mercaptides. A stronger one of these bases will displace a weaker one from its combinations with S^{++} and S_2^{++}.

Since that time the potential series has been extended.

From measurements of equilibria with iodine, the redox potentials of the systems:

$$2\ (RO)_2OPS^- \rightleftarrows ((RO)_2OPS)_2 + 2\ e$$

have been evaluated [18] for 9 different alkyl groups R.

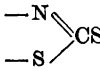
The equilibria of thiosulphonate ions with iodine [1, 2] have been found (unpublished work by the author) to obey the equation:

$$2\ RSO_2S^- + I_3^- \rightleftarrows (RSO_2S)_2 + 3\ I^-$$

with equilibrium constants (at room temperature) = 1 for ethanethiosulphonate and 50—60 for *p*-toluenethiosulphonate. The redox potentials of the systems:

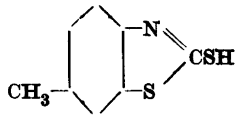
$$2\ RSO_2S^- \rightleftarrows (RSO_2S)_2 + 2\ e$$

calculated as in the case of the di-O-alkylmonothiophosphate systems [18], are —0.54 volts and —0.49 volts, respectively.

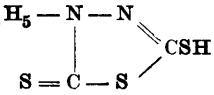
Three mercaptides containing the grouping 

have been examined (see the experimental part of this chapter), *viz.*, the anions of:

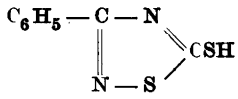
2-mercapto-6-methylbenzthiazol



2-mercapto-4-phenyl-5-thion-1-thia-3,4-diazol



2-mercapto-4-phenyl-1-thia-3,5-diazol



They are all oxidized by tetrathionate to the corresponding disulphides, as are other mercaptides [11], and xanthates [28], dithiocarbamates [29], and monothio [30] and dithio [28] carboxylates. The redox potentials of thiocarbonyl anion and mercaptide systems may be roughly estimated to ca + 0.3 volts (see, *e. g.*, the discussion by Remick [31] of the potential of the cysteine-cystine electrode).

In table I the listed values for the potentials of the thiocyanate-thiocyanogen electrode and the thiosulphate-tetrathionate electrode are those of Bjerrum and Kirschner [32] and of Zimmermann and Latimer [33], respectively.

Table 1. Redox potentials E_0 *(in volts) of systems* $2\ SX^- \rightleftarrows (SX)_2 + 2\ e$ *(at unit activity, against the hydrogen electrode).*

SX^-	E_0
SCN^-	— 0.77
$(CH_3O)_2OPS^-$	— 0.56
$C_2H_5SO_2S^-$	— 0.54
$(C_2H_5O)_2OPS^-$	— 0.53
$(n\text{-}C_3H_7O)_2OPS^-$	— 0.52
$(n\text{-}C_4H_9O)_2OPS^-$, $(iso\text{-}C_4H_9O)_2OPS^-$	— 0.51
$(iso\text{-}C_3H_7O)_2OPS^-$	— 0.50
$p\text{-}CH_3C_6H_4SO_2S^-$, $(sec\text{-}C_4H_9O)_2OPS^-$	— 0.49
$(iso\text{-}C_5H_{11}O)_2OPS^-$, $(C_2H_5(CH_3)CHCH_2O)_2OPS^-$	— 0.48
$S_2O_3^{--}$	— 0.10
RS^-, $-N\!=\!CS^-$ (−S), $ROC(S)S^-$, $R_2NC(S)S^-$, $RC(O)S^-$, $RC(S)S^-$	ca + 0.3

The base strengths (anionoid reactivities) towards RS^+ of the thio anions of table I increase with decreasing redox potentials, as will appear from the following series of displacement reactions, of the type:

$$RSSX' + SX''^- = RSSX'' + SX'^-$$

2-Nitro-5-methylbenzenesulphenyl thiocyanate reacts with di-O-ethylmonothiophosphate ion, ethanethiosulphonate ion, *p*-toluenethiosulphonate ion, and thiosulphate ion.

2-Nitro-5-methylbenzenesulphenyl di-O-methylmonothiophosphate reacts with di-O-*iso*-propylmonothiophosphate ion.

o-*Nitrobenzenesulphenyl di-O-methylmonothiophosphate* reacts with *p*-toluenethiosulphonate ion and thiosulphate ion and with the mercaptides  .

2-Nitro-5-methylbenzenesulphenyl ethanethiosulphonate reacts with *p*-toluenethiosulphonate ion and thiosulphate ion.

2-Nitro-5-methylbenzenesulphenyl di-O-ethylmonothiophosphate and *di-O*-iso-*propylmonothiophosphate* react with thiosulphate ion and ethylxanthate ion.

o-*Nitrobenzenesulphenyl* p-*toluenethiosulphonate* reacts with thiosulphate ion.

The parallelism between oxidability and base strength is logical from an electronic point of view. For the potentials the governing factor is the electron affinity of the thio sulphur atom of the concerned groups, whilst in the polar displacement reactions the driving force is the base strength of the thio sulphur atom of the anions, the reactive entity being a pair of electrons. The smaller electron affinity, the higher polarizability of the valence electrons and the greater portion of each unoccupied electron pair available for bond formation with kationoid centres.

Notice may be made to the statement of Branch and Calvin [34], on the basis of the transition state theory of reaction mechanisms, that in polar displacement reactions an increase in the number of electron shells (*i. e.*, increase in polarizability of the valence electrons) of either the reactant or the product base increases the rate.

EXPERIMENTAL

Reactions of mercaptides with tetrathionate

2-mercapto-6-methylbenzthiazol was prepared from 2,2'-dinitro-5,5'-dimethyldiphenyldisulphide (p. 311) in aqueous sodium disulphide suspension by treatment with hydrogen sulphide and carbon disulphide gas according to the method of Teppema and

Sebrell [26]. Recrystallized from glacial acetic acid, it showed the correct melting point [25], *viz.*, 181°. Its alkali salts may be titrated accurately with iodine (oxidation to disulphide):

0.4677 g 2-mercapto-6-methylbenzthiazol were dissolved in 10 ml ethanol and 5 ml 1 *N* sodium hydroxide. 200 ml water were added, and dilute acetic acid until colourless to phenolphtalein. The volume was adjusted to 250 ml, and 25 ml samples pipetted out and titrated with 0.01083 *N* iodine: 23.82 ml — 23.79 ml — 23.82 ml (theor. 23.82 ml).

Its potassium salt (I) was prepared from the mercaptan and potassium carbonate, by evaporation to dryness, and recrystallization from acetone-ether. 0.05987 g, dissolved in 25 ml water, consumed 25.16 ml 0.01083 *N* iodine (theor. 25.20 ml).

Potassium 2-mercaptido-4-phenyl-5-thion-1-thia-3.4-diazol (II) and 2-mercaptido-4-phenyl-1-thia-3.5-diazol (III) were prepared by the methods of Busch [35] and Schubart [36], respectively. Their aqueous solutions may be titrated accurately with iodine (oxidation to disulphide):

0.7183 g of (II) and 0.6339 g of (III) were each dissolved in 250 ml water, and 25 ml samples pipetted out and titrated with 0.01083 *N* iodine. (II) 25.11 ml — 25.09 ml — 25.10 ml (theor. 25.09 ml). (III) 25.14 ml — 25.18 ml — 25.17 ml (theor. 25.19 ml).

0.002 mole (0.60 g) potassium tetrathionate, dissolved in 20 ml water, were added to 0.005 mole of (I) (1.10 g), (II) (1.32 g), and (III) (1.16 g), respectively, dissolved in 20 ml water. Turbidity at once occurred. After 20 minutes' stirring the disulphides had flocculated, leaving the liquid clear, except for (II), in the case of which stirring was continued for 2 hours. The crystals were filtered off, dried, and weighed.

(I) 0.70 g (theor. 0.72 g). Colourless plates from chloroform, m. p. 202° (reported [25] 201—202°).

0.06914 g subst.: 0.1782 g $BaSO_4$. Calc. S 35.6. Found S 35.4.

(II) 0.82 g (theor. 0.90 g). Greenish-yellow plates from chloroform-ethanol, m. p. 124° (reported [35] 124—125°).

0.07129 g subst.: 0.2199 g $BaSO_4$. Calc. S 42.7. Found S 42.4.

(III) 0.75 g (theor. 0.77 g). Colourless needles from glacial acetic acid, m. p. 125° (reported [37] 121°).

0.06166 g subst.: 0.1495 g $BaSO_4$. Calc. S 33.2. Found S 33.3.

Displacement reactions

(1) To 1.13 g 2-nitro-5-methylbenzenesulphenyl thiocyanate, dissolved in 15 ml benzene, were added 1.2 g $C_2H_5SO_2SK$ (50 % excess), dissolved in 4 ml ethanol. After 5 minutes at room temperature the mixture was treated with water, the benzene layer separated, dried over anhydrous sodium sulphate, and the benzene evaported *in vacuo*. The crystalline residue was recrystallized from carbon disulphide, yield 0.94 g yellowish-green crystals, with the melting point of 2-nitro-5-methylbenzenesulphenyl ethanethiosulphonate, *viz.*, 91°, not depressed in mixture with a specimen obtained from the bromide (p. 000).

(2) To 1.13 g 2-nitro-5-methylbenzenesulphenyl thiocyanate, dissolved in 15 ml benzene, were added 1.6 g $(C_2H_5O)_2OPSK$ (50 % excess), dissolved in 4 ml ethanol. After 5 minutes was proceeded as in the case of (1). The residue was recrystallized from ether, yield 1.01 g yellowish-green crystals, with the melting point of 2-nitro-5-methylbenzenesulphenyl di-O-ethylmonothiophosphate, *viz.*, 53°, not depressed in mixture with a specimen obtained from the bromide (p. 311 f.).

(3) To 1.13 g 2-nitro-5-methylbenzenesulphenyl thiocyanate, dissolved in 20 ml ethylacetate and 20 ml ethanol, at 40—50°, were added 1.8 g p-$CH_3C_6H_4SO_2SK \cdot H_2O$ (50 % excess), dissolved in 40 ml water. On cooling, crystals began to separate. After 10 minutes they were filtered off (yield 1.65 g), and recrystallized from benzene. Yield 1.34 g yellowish-green crystals, m. p. 144°, not depressed in mixture with a specimen of 2-nitro-5-methylbenzenesulphenyl p-toluenethiosulphonate prepared from 2-nitro-5-methylbenzenesulphenyl bromide and potassium p-toluenethiosulphonate suspended in ether: Small yellowish-green crystals (from benzene), m. p. 144°.

0.08401 g subst.: 0.1650 g $BaSO_4$.

$O_2NC_7H_6SS_2O_2C_7H_7$ (355.4) Calc. S 27.1 Found S 27.0

(4) To 1.00 g 2-nitro-5-methylbenzenesulphenyl ethanethiosulphonate, dissolved in 25 ml ethanol, were added 1.7 g p-$CH_3C_6H_4SO_2SK \cdot H_2O$ (100 % excess), dissolved in 20 ml methanol, and the mixture was heated for 2 minutes. On cooling, crystals separated out. Yield 1.07 g crude product, m. p. (recrystallized from benzene, yield 0.86 g) 144°, not depressed in mixture with a specimen of 2-nitro-5-methylbenzenesulphenyl p-toluenethiosulphonate obtained from the bromide.

(5) To 1.54 g 2-nitro-5-methylbenzenesulphenyl di-O-methylmonothiophosphate, dissolved in 50 ml carbon disulphide, were added 1.65 g (iso-$C_3H_7O)_2OPSNa$ (50 % excess), dissolved in 50 ml carbon disulphide. A colourless salt at once separated out. After 5 minutes was proceeded as in the case of (1). The crystalline residue was recrystallized from petroleum, yield 1.72 g yellowish-green crystals, with the melting point of 2-nitro-5-methylbenzenesulphenyl di-O-iso-propylmonothiophosphate, *viz.*, 44°, not depressed in mixture with a specimen obtained from the bromide.

(6) To 1.55 g o-nitrobenzenesulphenyl di-O-methylmonothiophosphate, dissolved in 20 ml ethanol, were added 1.5 g p-$CH_3C_6H_4SO_2SK \cdot H_2O$ (20 % excess), dissolved in 20 ml water. A crystalline solid at once separated. Yield 1.74 g (theor. 1.73 g o-nitrobenzenesulphenyl p-toluenethiosulphonate), m. p. (recrystallized from glacial acetic acid) 141° (reported [8] 141°).

(7) 1 g 2-nitro-5-methylbenzenesulphenyl di-O-ethylmonothiophosphate, dissolved in 20 ml ether, was treated for 5 minutes with 2 g finely powdered potassium ethylxanthate. The suspension was then filtered and the ether partly evaporated off. On cooling (ice-sodium chloride), crystallization took place. Yield 0.78 g (theor. 0.82 g 2-nitro-5-methylphenyl-ethylxanthyldisulphide). Coarse green crystals, m. p. 69°, not depressed in mixture with a specimen prepared from 2-nitro 5-methylbenzenesulphenyl bromide and potassium ethylxanthate, as above: M. p. 69° (from ether).

0.1391 g subst.: 0.3379 g $BaSO_4$.

$O_2NC_7H_6SSC(S)OC_2H_5$ (289.4) Calc. S 33.2 Found S 33.4

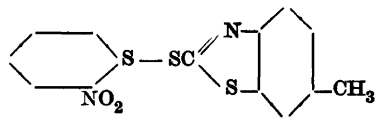
(8) 0.70 g 2-nitro-5-methylbenzenesulphenyl di-O-iso-propylmonothiophosphate, dissolved in 20 ml ether, were treated for 5 minutes with 1 g finely powdered potassium ethylxanthate. Water was added, the ethereal layer separated, dried over anhydrous sodium sulphate, and the ether partly evaporated off: Coarse green crystals, with the melting point of 2-nitro-5-methylphenyl-ethylxanthyldisulphide, *viz.*, 69°, not depressed in mixture with a specimen obtained from the bromide.

(9) To 1.5 g o-nitrobenzenesulphenyl di-O-methylmonothiophosphate, dissolved in 20 ml ethanol, were added 20 ml water containing

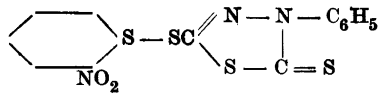
(a) 1.5 g potassium 2-mercaptido-6-methylbenzthiazol

(b) 1.5 g potassium 2-mercaptido-4-phenyl-5-thion-1-thia-3,4-diazol

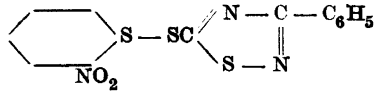
(c) 1.5 g potassium 2-mercaptido-4-phenyl-1-thia-3,5 diazol. Crystalline products at once separated out, and were further precipitated by addition of water. They were recrystallized from glacial acetic acid, from which they crystallize excellently.

(a)  Pale yellow, fatty leaves (thin plates), m. p. 147°.

0.08277 g subst.: 0.1729 g $BaSO_4$. Calc. S 28.8. Found S 28.7.

(b)  Pale yellow needles, m. p. 112°.

0.08433 g subst.: 0.2077 g $BaSO_4$. Calc. S 33.8. Found S 33.8.

(c)  Pale green, flat prisms, m. p. 121°.

0.08005 g subst.: 0.1618 g $BaSO_4$. Calc. S 27.7. Found S 27.8.

Aryl-benzthiazyldisulphides, prepared from sulphenyl chlorides and bromides, and mercaptobenzthiazols, are mentioned in patents [38], [39].

(10) 10 ml 0.1 *N* sodium thiosulphate were added to

(a) 0.283 g 2-nitro-5-methylbenzenesulphenyl ethanethiosulphonate (0.00096 mole) in 20 ml ethanol

(b) 0.287 g 2-nitro-5-methylbenzenesulphenyl di-O-ethylmonothiophosphate (0.00085 mole) in 10 ml ethanol

(c) 0.301 g 2-nitro-5-methylbencenesulphenyl di-O-*iso*-propylmonothiophosphate (0.00082 mole) in 10 ml ethanol

(d) 0.335 g *o*-nitrobenzenesulphenyl *p*-toluenethiosulphonate (0.00098 mole) in 50 ml ethanol

(e) 0.268 g *o*-nitrobenzenesulphenyl di-O-methylmonothiophosphate (0.00091 mole) in 10 ml ethanol.

The colour of the solutions rapidly changed to pure green. After 5 minutes the solutions were diluted to 100 ml. 10 ml were pipetted out, and 60 ml water, 1 g potassium iodide, and 10 ml 10 per cent acetic acid were added, and the solutions titrated with 0.01 *N* iodine for excess thiosulphate: (a) 0.6 ml (b) 1.8 ml (c) 2.1 ml (d) 0.6 ml (e) 1.0 ml. The values do not give the exact amounts of thiosulphate present, since thiosulphonate and di-O-alkylmonothiophosphate ions are attacked to a slight degree by iodine by the simultaneous oxidation of thiosulphate by iodine [3], even in the presence of much potassium iodide.

The experiments indicate that the reactions of thiosulphate ion with the above sulphenyl compounds are rapid and quantitative, as in the case of 2-nitro-5-methylbenzenesulphenyl thiocyanate.

To the remaining 90 ml were added 3 ml 1 *M* potassium cyanide. The green colour rapidly faded, and volumineous crystals separated out. After 10 minutes they were filtered off, and dried.

(a), (b), and (c): 0.14 g, 0.14 g, and 0.12 g, respectively. Pale yellow crystals (from glacial acetic acid), with the melting point of 2-nitro-5-methylphenyl thiocyanate, *viz.*, 135°.

(d) and (e): 0.12 g and 0.13 g, respectively. Pale yellow crystals (from glacial acetic acid), with the melting point of *o*-nitrophenyl thiocyanate, *viz.*, 130° [27].

III. DISPLACEMENT REACTIONS INVOLVING ANTHIO ANIONS

The term anthio anion was introduced by the author [2] as characteristic for anions capable of adding sulphur to give thio anions, *viz.*, sulphinate, sulphite, cyanide, and dialkylphosphite ions.

Displacement reactions from kationoid sulphur, involving thio anions and anthio anions as anionoid components, may be arranged into three classes (for an earlier discussion of the subject see the paper [2] mentioned above):

I. Displacements of thio anions by stronger basic thio anions.

II. Displacements of thio anions by anthio anions.

III. Displacements of anthio anions by stronger basic anthio anions.

The respective classes will be considered in detail on the following pages.

Class I. Hereto belong the reactions (displacements from RS^+) of the foregoing chapter, and the reactions [1,2] (displacements from S^{++} and S_2^{++}):

$$S(SCN)_2 + 2\ S_2O_2C_6H_4CH_3\text{-}p^- = S(S_2O_2C_6H_4CH_3\text{-}p)_2 + 2\ SCN^-$$
$$S_2(SCN)_2 + 2\ S_2O_2C_6H_4CH_3\text{-}p^- = S_2(S_2O_2C_6H_4CH_3\text{-}p)_2 + 2\ SCN^-$$
$$S(SPO(OCH_3)_2)_2 + 2\ S_2O_3^{--} = S(S_2O_3)_2^{--} + 2\ SPO(OCH_3)_2^-$$
$$S_2(SPO(OCH_3)_2)_2 + 2\ S_2O_3^{--} = S_2(S_2O_3)_2^{--} + 2\ SPO(OCH_3)_2^-$$
$$S(S_2O_3)_2^{--} + 2\ S_2COC_2H_5^- = S(S_2COC_2H_5 + 2\ S_2O_3^{--}$$
$$S_2(S_2O_3)_2^{--} + 2\ S_2COC_2H_5^- = S_2(S_2COC_2H_5)_2 + 2\ S_2O_3^{--}$$
$$S(S_2O_3)_2^{--} + 2\ S(O)CC_6H_5^- = S(S(O)CC_6H_5)_2 + 2\ S_2O_3^{--}$$
$$S_2(S_2O_3)_2^{--} + 2\ S(O)CC_6H_5^- = S_2(S(O)CC_6H_5)_2 + 2\ S_2O_3^{--}$$

Class II. If the displacing anthio anion is derived just from the thio anion which it displaces, the net reaction apparently (not actually) is a transfer of sulphur to the anthio anion, as, *e. g.*, in the reactions:

$$RSS_2O_3^- + \mathbf{SO_3^{--}} = RS\mathbf{SO_3^-} + S_2O_3^{--}$$
$$RSSCN + \mathbf{CN^-} = RS\mathbf{CN} + SCN^-$$

Examples of this class of reactions are:

(a) The reactions [2] of tetrathionate (and pentathionate and hexathionate) with cyanide (first step), sulphite, ethanesulphinate, and *p*-toluenesulphinate ions:

$$(S_2O_3)_2^{--} + CN^- = O_3SSCN^- + S_2O_3^{--}$$
$$(S_2O_3)_2^{--} + SO_3^{--} = O_3S_2SO_3^{--} + S_2O_3^{--}$$
$$(S_2O_3)_2^{--} + {}^{1}SO_2R^- = O_3S_2SO_2R^- + S_2O_3^{--}$$

(b) The reactions [14] of *o*-nitrobenzenesulphenyl *p*-toluenethiosulphonate with *p*-toluenesulphinate and *p*-bromobenzenesulphinate ions:

$$RSS_2O_2R' + SO_2R''^- = RSSO_2R'' + S_2O_2R'^-$$

(c) The reactions [40, 41], of cystine and glutatione with cyanide and sulphite ions:

$$(RS)_2 + CN^- = RSCN + SR^-$$
$$(RS)_2 + SO_3^{--} = RSSO_3^- + SR^-$$

It should be noted that mercaptides are not always displaced by anthio anions. Thus the following reactions go (R and R′ = aryl or alkyl [9, 10, 11]):

$$RSSO_3^- + SR'^- = RSSR' + SO_3^{--}$$
$$RSSO_2R + SR'^- = RSSR' + SO_2R^-$$

(d) The reactions [2, 30, 42, 43], of xanthyldisulphides and thiocarbamyldisulphides with cyanide ion (first step):

$$(ROC(S)S)_2 + CN^- = ROC(S)SCN + ROC(S)S^-$$
$$(R_2NC(S)S)_2 + CN^- = R_2NC(S)SCN + R_2NC(S)S^-$$

(e) The reactions of 2-nitro-5-methylbenzenesulphenyl thiosulphate with cyanide and sulphite ions:

$$RSS_2O_3^- + CN^- = RSCN + S_2O_3^{--}$$
$$RSS_2O_3^- + SO_3^{--} = RSSO_3^- + S_2O_3^{--}$$

(f) The following reactions, demonstrated in the experimental part of this chapter:

Of 2-nitro-5-methylbenzenesulphenyl thiocyanate and di-O-methyl-monothiophosphate with cyanide and sulphite ions:

$$RSSCN + CN^- = RSCN + SCN^-$$
$$RSSCN + SO_3^{--} = RSSO_3^- + SCN^-$$
$$RSSPO(OCH_3)_2 + CN^- = RSCN + SPO(OCH_3)_2^-$$
$$RSSPO(OCH_3)_2 + SO_3^{--} = RSSO_3^- + SPO(OCH_3)_2^-$$

Of 2-nitro-5-methylbenzenesulphenyl thiocyanate, di-O-methylmonothiophosphate, and methanethiosulphonate with sulphinate ions:

$$RSSCN + SO_2C_6H_4CH_3\text{-}p^- = RSSO_2C_6H_4CH_3\text{-}p + SCN^-$$
$$RSSPO(OCH_3)_2 + SO_2C_6H_4Br\text{-}p^- = RSSO_2C_6H_4Br\text{-}p + SPO(OCH_3)_2^-$$
$$RSS_2O_2CH_3 + SO_2C_6H_4CH_3\text{-}p^- = RSSO_2C_6H_4CH_3\text{-}p + S_2O_2CH_3^-$$

Class III. Reactions of this type are:
(a) The reactions of thiosulphate ion [44, 45] and aromatic thiosulphonate ions [46] with cyanide ion:

$$SSO_3^{--} + CN^- = SCN^- + SO_3^{--}$$
$$SSO_2R^- + CN^- = SCN^- + SO_2R^-$$

(b) The reactions [2] of ethanethiosulphonate and *p*-toluenethiosulphonate ions with sulphite ion:

$$SSO_2R^- + SO_3^{--} = SSO_3^{--} + SO_2R^-$$

p-Toluenethiosulphonate reacts more rapidly than does ethanethiosulphonate.
(c) The reactions [11] of sulphenyl suphites and sulphinates with cyanide ion:

$$RSSO_3^- + CN^- = RSCN + SO_3^{--}$$
$$RSSO_2R + CN^- = RSCN + SO_2R^-$$

(d) The reaction [2] of trithionate with cyanide ion (first step):

$$O_3S_2SO_3^{--} + CN^- = O_3SSCN^- + SO_3^{--}$$

(e) The reaction [47] of cystine disulphoxide with sulphite ion:

$$RSSO_2R + SO_3^{--} = RSSO_3^- + SO_2R^-$$

and the analogous reaction (see the experimental part of this chapter) of 2-nitro-5-methylbenzenesulphenyl *p*-toluenesulphinate with sulphite ion.
(f) The equilibria of sulphenyl sulphinates with sulphinate ions [14, 15]:

$$RSSO_2R' + SO_2R''^- \rightleftarrows RSSO_2R'' + SO_2R'^-$$

According to Gibson and Loudon [15] the equilibria are progressively displaced to the right in the order R″ = 2,5-dichlorophenyl, *o*-tolyl, *o*-nitrophenyl,

m-nitrophenyl, *p*-chlorophenyl, *p*-bromophenyl, β-naphtyl, α-naphtyl, *p*-fluorophenyl, 4-methoxy-*m*-tolyl, phenyl, *p*-acetamido, *p*-tolyl, ethyl, and *n*-pentyl.

It appears that the anthio anions may be arranged into the following sequence of increasing base strength towards kationoid sulphur: Aromatic sulphinates, aliphatic sulphinates, sulphite, and cyanide.

From the great affinity of dialkylphosphites to sulphur [18] their base strength should be expected to be of the same order of magnitude, or even greater, than that of cyanide.

It is interesting to note that the base strengths of sulphinate ions towards I^+, as expressed through the relative positions of the equilibria:

$$RSO_2^- + I_3^- \rightleftarrows RSO_2I + 2\ I^-$$

follow the same sequence [48] as that found by Gibson and Loudon [15] in the quoted case of sulphenyl sulphinate equilibria.

Experimental

(1) To 1.13 g 2-nitro-5-methylbenzenesulphenyl thiocyanate, dissolved in 10 ml ethylacetate and 20 ml ethanol, were added 100 ml 1 *M* potassium cyanide. Crystals immediately began to separate. 10 ml water were added and the crystals filtered off and dried: 0.80 g (theor. 0.97 g 2-nitro-5-methylphenyl thiocyanate). M. p. (recrystallized from glacial acetic acid) 135°. Greenish-yellow prisms.

0.1362 g subst.: 0.1649 g $BaSO_4$.

$O_2NC_7H_6SCN$ (194.2) Calc. S 16.5 Found S 16.6

(2) 0.75 g 2-nitro-5-methylbenzenesulphenyl di-O-methylmonothiophosphate, dissolved in 10 ml ethanol, 5 ml 1 *M* potassium cyanide. The reaction took place and the product was isolated as in the case of (1). Yield 0.45 g (theor. 0.47 g) crude product, m. p. (recrystallized from glacial acetic acid) 135°, not depressed in mixture with a specimen of 2-nitro-5-methylphenyl thiocyanate obtained in exp. (1).

(3) To 1.6 g 2-nitro-5-methylbenzenesulphenyl thiocyanate, dissolved in 20 ml ethylacetate and 20 ml ethanol, at 50—60°, were added 2 g p-$CH_3C_6H_4SO_2Na \cdot H_2O$, dissolved in 20 ml water. On cooling, crystallization took place. After 20 minutes, 200 ml water were added, and the product filtered off and recrystallized from methanol. Yield 1.7 g yellowish-green crystals, m. p. 104°.

0.1496 g subst.: 0.2161 g $BaSO_4$.

$O_2NC_7H_6SSO_2C_7H_7$ (323.4) Calc. S 19.8 Found S 19.8

(4) 0.70 g 2-nitro-5-methylbenzenesulphenyl methanethiosulphonate, in 20 ml ethanol, 1 g p-$CH_3C_6H_4SO_2Na \cdot 2H_2O$, in 10 ml water. On addition of water (after 10 minutes) a solid separated, which, recrystallized from methanol, had the melting point of 2-nitro-5-methylbenzenesulphenyl *p*-toluenesulphinate, *viz.*, 104°, not depressed in mixture with a specimen obtained in exp. (3).

(5) To 1.6 g *o*-nitrobenzenesulphenyl di-O-methylmonothiophosphate, in 20 ml ethanol, were added 1.5 g p-$BrC_6H_4SO_2Na \cdot 2H_2O$, in 20 ml water. Crystals rapidly separated out. 200 ml water were added, and the product filtered off, and dried: 1.9 g (theor. 2.0 g *o*-nitrobenzenesulphenyl *p*-bromobenzenesulphinate). M. p. (recrystallized from ethanol) 139° (reported [14] 137°).

0.1195 g subst.: 0.1494 g $BaSO_4$.

$O_2NC_6H_4SSO_2C_6H_4Br$ (374.2) Calc. S 17.1 Found S 17.2

(6) 10 ml 0.1 *M* sodium sulphite (in freshly boiled water containing 5 % ethanol as an antioxidant) were added to

(a) 0.181 g 2-nitro-5-methylbenzenesulphenyl thiocyanate (0.00080 mole) in 10 ml ethylacetate and 20 ml ethanol

(b) 0.247 g 2-nitro-5-methylbenzenesulphenyl di-O-methylmonothiophosphate (0.00080 mole) in 10 ml ethanol

(c) 0.266 g 2-nitro-5-methylbenzenesulphenyl *p*-toluenesulphinate (0.00082 mole) in 20 ml ethanol (at 40—50°).

After one hour the green solutions were diluted to 100 ml with freshly boiled water (they thereby remained clear), and 10 ml were pipetted out. 60 ml freshly boiled water, 1 g potassium iodide, and 5 ml 10 per cent acetic acid were added, and the solutions were titrated with 0.01 *N* iodine for excess sulphite: (a) 6.0 ml (b) 5.4 ml (c) 4.9 ml. Blind run (treated as above): 20.4 ml.

To the remaining 90 ml were added 5 ml 1 *M* potassium cyanide. After standing for 3 hours, water was added, and the formed crystals filtered off, and dried: (a) 0.12 g (b) 0.12 g (c) 0.14 g. Recrystallized from glacial acetic acid, they had the melting point of 2-nitro-5-methylphenyl thiocyanate, *viz.*, 135°, not depressed in mixture with a specimen obtained in exp. (1).

SUMMARY

Some new types of sulphenyl compounds are described, *viz.*, sulphenyl di-O-alkylmonothiophosphates, sulphenyl alkanethiosulphonates, and sulphenyl thiosulphates (in solutions).

Compounds containing the 2-nitro-5-methylphenylsuphur group are prepared for the first time.

Methods are devised for the iodometric analysis of sulphenyl thiocyanates, sulphenamides, and sulphenyl thiosulphates.

Displacement reactions are demonstrated, of the type:

$$RSSX' + SX''^- = RSSX'' + SX'^-$$

It is shown that the base strengths (anionoid reactivities) towards RS^+ of thio anions SX^- increase with decreasing redox potentials of the systems:

$$2\ SX^- \rightleftarrows (SX)_2 + 2\ e$$

as do their base strengths towards S^{++} and S_2^{++}.

The different types of displacement reactions from kationoid sulphur are discussed, and some new reactions of sulphenyl compounds are demonstrated.

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The Diffusion Constant of Danish Penicillin and its Application to the Determination of the Molecular Weight

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Frieden [1] has determined the diffusion constant of American penicillin and found the value $D = 0.176$ at a temperature of 0.5° C. From this value the molecular weight was calculated, applying Einstein-Stokes' equation for the relation between D and the radius of the molecules. The value obtained was $M = 490$.

It has been the aim of the present work to determine the diffusion constant of Danish penicillin and its approximate molecular weight. These investigations can be carried out, although the substance is not so pure as it should be for the generally used methods of determination of the molecular weight.

EXPERIMENTAL

Fig. 1 shows the apparatus used. Concerning the principle of the apparatus and its application in practice the reader may be referred to Brodersen and Klenow [2].

In order to eliminate the disturbing influence of the electric charge of the penicillin ions, about 1 M KCl was used as a solvent. The lower liquid consisted of a Leo penicillin solution to which a corresponding quantity of KCl was added. The liquid was diluted to a concentration of 5 000—10 000 units per ml, and the molarity was 1 M with regard to KCl.

At the end of the diffusion period the diffusion liquid was displaced by mercury and collected from the outlet in fractions of about 1.5 g.

After several preliminary experiments the necessary period of diffusion was found to be about 72 hours, depending *i. a.* upon the strength of the penicillin solution and the dimensions of the apparatus.

The relative activity of the penicillin solutions was determined by testing their inhibition of growth of staphylococcus aureus seeded on agar bouillon plates. This method, the so-called cup method, has been fully described by Jensen, Møller, and Overgaard [3], and it has been further investigated by Vesterdal [4].

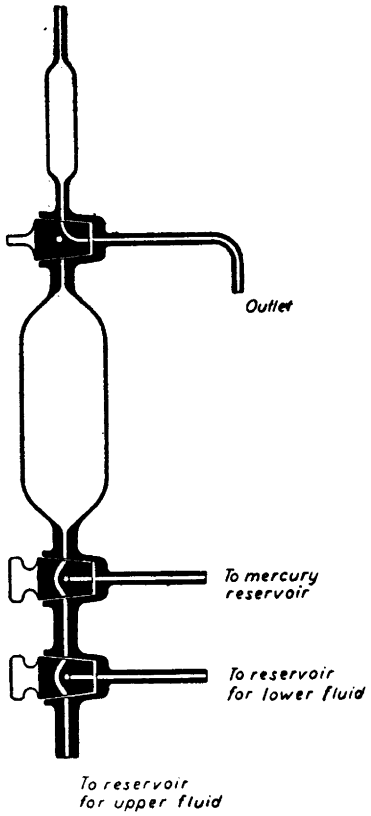

Fig. 1. The apparatus employed.

The activity of the penicillin samples was determined on the basis of a standard curve representing the inhibition produced by a series of dilutions of the last fraction of the diffusion liquid which had been removed from the apparatus. The diameters of the inhibition circles obtained from the diluted samples are plotted against the degree of dilution on logarithmic graph paper.

The curve obtained allows us to determine the relative concentrations of the individual fractions from the diameters of the inhibition circles. The mean of the two perpendicular inhibition diameters is used as a measure of the inhibitory strength.

In order to reduce the uncertainty of these determinations, the activity of each fraction is measured at several dilutions and, in the final calculation, the mean values are used.

Since both the diameter of the cylinder, the volumes of the individual fractions, and the volume of the displaced quantity of solvent are known, the mean distance x from the original boundary in the cylinder can be calculated for each fraction.

Table 1. First experiment. Temperature: 11.8—12.1° C. The penicillin solution displaced 6.50 g of the upper fluid. By testing the strength of fraction no. 15, it was found that the concentration in the region of the cylinder where the original boundary had been, actually was c_s.

Fraction no.	Weight of fraction in g	Distance from boundary in cm	Dilution	Inhibition in mm	Relative concentration $\frac{c}{c_s}$	$\log\frac{c}{c_s}$		Dt
1	2.66							
2	1.50							
3	1.47							
4	1.22	5.17	1 : 1	29.3	$2 \times 0.30 \times 10^{-5}$	—4.22		0.80
5	1.68	4.73	1 : 1	34.0	$2 \times 0.95 \times 10^{-4}$	—3.72	—3.72	0.79
			1 : 2	30.5	$4 \times 0.44 \times 10^{-4}$	—3.74		
			1 : 4	28.3	$8 \times 0.25 \times 10^{-4}$	—3.70		
6	1.40	4.27	1 : 1	41.0	$2 \times 0.46 \times 10^{-3}$	—3.04	—3.12	0.80
			1 : 2	37.5	$4 \times 0.22 \times 10^{-3}$	—3.06		
			1 : 4	33.8	$8 \times 0.97 \times 10^{-4}$	—3.11		
			1 : 8	29.6	$16 \times 0.34 \times 10^{-4}$	—3.26		
7	1.69	3.81	1 : 1	43.5	$2 \times 0.77 \times 10^{-3}$	—2.81	—2.77	0.74
			1 : 2	40.5	$4 \times 0.40 \times 10^{-3}$	—2.80		
			1 : 4	37.8	$8 \times 0.23 \times 10^{-3}$	—2.74		
			1 : 8	34.2	$16 \times 0.10 \times 10^{-3}$	—2.80		
			1 : 16	33.0	$32 \times 0.63 \times 10^{-4}$	—2.70		
8	1.42	3.34	1 : 4	44.0	$8 \times 0.84 \times 10^{-3}$	—2.18	—2.21	0.74
			1 : 8	40.0	$16 \times 0.37 \times 10^{-3}$	—2.23		
			1 : 16	36.9	$32 \times 0.19 \times 10^{-3}$	—2.23		
			1 : 32	34.0	$64 \times 0.95 \times 10^{-4}$	—2.20		
9	1.95	2.84	1 : 10	46.0	$20 \times 0.13 \times 10^{-2}$	—1.60	—1.46	0.90
			1 : 20	43.0	$40 \times 0.72 \times 10^{-3}$	—1.54		
			1 : 40	41.0	$80 \times 0.42 \times 10^{-3}$	—1.48		
			1 : 80	39.0	$160 \times 0.29 \times 10^{-2}$	—1.33		
			1 : 160	35.7	$320 \times 0.14 \times 10^{-3}$	—1.34		
10	1.73	2.28						
11	1.60	1.78						

Fraction no.	Weight of fraction in g	Distance from boundary in cm	Dilution	Inhibition in mm	Relative concentration $\frac{c}{c_s}$	$\log\frac{c}{c_s}$		Dt
12	1.78	1.28						
13	1.43	0.80						
14	1.21	0.40						
15	1.77	—0.05	1 : 1000	41.5	$2000 \times 0.50 \times 10^{-3}$	0.00	—0.11	
			1 : 2000	37.7	$4000 \times 0.83 \times 10^{-3}$	—0.08		
			1 : 4000	33.0	$8000 \times 0.67 \times 10^{-3}$	—0.21		
			1 : 8000	30.6	$16000 \times 0.70 \times 10^{-3}$	—0.16		
16	1.73							
17	1.48							
18	1.64							
19	0.61		1 : 1000	45.0	Series of standard dilutions			
			1 : 2000	40.8				
			1 : 4000	38.5				
			1 : 6000	36.3				
			1 : 8000	35.3				
			1 : 12000	34.0				
			1 : 16000	32.0				
			1 : 32000	29.2				
							Mean:	0.79_5

$D_{12}t = 0.79_5$, time $t = 2.90$ days, $D_{12} = 0.27_4$, $D_{10} = 0.26_1 \pm 0.00_6$ cm²/day.

Table 2. Second experiment. Temperature: 10.7—11.3° C.

Fraction no.	Distance from boundary in cm	$\log\frac{c}{c_s}$	Dt
5	4.92	—4.00	0.78
6	4.44	—3.39	0.78
7	4.05	—3.00	0.73
8	3.62	—2.47	0.77
9	3.15	—1.76	0.89
10	2.72	—1.68	0.70
		Mean:	0.775

$D_{11}t = 0.77_5$, time $t = 2.75$ days, $D_{11} = 0.28_2$, $D_{10} = 0.27_5 \pm 0.00_9$ cm²/day.

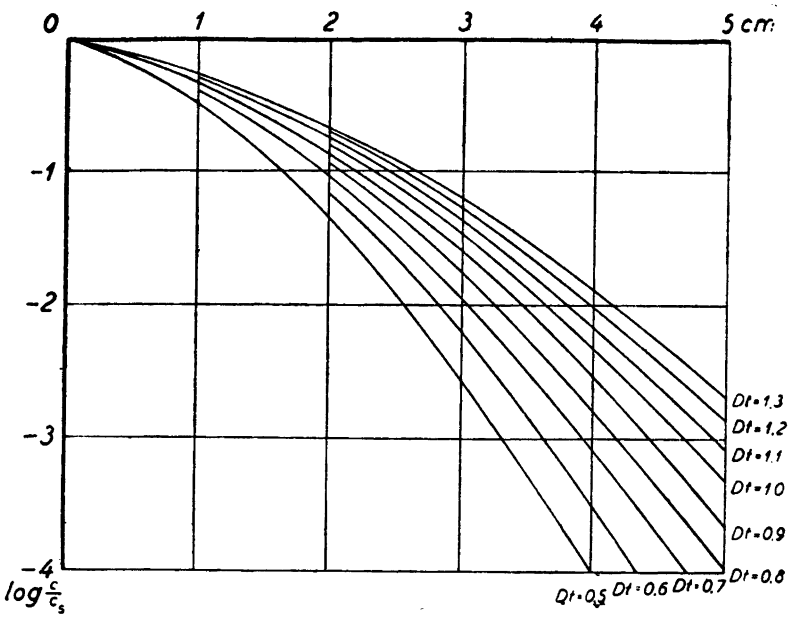

Fig. 2. The interdependence between the distance from boundary and $\log \frac{c}{c_s}$, *for different values of Dt.*

METHOD OF CALCULATION

By means of tables for the error integral which resembles the equation describing the dependence of the concentration on the path of diffusion after the lapse of time t, curves are drawn for different values of Dt; the path of diffusion is plotted as abscissa and the logarithm of the relative penicillin concentration as ordinate; here, half of the concentration of the initial penicillin solution (c_s) is taken as a unit. In this way, we arrive at the curves of fig. 2 (Brodersen and Klenow [2]).

When the points representing the experimental results are plotted, the corresponding values of Dt may be found by interpolation. The shape of the curves indicates that the most accurate determinations are obtained when the fractions of weakest concentration are used, because, in this case, a lower accuracy of the method of testing is required. Therefore, only the first ten fractions were applied; the first three or four figures were, however, discarded in view of the fact that these samples originated from the upper conical part of the cylinder, where diffusion did not take place according to Fick's law.

From the mean values of Dt for different fractions, D is calculated, the time of diffusion being known. For the sake of comparison, the experimental

results must be converted to the same temperature, *viz.* 10° C. The temperature coefficient was assumed to be 2.6 % per degree, as found by Jander and Winkel [5].

As the two experiments were performed analogously, the measurements from the first experiment only are given (table 1), while the results of the second experiment can be found from table 2. Mean value of the two experiments: $D_{10} = 0.26_8 \pm 0.00_9$ cm²/day.

ESTIMATION OF THE MOLECULAR WEIGHT

No unambiguous relation between the diffusion constant of a substance and its molecular weight M (ionic weight) is known. Several methods of calculation have been used in order to find M, when D is known. The empiric formula given by Riecke [6], *i. e.* $D\sqrt{M} = k$, where k is constant, leads to the best results for molecular weights below 500 (Stumpf [7]; Brodersen and Klenow [2]). The value for k was found by Øholm [8] to be 7.0—7.8 at 20° C, corresponding to 5.2—5.8 at 10° C. More recently, other investigators arrived at similar results. Brodersen and Klenow [2], in experiments with various substances, performed with the method and the apparatus described, found $D_{10}\sqrt{M}$ to be about 5.0.

Using this value for $D_{10}\sqrt{M}$, the ionic weight of Danish penicillin will turn out to be 346. The correctness of this figure depends upon whether 5.0 is the correct value for $D_{10}\sqrt{M}$ in the case of penicillin. In the experimental series just mentioned, this value varied for the different substances tested so that the value obtained for M (in our case the ionic weight) is encumbered with a greater uncertainty than that of the determination of D.

On the basis of the constitutional formulas stated by British and American authors (Committee on Medical Research, and the Medical Research Council') the ionic weights of various penicillins are from 312 to 350.

By means of decomposition experiments, Brodersen [10] showed that Danish penicillin consists primarily of one component, the other components being present in so minute quantities that they can be regarded as insignificant in the present experiments. It was also shown that decomposition takes place according to a reaction of the first order. Therefore, a decomposition of penicillin, if at all occurring, need not be taken into consideration.

SUMMARY

In diffusion experiments with a solution of Danish penicillin (Leo), using the cup method for the concentration determinations, the diffusion constant

at 10° C was found to be $D_{10} = 0.26_8 \pm 0.00_9$ cm²/day. Applying the formula $D_{10} \sqrt{M} = 5.0$, the ionic weight was found to be 346.

From the Anglo-American formulas for the different penicillins the values 312—350 were calculated.

The present work was performed with samples of Leo penicillin most kindly furnished by Professor K. A. Jensen, M. D.

The work was made possible by a University scholarship placed at the disposal of Rolf Brodersen, A. M., to whom my thanks are furthermore due for valuable advice and suggestions during the performance of the experiments.

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On the Determination of Polydispersity from Ultracentrifugal Sedimentation

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In the physico-chemical characterization of polydisperse, high molecular compounds it is very important to get information about the degree of heterogeneity. If an ultracentrifugal sedimentation * of a polydisperse substance is performed, a separation of the components always occurs because molecules of different sizes have different sedimentation velocities. Sedimentation in the ultracentrifuge therefore is a very convenient way to get the required information. Gralén [1] using this method as a measure of the polydispersity, determined the width (B) of the sedimentation curve (obtained by means of the scale method according to Lamm [2]) as the quotient between the area (A) and the maximum height (H), *i. e.*

$$B = \frac{A}{H} \tag{1}$$

If the molecular weight of the particles is not too small, the diffusion in case of substances with threadlike molecules can be neglected. Hence it can be assumed, that the variation in width of the curve is due only to the polymolecularity of the substance. As the large molecules always sediment more rapidly than the small ones, the width B will increase with time and have different values at different distances (x) from the centre of rotation. In this way Gralén has studied the derivative dB/dx and as the final measure of the polydispersity introduced dB/dx extrapolated to zero concentration.

Gralén assumes that B varies linearly with x, and points out that this is evident from the experiments. The purpose of this study is to discuss this

* For details about the sedimentation *cf.* T. Svedberg and K. Pedersen, The Ultracentrifuge Oxford (1940); subsequently referred to as UC.

relationship more thoroughly, and to show a way in which dB/dx can be calculated in order to get a close relationship between the value, B, and quantities which characterize the frequency-distribution function of the substance. The discussions have hitherto been limited to the case where the sedimentation is not dependent upon the concentration of the solution.

There are mainly three factors which may have an influence on the calculation of dB/dx and for which corrections have to be introduced. These factors arise from the changes occuring in the curve during the course of the sedimentation. Firstly, the width of the sedimentation curve is not only determined from the different sizes of the molecules but also from the increase in the centrifugal field with the distance from the centre of rotation. The width of the curve is larger then what would be expected from the polydispersity. Secondly, the value of dB/dx may change, depending upon whether the calculation is carried out in the originally plotted sedimentation diagram or whether it is made in a diagram where a correction has been made for the dilution due to the sector shape of the centrifuge cell. Thirdly, dB/dx may be affected by the characteristic displacement of the maximum point of the sedimentation curve of polydisperse substances (Kinell [3]).

In order to study the relation between B and the distance x, we assume that the substance is characterized by the following frequency-distribution function for the sedimentation constants (s)

$$\frac{dc_0}{ds} = \begin{cases} f(s) & \text{for } s_1 \leq s \leq s_2 \\ 0 & \text{for } s < s_1 \text{ and } s > s_2 \end{cases} \tag{2}$$

where c_0 is the analytical concentration. Further we assume that this function has a single maximum for $s = \bar{s}$. We get the following sedimentation curve:

$$\frac{dc}{dx} = \begin{cases} \varphi(x) & \text{for } x_1 \leq x \leq x_2 \\ 0 & \text{for } x_0 \leq x < x_1 \text{ and } x > x_2 \end{cases} \tag{3}$$

or correcting for the sector shape of the cell:

$$\frac{dc_0}{dx} = \begin{cases} \psi(x) & \text{for } x_1 \leq x \leq x_2 \\ 0 & \text{for } x_0 \leq x < x_1 \text{ and } x > x_2 \end{cases} \tag{4}$$

These curves too have only one maximum, for x_m and x'_m respectively. Between dc/dx and dc_0/dx the following relation is valid (*cf.* Rinde [4]):

$$\frac{dc_0}{dx} = \frac{dc}{dx}\left(\frac{x}{x_0}\right)^2 \tag{5}$$

For the transformation of the dc_0/ds-curve to the dc/dx- and dc_0/dx-curves respectively, the following equations have to be used:

$$x = x_0 e^{\omega^2 st} \tag{6}$$

$$\frac{dc}{dx} = \frac{dc_0}{ds} \frac{e^{-3\omega^2 st}}{x_0 \omega^2 t} \tag{7a}$$

$$\frac{dc_0}{dx} = \frac{dc_0}{ds} \frac{e^{-\omega^2 st}}{x_0 \omega^2 t} \tag{7b}$$

where x_0 is the position of the meniscus in the cell and ω is the angular velocity of the rotor.

From equ. (7) it is easy to obtain the following equations, which determine the s-values corresponding to the maximum points of the dc/dx- and dc_0/dx-curves (*cf.* Kinell *l. c.*)

$$\frac{df(s)}{ds} - 3\omega^2 t f(s) = 0 \tag{8a}$$

$$\frac{df(s)}{ds} - \omega^2 t f(s) = 0 \tag{8b}$$

The solution of these equations gives $s_m(t)$ and $s'_m = s'_m(t)$. It is easily seen, that these functions satisfy the relation

$$s_m(t) \leq s'_m(t) \leq \bar{s} \tag{9}$$

where the equality sign has to be used for $t = 0$. The displacement of the maximum point is therefore largest for the original sedimentation curve.

The calculation of dB/dx from the original and corrected sedimentation curves is now possible by means of equ.s (1), (6) and (7). We get the following expressions for B and B_0 as functions of the time:

$$B = \frac{\int_{x_1}^{x_2} \frac{dc}{dx}\, dx}{\left(\frac{dc}{dx}\right)_{x = x_m}} = \Phi_1(t)\ x_0 \omega^2 t \tag{10a}$$

$$B_0 = \frac{\int_{x_1}^{x_2} \frac{dc_0}{dx}\, dx}{\left(\frac{dc_0}{dx}\right)_{x = x'_m}} = \Phi_2(t)\ x_0\omega^2 t \tag{10b}$$

where

$$\Phi_1(t) = \frac{\int_{s_1}^{s_2} \frac{dc_0}{ds} e^{-2\omega^2 s t} ds}{\left(\frac{dc_0}{ds}\right)_{s = s_m}} e^{3\omega^2 s_m t} \tag{11a}$$

$$\Phi_2(t) = \frac{\int_{s_1}^{s_2} \frac{dc_0}{ds}\, ds}{\left(\frac{dc_0}{ds}\right)_{s = s'_m}} e^{\omega^2 s'_m t} \tag{11b}$$

By differentiating with respect to x_m and x'_m and observing that s_m and s'_m are functions of the time, we get

$$\frac{dB}{dx_m} = \Phi_1(t)\ \frac{\left[1 + t\,\frac{d\ \ln\ \Phi_1(t)}{dt}\right] e^{-\omega^2 s_m t}}{s_m\left[1 + \frac{t}{s_m}\,\frac{ds_m}{dt}\right]} \tag{12a}$$

$$\frac{dB_0}{dx'_m} = \Phi_2(t)\ \frac{\left[1 + t\,\frac{d\ \ln\ \Phi_2(t)}{dt}\right] e^{-\omega^2 s'_m t}}{s'_m\left[1 + \frac{t}{s'_m}\,\frac{ds'_m}{dt}\right]} \tag{12b}$$

These expressions are evidently dependent on the time t and hence dB/dx generally cannot be a linear function of x. It also follows, that dB/dx does not give any simple characterization of the frequency-distribution curve. However, if we regard the case $t = 0$, we have

$$\left(\frac{dB}{dx_m}\right)_{t=0} = \frac{\Phi_1(0)}{\bar{s}} \tag{13a}$$

$$\left(\frac{dB_0}{dx'_m}\right)_{t=0} = \frac{\Phi_2(0)}{\bar{s}} \tag{13b}$$

Now according to equ.s (11) and (9)

$$\Phi_1(0) = \Phi_2(0) = \frac{\int_{s_1}^{s_2} \frac{dc_0}{ds}\, ds}{\left(\frac{dc_0}{ds}\right)_{s=\bar{s}}}$$

and hence

$$\left(\frac{dB}{dx_m}\right)_{t=0} = \left(\frac{dB_0}{dx'_m}\right)_{t=0} = \frac{\int_{s_1}^{s_2} \frac{dc_0}{ds}\, ds}{\bar{s}\left(\frac{dc_0}{ds}\right)_{s=\bar{s}}} \tag{14}$$

If an extrapolation is made to the moment when the sedimentation starts, we obviously get a quantity, which in a very simple manner is related to the frequency-distribution function. This expression is already given by Gralén, but from the treatment here, it is evident that it is valid only at the time $t = 0$, and further, that the same expression is obtained independently, whether or not the correction for the dilution with the sector shape of the cell has been made.

In order to understand thoroughly the deviations from the linear relationship between B and x, it would be necessary to give an involved discussion of equ. (10). This is however rather laborious and not necessary for the practical calculations as will be shown later. It is only of interest to point out that the following expression is obtained by expansion of equ. (12a) in a power serie of the time t:

$$\left(\frac{dB}{dx_m}\right) \Big/ \left(\frac{dB}{dx_m}\right)_{t=0} = 1 + \left(5\bar{s} - 4\bar{s}_w - \frac{6f(\bar{s})}{sf''(\bar{s})}\right)\omega^2 t + \ldots.$$

where $\bar{s}_w$ is the weight average value of s corresponding to the original frequency-distribution function. It is evident that for small values of $\omega^2 t$, the

curve $B(x)$ can be situated either below or above its tangent at the origin depending upon the sign of the expression within the brackets in the right member. This sign depends upon the nature of the frequency-distribution function. For a high polydispersity $\bar{s}_w$ may have such a large value that $dB/dx_m < (dB/dx_m)_{t=0}$, and for low polydispersity the difference between $\bar{s}$ and $\bar{s}_w$ may be so small that $dB/dx_m > (dB/dx_m)_{t=0}$ For equ. (12b) it is possible to show that $dB_0/dx'_m > (dB_0/dx'_m)_{t=0}$ for all values of t and for every frequency-distribution function. The reason for the difference between the two cases is the dilution with the sector shape of the cell. This brief discussion elucidates the necessity of having complete information about all the phenomena occurring during the sedimentation before judging the results obtained by calculating dB/dx.

The relation between B and x for a special frequency-distribution function is shown below.

Let us assume, that the substance is characterized by the logaritmic frequency-distribution function given by Gralén (*l. c.*):

$$\frac{dc_0}{ds} = K_s e^{-\frac{1}{\gamma_s^2}\ln^2\frac{s}{\bar{s}}} \tag{15}$$

where K_s is the maximum height and γ_s is a distribution coefficient. The range for the s-values is $0 \leq s \leq \infty$. If we apply equ.s (8), (10) and (11) we get

$$B = x_0\left(\frac{x_m}{x_0}\right)^3 + \frac{3}{2}\gamma_s^2 \ln\frac{x_m}{x_0} e^{\frac{9}{4}\gamma_s^2 \ln^2\frac{x_m}{x_0}} \cdot I_1 \tag{16a}$$

where

$$I_1 = \int_0^\infty e^{-\frac{1}{\gamma_s^2}\ln^2\frac{s}{\bar{s}} - \frac{2s}{\bar{s}}\left(\frac{x_m}{x_0}\right)^{\frac{3}{2}\gamma_s^2} \ln\frac{x_m}{x_0}} d\left(\frac{s}{\bar{s}}\right)$$

and

$$B_0 = x_0\gamma_s\sqrt{\pi}\left(\frac{x'_m}{x_0}\right)^1 + \frac{1}{2}\gamma_s^2 \ln\frac{x'_m}{x_0} e^{\frac{1}{4}\gamma_s^2 \ln^2\frac{x'_m}{x_0} + \frac{1}{4}\gamma_s^2} \tag{16b}$$

For comparison we have the following expressions, if the displacement of the maximum point is neglected:

$$B = x_0\left(\frac{x_m}{x_0}\right)^3 \ln\frac{x_m}{x_0} \cdot I_2 \tag{17a}$$

where

$$I_2 = \int_0^\infty e^{-\frac{1}{\gamma_s^2}\ln^2\frac{s}{\bar{s}} - \frac{2s}{\bar{s}}\ln\frac{x_m}{x_0}} d\left(\frac{s}{\bar{s}}\right)$$

and

$$B_0 = x_0\,\gamma_s\sqrt{\pi}\left(\frac{x_m'}{x_0}\right)\ln\frac{x_m'}{x_0}\,e^{\frac{1}{4}\gamma_s^2} \tag{17b}$$

The numerical calculations have been made for $5.0 \leq x \leq 6.0$ cm and for $\gamma_s = 0.1$, 0.5 and 1.0 and the results are given in figs. 1 and 2.

Firstly it is seen that the effect of the displacement of the maximum point cannot be neglected when the polydispersity is high. It is also evident that for high values of γ_s, we get curves which deviate rather much from the straight lines corresponding to the tangents of the curves at the origin. An exception is the curve for $\gamma_s = 1$ in fig. 1. Theoretically, at low values of $\omega^2 t$, the change of the slope shall occur for a γ_s-value determined from the equation

$$5 - 4\,e^{0.75\gamma_s^2} + 3\gamma_s^2 = 0$$

Solving this we get $\gamma_s = 0.919$. Secondly we see that the curvature of the curves is rather low, and it is easy to make serious mistakes about the nature of the curves. When for instance the points A, B, C and D (fig. 2) were experimental points, it is consistent with a rather high degree of accuracy to assume that they represent a linear relationship between B and x. Taking the slope of such a line we will get too high values of dB/dx.

In order to avoid all the difficulties associated with the dB/dx-calculation according to equ. (10), we have to change the method essentially with respect to the displacement of the maximum point. The first quantity we need is an area A. For this we choose the area of the corrected sedimentation curve. But since the correction means that this area will be equal to the analytical concentration at every time, it is not necessary to make this correction. We have only to use the analytical concentration (c_0). As Gralén points out, it is obviously correct to assume a constant concentration throughout the experiment. At least this must be the case for a pure substance in a good solvent. If there is any doubt about this, the corrected curve can be traced by means of equ. (5)*. The areas are measured and compared with the analytical

* It is to be noted, that the use of equation $c/c_0 = (x_0/x)^2$ gives a wrong result, because this equation is valid only for a monodisperse substance with a sedimentation, which is not concentration dependent. Using this equation we get

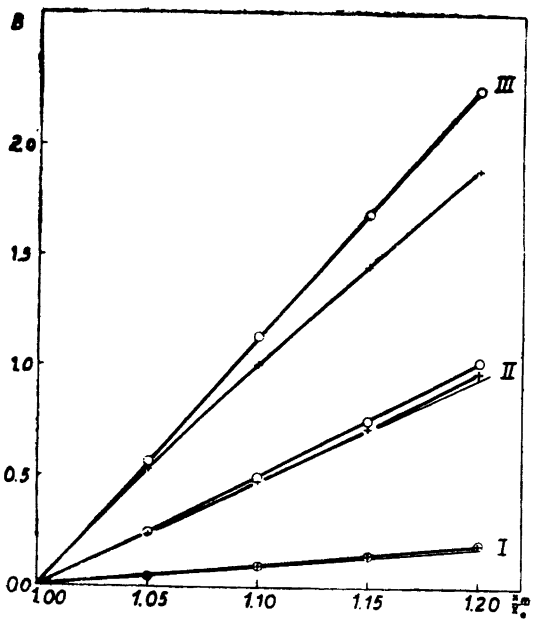

Fig. 1. B as a function of x_m/x_0 for different values of γ_s. I : $\gamma_s = 0.1$; II : $\gamma_s = 0.5$; III : $\gamma_s = 1.0$. ○ values calculated from equ. (16a), + values calculated from equ. (17a). The thin lines are the tangents of the curves at the origin.

concentration. The proper height H to be used must be a quantity corresponding to the maximum height of the frequency-distribution curve. Firstly, it is advantageous from an experimental point of view to take this height from the original sedimentation diagram and secondly, it is necessary to take the height corresponding to a point $\bar{x}$ determined from

$$\bar{x} = x_0 \, e^{\omega^2 \bar{s} \, t}. \tag{18}$$

$$\Delta c = \int_{x_1}^{x_2} \frac{dc_0}{dx} \, dx - \left(\frac{x_m}{x_0}\right)^2 \cdot \int_{x_1}^{x_2} \frac{dc}{dx} \, dx$$

and if we express this in the frequency-distribution function:

$$\Delta c = \int_{s_1}^{s_2} \frac{dc_0}{ds} \left\{ 1 - e^{-2\omega^2 (s - s_m) t} \right\} ds$$

thus Δc is not equal to zero but dependent upon the polydispersity and the position in the cell.

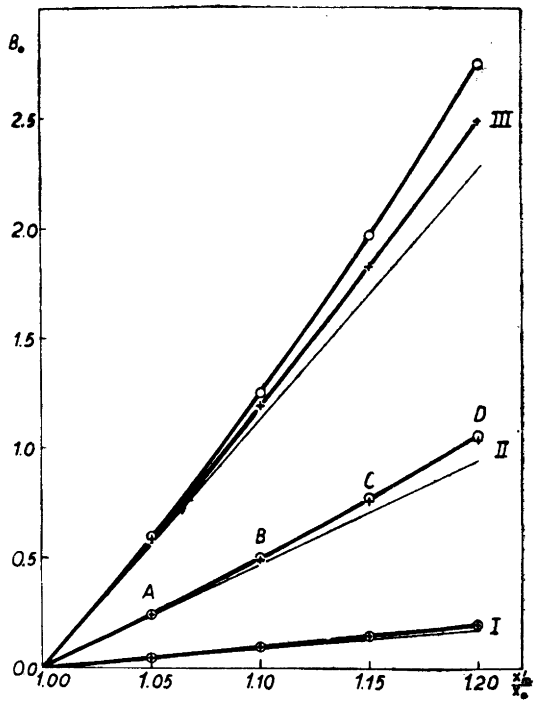

Fig. 2. B_0 as a function of x'_m/x_0 for different values of γ_s. I : γ_s = 0.1; II : γ_s = 0.5; III : γ_s = 1.0. ○ values calculated from equ. (16b); + values calculated from equ. (17b). The thin lines are the tangents of the curves at the origin.

The value $\bar{s}$ can be found if we plott the s-values obtained at different times t in the ordinary way against t and then extrapolate to $t = 0$. Then $\bar{x}$ can be calculated from equ. (18) for different times, and the heights corresponding to these points can be measured in the sedimentation diagram. Thus we get for H:

$$H = \left(\frac{\bar{x}}{x_0}\right)^2 \left(\frac{dc}{dx}\right)_{x=\bar{x}} = \left(\frac{dc_0}{ds}\right)_{s=\bar{s}} \cdot \frac{e^{-\omega^2 \bar{s} t}}{x_0 \omega^2 t} \tag{19}$$

and thus for the width B:

$$B = \frac{c_0}{\left(\frac{\bar{x}}{x_0}\right)^2 \left(\frac{dc}{dx}\right)_{x=\bar{x}}} = \frac{\int_{s_1}^{s_2} \frac{dc_0}{ds}\, ds}{\left(\frac{dc_0}{ds}\right)_{s=\bar{s}}} x_0\, \omega^2 t\, e^{\omega^2 \bar{s} t} \tag{20}$$

If we further apply equ. (18), we get as the final expression:

$$\bar{B} = \frac{c_0}{\left(\frac{\bar{x}}{x_0}\right)^2 \left(\frac{dc}{dx}\right)_{x=\bar{x}} \ln\frac{\bar{x}}{x_0}} = \frac{\int_{s_1}^{s_2} \frac{dc_0}{ds}\, ds}{\bar{s}\left(\frac{dc_0}{ds}\right)_{s=\bar{s}}} \bar{x}, \tag{21}$$

and taking the derivative with respect to $\bar{x}$:

$$\frac{d\bar{B}}{d\bar{x}} = \frac{\int_{s_1}^{s_2} \frac{dc_0}{ds}\, ds}{\bar{s}\left(\frac{dc_0}{ds}\right)_{s=\bar{s}}}. \tag{22}$$

This expression is independent of $\bar{x}$, and is in a very simple way related to quantities characterizing the frequency-distribution function. If the displacement of the maximum point can be neglected, this method of calculation mainly corresponds to Gralén's, except that the height is corrected by multiplying with the factor $\ln x/x_0$.

One of the main problems in most of the calculations based on the sedimentation diagrams is to obtain good base lines. The peak in the diagram is often rather good but the horizontal parts of the curve, from which the base line have to be drawn, are in many cases irregular and hence the drawing of this line may be a little arbitrary. Gralén has tried to avoid this difficulty by drawing a base line and adjust its position in such a way that the area of the curve corresponds to the analytical concentration. Of course even this involves some arbitrariness, because the lowest part of the curve has to be constructed as extensions of the original curve. Furthermore, the shape of the curve is not in agreement with that one which would be obtained if the correction were made according to equ. (5). This may have some influence on the height of the curve. The method suggested here does not involve any arbitrariness, but does depend upon the ability to draw a good base line.

It is difficult to say anything about the application of equ. (21) for a substance with concentration dependent sedimentation. The formulas for the correction of the dilution due to the sector shape of the cell etc. involve in this case functions which characterize the concentration dependence. For

concentration dependences, not greatly pronounced, however, equ. (21) may be expected to give useful results.*

In order to show the application of the new method, a calculation of $d\bar{B}/d\bar{x}$ has been carried out on a fraction of commercial polymeric methyl methacrylate. The fractionation was made in the ordinary way by precipitation with cyclohexane from a benzene solution. One of the middle fractions was run in the ultracentrifuge in an acetone solution. The sedimentation was studied by means of the scale method of Lamm (*l.c.*); the following concentrations were used: 0.396, 0.298, 0.198, 0.104 and 0.047 g/100 ml. Lower concentrations could not be used, because they did not give any observable peak.

According to Svedberg [5] the sedimentation constant at the temperature T and the distance x from the centre of rotation (*cf.* also Kinell, *l. c.***) $s_T(x)$ is given by the expression:

$$s_T(x) = \frac{M[1 - V_T \varrho_T(x)]}{f_T(x)} \tag{23}$$

where M is the molecular weight, V_T the partial specific volume of the solute, $\varrho_T(x)$ the density of the solvent, and $f_T(x)$ the molar frictional coefficient of the solute at the distance x from the centre of rotation. The dependence on x for these last two quantities has been introduced, because they are dependent on the hydrostatic pressure in the cell. We write $f_T(x) = \eta_T(x) \cdot F\ (M, V, h)$, where $\eta_T(x)$ is the viscosity of the solvent and F a function which is determined by the size, shape, and solvation of the molecule. In a first approximation, we can assume this function to be independent of small changes in temperature and pressure. We now reduce $s_T(x)$ to a standard condition $T = 20°$ C and the pressure 1 atm, then we get (*cf.* UC p. 35 and Mosimann and Signer [6]) the following expression:

$$s_T(x) = s_{1,20} \frac{\eta_{20}(x)\ [1 - V_T \varrho_T(x)] \cdot \eta_{20}(x_0)\ [1 - V_{20}\varrho_{20}(x)]}{\eta_T(x)\ [1 - V_{20}\varrho_{20}(x)] \cdot \eta_{20}(x)\ [1 - V_{20}\varrho_{20}(x_0)]}$$

* Gralén's treatment of the sedimentation curves before the dB/dx-calculation makes it probable that his calculations theoretically should correspond to a case between our cases representad by equ.s (10a) and (10b). A linear relationship is not then excluded with regard to the discussions on p. 340. Furthermore the concentration dependence may have an influence upon the deviations from the linear relationship but a final decision cannot be made before the mathematical calculations for the concentration dependent case have been carried out.

** The dependence of s on the changes of concentration in the cell has been disregarded in the following treatment.

$$= \frac{s_{1,20}}{[1 + \delta\,(T)]\,[1 + \varepsilon\,(x)]} \tag{24}$$

The correction for the temperature is made under the assumption that the pressure is constant. In the equation above $1 + \delta\,(T)$ may be regarded as independent of the time and the distance x. This is true only as an approximation, but if the centrifuge run has been carried out carefully the variations in the temperature are rather small and serious disturbances can occur only at very low concentrations (*cf.* Jullander [7] p. 54). The function $1 + \varepsilon\,(x)$ is at low speeds of rotation a linear function of x and can be written in the form $1 + R(x - x_0)/x_0$. According to Svedberg (*l. c.*) we have

$$s_T(x) = \frac{1}{\omega^2 x}\frac{dx}{dt} \tag{25}$$

and hence we get from equ. (24)

$$\left[1 + \frac{R}{x_0} \cdot (x - x_0)\right]\frac{dx}{x} = \frac{s_{1,20}\omega^2 dt}{1 + \delta\,(T)} \tag{26}$$

Integrating this and assuming that ω is independent of time we get

$$(1 - R)\,\ln\frac{x}{x_0} + \frac{R}{x_0} \cdot (x - x_0) = \frac{s_{1,20}\omega^2 t}{1 + \delta\,(T)} \tag{27}$$

Here we can develop $R\,\ln x/x_0$ in a power serie of $\Delta x/x_0$, where $\Delta x = x - x_0$. In our case, where R has a rather low value, it is sufficient to have only two terms and then we get

$$[1 + \delta\,(T)]\left[\ln\frac{x}{x_0} + \frac{R}{2}\left(\frac{\Delta x}{x_0}\right)^2\right] = s_{1,20}\omega^2 t \tag{28}$$

This formula is valid with an accuracy of a few tenth of a percent. If we plott the left member of this equation against $\omega^2 t$, we obtain straight lines if s is not affected by the decrease of concentration with the sector shape of the cell and by the polydispersity.

For the substance investigated here, the speed of the rotor was 30000 r. p. m., the correction for the changes in temperature was less than 2 %, and the correction for the pressure increased to about 5 % for a sedimented distance of about 10 mm. The R-value was calculated to 0.232 for all concen-

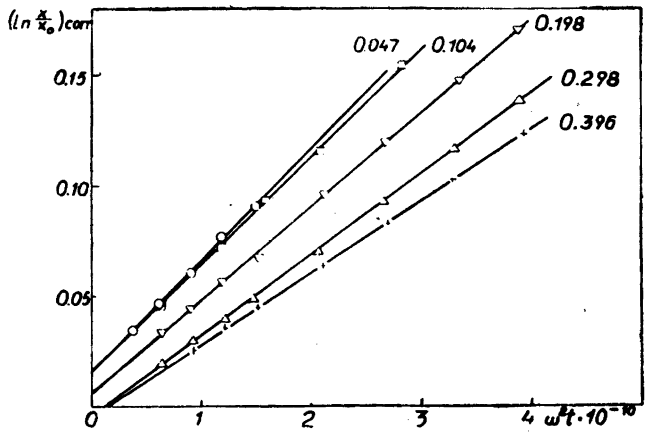

Fig. 3. Diagram according to equ. (28) for different concentrations.

trations used. In fig. 3 the left member of equ. (28) is plotted against $\omega^2 t$. The lines (calculated from the experimental values) do not intersect the $\omega^2 t$-axis in the origin. The reason for this is the difficulty to determine the exact starting time, because the substance always starts its sedimentation before the rotor has reached its full speed. Further the meniscus can have an effect on the sedimentation during the first moments. The following intersection points have been calculated: $\omega^2 t = + 147.3 \cdot 10^7$, $+ 134.3 \cdot 10^7$, $- 143.0 \cdot 10^7$, $- 310.9 \cdot 10^7$ and $- 317.6 \cdot 10^7$. Correcting the $\omega^2 t$-values used in fig. 3 for these values we can use equ. (28) to calculate the s-values corresponding to each point in the cell. The values obtained follow from table 1 where x is in cm and s in S-units. It is evident that we do not have any changes in the s-values

Table 1. Values of $s_{1,20}$ for different concentrations.

$c_0 = 0.396$		$c_0 = 0.298$		$c_0 = 0.198$		$c_0 = 0.104$		$c_0 = 0.047$	
x	$s_{1,20}$	x	$s_{1,20}$	x	$s_{1,20}$	x	$s_{1,20}$	x	$s_{1,20}$
5.840		5.838		5.837		5.839		5.874	
5.991	32.6	5.954	39.4	6.031	43.3	6.109	48.6	6.074	50.4
6.045	32.9	6.018	38.0	6.095	42.8	6.207	50.5	6.146	50.1
6.101	32.7	6.076	36.5	6.170	42.8	6.274	48.4	6.228	48.5
6.213	32.2	6.133	36.3	6.251	41.0	6.404	49.1	6.332	51.4
6.333	32.3	6.262	36.1	6.407	42.3	6.548	48.4	6.414	49.7
6.462	32.7	6.400	36.5	6.556	42.6	6.778	49.3		50.0
6.595	32.6	6.549	36.8	6.735	42.5		49.0		
	32.5	6.688	37.3	6.881	42.4				
			36.9		42.4				

which can be due to decrease in the concentration or to the polydispersity of the substance. In any case these changes are less than the experimental accuracy.

Plotting s against $c.s$, we get for s at zero concentration, $s_0 = 59.0\ S$, and for the characterization of the concentration dependence, $k = 2.0$ (*cf.* Gralén, *l. c.* p. 12). Thus the dependence of concentration is rather low.

Having characterized our substance and shown that there is no observable displacement of the maximum point of the sedimentation curve, we can proceed to the calculations according to equ. (21). Since this equation is valid only in a case where we do not have any changes in the hydrostatic pressure, we have to introduce the above mentioned corrections. At the same time, we can reduce the $\bar{B}$-values to the temperature 20° C and the pressure 1 atm. For equ. (28) we easily get the necessary corrections. The final result is:

$$\bar{B} = \frac{c_0}{\left\{\left(\frac{\bar{x}}{x_0}\right)\left[1 + \frac{R}{2}\left(\frac{\Delta\bar{x}}{x_0}\right)^2\right]\right\}^{2[1+\delta\ (T)]}\left(\frac{dc}{d\bar{x}}\right) \cdot \frac{\ln \frac{\bar{x}}{x_0} + \frac{R}{2}\left(\frac{\Delta\bar{x}}{x_0}\right)^2}{1 + R \cdot \frac{\Delta\bar{x}}{x_0}}} \tag{29}$$

or in an abbreviated form

$$\bar{B} = \frac{c_0}{\left\{\left(\frac{\bar{x}}{x_0}\right)^2\right\}_{\text{corr.}}\left(\frac{dc}{d\bar{x}}\right)\left\{\ln \frac{\bar{x}}{x_0}\right\}_{\text{corr.}}} \tag{30}$$

Since there is no change in the s-values, $\bar{x}$ is equal to the x previously used. $dc/d\bar{x}$ can be calculated from the heights (Z) measured in the sedimentation diagrams by means of the formula (*cf.* UC p. 259)

$$\frac{dc}{d\bar{x}} = \frac{Z}{Gaba} \tag{31}$$

where a is the refractive index increment, G the photographic enlargement, a the thickness of the cell, and b the scale distance. For the case of simplicity, $dn/d\bar{x}$ can be calculated instead of $dc/d\bar{x}$, and at the same time, c_0 in the numerator of equ. (30) can be expressed in refractive index units by multiplication with a. The value of a for the substance used here, determined in acetone, was $134 \cdot 10^{-5}$.

Table 2. Values of $d\bar{B}/d\bar{x}$ calculated according to equ. (21).

$c_0 = 0.396$			$c_0 = 0.298$			$c_0 = 0.198$		
$\bar{x}$	$\bar{B}$	$d\bar{B}/d\bar{x}$	$\bar{x}$	$\bar{B}$	$d\bar{B}/d\bar{x}$	$\bar{x}$	$\bar{B}$	$d\bar{B}/d\bar{x}$
5.840			5.838			5.837		
5.991	1.46	0.24	5.954	2.40	0.40	6.031	2.83	0.47
6.045	1.38	0.23	6.018	2.06	0.34	6.095	2.47	0.41
6.101	1.28	0.21	6.076	1.72	0.28	6.170	2.57	0.42
6.213	1.43	0.23	6.133	1.95	0.32	6.251	2.69	0.43
6.333	1.31	0.21	6.262	1.99	0.32	6.407	2.69	0.42
6.462	1.47	0.23	6.400	2.06	0.32	6.556	2.42	0.37
6.595	1.37	0.21	6.549	2.00	0.31	6.735	2.47	0.37
			6.688	1.93	0.29	6.881	2.96	0.43
$c_0 = 0.104$			$c_0 = 0.047$					
$\bar{x}$	$\bar{B}$	$d\bar{B}/d\bar{x}$	$\bar{x}$	$\bar{B}$	$d\bar{B}/d\bar{x}$			
5.839			5.874					
6.109	3.37	0.55	6.074	2.95	0.49			
6.207	3.00	0.48	6.146	3.07	0.50			
6.274	3.41	0.54	6.228	3.01	0.48			
6.404	3.53	0.55	6.332	3.88	0.61			
6.548	3.25	0.50	6.414	4.37	0.68			
6.778	3.50	0.52						

The performance of all the numerical calculations is omitted here. The final values follow from table 2. Theoretically the $\bar{B}$-values should increase with increasing distance from the centre of rotation. This is not quite obvious from the values given in the table, but the $d\bar{B}/d\bar{x}$-values show a rather good consistency for each concentration. Notwithstanding the difficulties in measuring the heights of the sedimentation curves, the result is quite satisfactory. The following mean values (with mean deviations from the mean) are obtained

c_0	$d\bar{B}/d\bar{x}$
0.396	0.22 ± 0.01
0.298	0.32 ± 0.03
0.198	0.41 ± 0.02
0.104	0.52 ± 0.03
0.047	0.55 ± 0.07
0	0.61

The last value is extrapolated to zero concentration.

This example shows that the new formula derived for a non concentration dependent sedimentation can be used even in the case of concentration dependence. One advantage of this method over Gralén's is, that the values of $d\bar{B}/d\bar{x}$ obtained for different distances from the centre of rotation theoretically shall be independent of this distance. The example given presents experimental evidence that this is the case even for substances with concentration dependent sedimentation. It is consequently possible to get evidence that the quantities measured in the sedimentation diagrams are correct.

A further use of the method will show if it is possible to apply the simple formula (21) even to substances having greater concentration dependence.

SUMMARY

The dB/dx-method introduced by Gralén to characterize polydispersity of high molecular compounds has been studied as regards the influence of 1) increase in centrifugal field with the distance from the centre of rotation, 2) dilution with the sector shape of the cell, and 3) displacements in the maximum point of the sedimentation curve. The result is that generally the width B is not a linear function of x, however, it is shown that a linear relationship can be obtained, if corrections are introduced for the above mentioned influences. The new method has been applied with a good result to a polymeric methyl methacrylate.

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The Use of Dialysis for the Preparation of Enterogastrone

Preliminary Communication

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The mechanism for the regulation of the gastric secretion is still in many respects unexplained in spite of a good deal of intensive studies. It is known that enterogastrone is a factor which inhibits the secretion of gastric juice. This factor is obtained from the first 2 m of the upper part of the small intestine of the swine. A method for its preparation has been worked out by Ivy and his collaborators[1]. Only very incomplete data concerning the chemical nature of the enterogastrone are available. Inactivation of enterogastrone by pepsine indicates that it may be of a protein nature. Urogastrone has a physiological effect which is similar to that of enterogastrone. It derives its name from the fact that it may be obtained from human or animal urine. Urogastrone, however, is not inactivated by pepsine. Gray *et al.*[2] have claimed that urogastrone is a complex organic base. According to the literature, urogastrone inhibits the gastric secretion in considerably smaller doses than does enterogastrone.

For the last years the author has worked on the preparation of urogastrone and enterogastrone. For the preparation of enterogastrone Ivy's method has been used. As the results have varied and the preparations often have been inactive, some attempts have been made to improve the procedure. By the improvements it has been possible to obtain consistently a enterogastrone preparation of a high activity.

Dialysis is an essential step of the method. Enterogastrone has proved to be dialysable through a cellophane membrane. By this new method we obtained a yield of ca. 10 mg of substance per 2 m of intestine. 25 mg of the preparation inhibit very strongly the gastric secretion in the dog. Several inactive preparations, which had been prepared according to Ivy's method, could be activated and purified by dialysis. However, by this procedure

only fairly small amounts of active material were obtained. Thus in one case we obtained 1.76 g of active preparation from 18 g of inactive material. The new method will be published in its details later on.

One of the difficulties in the preparation of enterogastrone results from the fact that the mucosa contains substances which stimulate the secretion of gastric juice. According to Harper [3] they do not dialyse through a cellophane membrane. Thus dialysation is a good method for the separation of the stimulatory substances of high molecular weight from the inhibitory substances of low molecular weight.

The reported investigation has been performed in collaboration with Dr. Karl Johan Öbrink, *Fysiologiska Institutionen*, Uppsala, who has carried out the activity determinations and the investigations of the physiological effect of the preparations.

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The Kinetics of 1-Chloroether Alcoholysis

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The exceptional reactivity of 1-chloroethers has called the attention of numerous investigators and several explanations for this have been offered [1]. Whereas a number of theoretical approaches to the mechanisms of the reactions of the 1-chloroethers may be found in the literature [2], in only a few instances have these compounds been subjected to kinetic investigation. Clarke's [3] attempts to follow the reaction of methyl chloromethyl ether in alcoholic pyridine failed due to the rapid rate of reaction. Conant, Kirner and Hussey [4], however, were able to appraise the rate of reaction between methyl chloromethyl ether and potassium iodide in acetone solution. More interesting are the results of Böhme[2c], who followed the rate of hydrolysis of ethyl chloromethyl ether in aqueous dioxane solution. He noted that the hydrogen chloride produced catalyzed the reaction but did not take into account the concentration of the hydrogen chloride in calculating the rate constants. On the contrary, he attempted to show that, except for a short initial period, the reaction follows the first order law.

A conflicting view of the reaction in this respect is given by the results of Leimu [5] for the rates of alcoholysis of 1-chloroethers in dioxane solution containing one and two moles per liter of *n*-propyl alcohol. Catalysis by hydrogen chloride was also observed in these reactions and to such an extent that constants calculated from the first order equation, neglecting autocatalysis, increased toward the end of the reaction. Reactions carried out in solutions initially containing hydrogen chloride indicated that the rate is proportional to the concentration of this substance. The data obtained were found to fit the integrated form (2) of the differential equation (1):

$$\frac{dx}{dt} = k_s a\ (b-x)\ (c+x) \qquad (1)$$

* The authors wish to thank the *Suomen Kulttuurirahasto* for financial aid.

$$k_s = \frac{1}{a(b+c)t} \ln \frac{b(c+x)}{c(b-x)} \quad (2)$$

In these equations the relatively small change in the alcohol concentration a is disregarded; b and c are the initial concentrations of chloroether and hydrogen chloride, respectively, and x the amount of chloroether reacted at time t.

Certain observations led us to suspect that the reaction is reversible in this medium and this has been confirmed in the present work. In order to ascertain the nature of the reaction, it was followed in solvents containing various amounts of the lower aliphatic alcohols in dioxane and in the pure alcohols. The results reveal that the alcoholysis of 1-chloroethers is very susceptible to changes in medium.

THE RATE EQUATIONS

The method of analysis used to follow the reaction

$$ROCH_2Cl + HOR' \longrightarrow ROCH_2OR' + HCl \quad (I)$$

was the same as that used in the previous work [5]. Samples were withdrawn from the reaction solution with an automatic pipette and run into aqueous sodium hydroxide solution. The liberated formaldehyde was determined iodometrically following the method originally developed by Ripper [6].

In dioxane solutions containing small amounts of alcohol, reaction (I) was found to be catalyzed. The rate is approximately directly proportional to the hydrogen chloride concentration. The reaction did not proceed to completion in these solvents but attained a state of equilibrium. The existence and nature of the reverse reaction was confirmed by experiments in which the corresponding formal was substituted for the chloroether. The numerical data obtained in these experiments were found to fit satisfactorily the rate equation derived on the assumption that the rate of the reverse reaction was proportional to the first power of the formal concentration and to the square of the concentration of the hydrogen chloride.

Following these considerations, the differential equation for the reaction in dioxane solutions containing small amounts of alcohol and added hydrogen chloride, but no initial formal, may be written

$$\frac{dx}{dt} = k_1(a-x)(b-x)(c+x) - k_2 x(c+x)^2 \quad (3)$$

where a, b and c are the initial concentrations of alcohol, chloroether and hydrogen chloride, respectively, x the amount of chloroether reacted at time t, k_1 and k_2 the rate constants for the alcoholysis and the reverse reaction.

* An error appearing in this equation in the previous paper [5] is here corrected.

Experiments carried out with different initial concentrations of chloroether and hydrogen chloride gave values of the equilibrium constant conforming with equation (3). The equilibrium constant K is in this case given by

$$\frac{k_1}{k_2} = \frac{\xi\ (c + \xi)}{(a - \xi)\ (b - \xi)} = K \tag{4}$$

where ξ is the value of x at equilibrium ($dx/dt = 0$).

The integrated form of equation (3) suitable for the application of the numerical results may be derived as follows[7]:

If $k_1 \neq k_2$, equation (3) may be written

$$\frac{dx}{dt} = (k_1 - k_2)\ (c + x)\ (x^2 + px + q), \text{ where}$$

$$p = -\frac{k_1(a + b) + k_2 c}{k_1 - k_2} = -\frac{K(a + b) + c}{K - 1} \text{ and}$$

$$q = \frac{k_1 ab}{k_1 - k_2} = \frac{Kab}{K - 1}$$

$(\xi - x)$ is one factor of the expression $x^2 + px + q$. If the other factor is designated by $(\zeta - x)$, the value of ζ is given by

$$\zeta = \frac{Kab}{(K - 1)\,\xi} \tag{5}$$

The differential equation now takes the form

$$\frac{dx}{dt} = (k_1 - k_2)\ (c + x)\ (\xi - x)\ (\zeta - x) \tag{3'}$$

which is easily integrated by the method of partial fractions.

With the condition $t = 0$, $x = x_0$, we have then

$$(k_1 - k_2)t = \frac{1}{(c + \xi)(c + \zeta)} \ln \frac{c + x}{c + x_0} + \frac{1}{(c + \xi)\ (\zeta - \xi)} \ln \frac{\xi - x_0}{\xi - x} + \frac{1}{(c + \zeta)(\xi - \zeta)} \ln \frac{\zeta - x_0}{\zeta - x} \tag{6}$$

The individual constants k_1 and k_2 are calculated from the difference and the value of K from (4). In certain cases, where the absolute value of ζ is found to be relatively large, the last term of (6) is of a much smaller magnitude than the others and may be disregarded in the calculations. In particular, when K is approximately unity, the absolute value of ζ is so high that the following approximation holds:

$$\zeta \sim c + \zeta \sim \zeta - \xi$$

The equation (6) may then be replaced by the approximate equation

$$k_1 t = \frac{\xi}{ab(c+\xi)} \cdot \ln \frac{(\xi - x_0)\ (c+x)}{(c+x_0)\ (\xi - x)} \tag{7}$$

This equation is easily shown to be the solution of equation (3) in the special case of $k_1 = k_2$ $(K = 1)$.

Equations for the reverse reaction between the formal and hydrogen chloride may be derived in a similar manner. Following the considerations outlined above, the differential equation corresponding to (3) is

$$\frac{dx}{dt} = k_2(d-x)\ (c-x)^2 - k_1 x(a+x)\ (c-x) \tag{8}$$

in which a, c, k_1 and k_2 have the same significance as above, d is the initial concentration of the formal and x the amount of formal reacted at time t. If the equilibrium value of x is again denoted by ξ, the equilibrium constant is obtained from

$$\frac{k_1}{k_2} = \frac{(d-\xi)\ (c-\xi)}{\xi(a+\xi)} = K \tag{9}$$

For the condition $k_1 \neq k_2$, (8) may be transformed into

$$\frac{dx}{dt} = (k_2 - k_1)\ (c-x)\ (\xi - x)\ (\zeta - x) \tag{8'}$$

where ζ is calculated from

$$\zeta = \frac{cd}{(1-K)\xi} \tag{10}$$

On substituting the limit $x = x_0$, when $t = 0$, the integrated form of (8′) is

$$(k_2 - k_1)t = \frac{1}{(c-\xi)\ (c-\zeta)} \ln \frac{c-x_0}{c-x} + \frac{1}{(c-\xi)\ (\zeta-\xi)} \ln \frac{\xi - x_0}{\xi - x} + \frac{1}{(c-\zeta)\ (\xi-\zeta)} \ln \frac{\zeta - x_0}{\zeta - x} \tag{11}$$

When K is approximately unity, the following approximate equation corresponding to (7) is obtained

$$k_2 t = \frac{\xi}{cd(c-\xi)} \ln \frac{(\xi - x_0)\ (c-x)}{(c-x_0)\ (\xi - x)} \tag{12}$$

This is the exact solution of (8) when k_1 is equal to k_2 $(K = 1)$.

These equations were found to agree fairly well with the numerical values obtained in following the reactions in the dioxane solutions containing one mole of alcohol per

liter. Since the agreement was not so satisfactory for the values obtained for the higher concentrations of alcohol, experiments were extended to include the pure alcohols as reaction media. Under these conditions the reactions were found to exhibit no catalysis by hydrogen chloride, and no reverse reaction was detected. The rate constants were calculated from the simple first order equation

$$kt = \ln \frac{b}{b - x} \tag{13}$$

DISCUSSION

A survey of the experimental data obtained is presented in the following tables. As above, the letters a, b, c and d denote the concentrations in moles per liter of the various components of the reaction mixtures: a alcohol, b chloroether, c hydrogen chloride and d formal. k_1 is the alcoholysis rate constant in $l^2 \cdot mole^{-2} \cdot min^{-1}$ and $K = k_1/k_2$ the equilibrium constant. k is the first order rate constant of the uncatalyzed reaction in pure alcohol, in min^{-1}.

The values of the equilibrium constant given in the tables reveal that the state of equilibrium is adequately defined by equations (4) and (9) in that experiments conducted with various initial concentrations give identical values of this constant. Worth notice in this respect is the fact that the same state of equilibrium is attained in both directions starting from ethyl chloromethyl ether and from ethylal, giving 1.22 for the average of the equilibrium constant for the former reaction and 1.28 for the latter in 1 M ethanol solution at 15° C (table 4). In 2 M alcohol solution the alcoholysis reaction proceeds almost to completion, due to which the values of the equilibrium constants of table 1 are relatively inaccurate *. This pertains particularly to the data for the reaction of methyl chloromethyl ether as the reverse reaction in 2 M solution is very slight. In these cases the error in the value of the equilibrium constant does not, however, noticeably affect the value of the alcoholysis rate constant when this is calculated from the equations given above. The equilibrium constant is, contrary to that observed in the esterification of carboxylic acids [8], very sensitive to changes in temperature, the temperature coefficients being much larger for the reverse reaction than for the alcoholysis. This is observed as a sometimes steep fall in the values of the equilibrium constant as the temperature is increased.

* In comparing the values of the equilibrium constant it must be noted that the state of equilibrium is not so simple for those reactions in which the alkyl components of the chloroether and alcohol are different and the final equilibrium mixture may be composed of two chloroethers and three formals.

Table 1. Alcoholysis in 2 M ethanol in dioxane (a = 2). Rate constants calc. from (6) and (7).

$ROCH_2Cl$	$t°$ C	b	c	K	k_1
CH_3OCH_2Cl	15	0.119	0.125	5.1	0.089
—»—	»	0.121	0.229	6.7	0.083
—»—	»	0.119	0.377	6.3	0.079
—»—	25	0.117	0.125	4.2	0.139
—»—	»	0.110	0.229	4.6	0.135
—»—	»	0.117	0.377	4.7	0.127
—»—	35	0.0473	0.125	2.7	0.216
—»—	»	0.127	0.125	3.5	0.209
—»—	»	0.105	0.229	3.3	0.202
—»—	»	0.0724	0.377	3.7	0.186
$C_2H_5OCH_2Cl$	15	0.099	0.0663	0.96	0.228
—»—	»	0.120	0.0663	1.05	0.219
—»—	»	0.156	0.114	1.04	0.226
—»—	»	0.163	0.244	0.98	0.207
—»—	25	0.135	0.114	0.71	0.365
—»—	»	0.101	0.133	0.73	0.357
—»—	35	0.153	0.114	0.46	0.576
—»—	»	0.121	0.114	0.42	0.543
—»—	»	0.096	0.311	0.50	0.527
n-$C_3H_7OCH_2Cl$	15	0.135	0.098	1.22	0.232
—»—	»	0.112	0.243	1.23	0.213
—»—	25	0.118	0.098	1.00	0.392
—»—	»	0.111	0.243	1.09	0.334
—»—	35	0.129	0.098	0.87	0.587
—»—	»	0.094	0.243	0.88	0.510
i-$C_3H_7OCH_2Cl$	15	0.111	0.0596	0.43	1.13
—»—	»	0.156	0.0596	0.46	1.03
—»—	25	0.109	0.0596	0.38	1.68
—»—	»	0.160	0.0596	0.42	1.56
—»—	35	0.101	0.0596	0.39	2.34
—»—	»	0.140	0.0596	0.38	2.21

Table 2. Alcoholysis in dioxane solutions containing different alcohols. The alcoholysis of methyl chloromethyl ether in 1 M solutions of alcohol in dioxane (a = 1) at 15° C. Constants calc. from (6) and (7).

ROH	b	c	K	k_1
CH_3OH	0.130	0.164	2.42	0.0375
C_2H_5OH	0.131	0.492	1.13	0.0296
$ClCH_2CH_2OH$	0.128	0.241	0.45	0.0047

Table 3. Alcoholysis in dioxane solutions containing different concentrations of ethyl alcohol. The alcoholysis of methyl chloromethyl ether at 15° C. Constants calc. from (6) and (7).

a	b	c	K	k_1
2	0.121	0.229	6.7	0.083
1	0.131	0.492	1.13	0.0296
1	0.184	0.607	1.17	0.0300
0.5	0.120	0.169	1.26	0.0154
0.25	0.191	0.219	0.94	0.0055
0.125	0.202	0.349	0.205	0.0014

Table 4. Equilibrium constant determination. A. The alcoholysis of ethyl chloromethyl ether in 1 M solutions of ethanol in dioxane at 15° C. ($a = 1$). Constants calc. from (6) and (7).

b	c	K	k_1
0.177	0.209	1.29	0.086
0.181	0.325	1.15	0.092
0.193	0.410	1.26	0.101
0.214	0.567	1.17	0.103

B. The reaction between ethylal and hydrogen chloride in 1 M solutions of ethanol in dioxane ($a = 1$). Constants calc. from (11) and (12).

t° C	d	c	K	k_1
15	0.166	0.144	1.33	0.100
»	0.193	0.210	1.32	0.114
»	0.204	0.255	1.30	0.105
»	0.197	0.410	1.27	0.107
»	0.192	0.567	1.17	0.119
25	0.213	0.431	1.08	0.166
35	0.212	0.431	0.96	0.257

The proportionality between the reaction rate and the hydrogen chloride concentration is not explicit and due to this the equations used in the calculations of the results obtained in the experiments in dioxane solutions containing higher concentrations of alcohol are only approximate. It may be seen from table 1 that the rate constants calculated from the results of experiments in 2 *M* alcohol solutions increase slightly with decreasing hydrogen chloride

concentration; this is also evident in the results of the previous investigation [5]. This is paralleled by the increase in the values of the rate constants when the initial concentration of the chloroether is decreased. In 1 *M* solutions of alcohol the state of affairs is the opposite, the decrease in the rate of alcoholysis being less than the decrease in catalyst concentration, as shown by the data of table 4.

Table 5. Alcoholysis in pure alcohol. Equation (13).

Solvent	$ROCH_2Cl$	k_5	k_{15}	k_{25}	k_{35}	k_{15}/k_5	k_{25}/k_{15}	k_{35}/k_{25}
CH_3OH	CH_3OCH_2Cl	10.2	22.3			2.19		
—»—	$C_2H_5OCH_2Cl$	19.4						
C_2H_5OH	CH_3OCH_2Cl	1.53	3.86	9.0	19.7	2.52	2.33	2.19
—»—	$C_2H_5OCH_2Cl$	5.05	10.4			2.06		
$(CH_3)_2CHOH$	CH_3OCH_2Cl	0.520	1.29	3.08	6.88	2.48	2.39	2.23
—»—	$C_2H_5OCH_2Cl$		4.03					
$CH_3O(CH_2)_2OH$	CH_3OCH_2Cl		3.12	6.96			2.23	
—»—	$C_2H_5OCH_2Cl$	4.84	10.4			2.15		
$Cl(CH_2)_2OH$	CH_3OCH_2Cl	7.83	15.9			2.03		

The rate constants of the different chloroethers given in table 1 show the same sequence for the effect of structure on the velocity of reaction as that found in the acid catalyzed hydrolysis of dialkylformals [6b], namely, that the rate increases in the following order respecting the alkyl groups:

$$CH_3 \leqq C_2H_5 < n\text{-}C_3H_7 < i\text{-}C_3H_7.$$

A remarkable fact is revealed by the small values of the temperature coefficients of the alcoholysis in dioxane solution, which vary from 1.4 to 1.7 and are of the same magnitude as those observed in the alcoholysis of acid chlorides in similar media [9].

Table 2 shows that the rates in dioxane containing the same concentration of various alcohols vary in the following order:

$$ClCH_2CH_2OH < C_2H_5OH \leqq CH_3OH$$

This order is the same as that observed in other reactions of alcohols involving an electrophilic attack on oxygen [10]. The same order has been observed in the alcoholysis of acid chlorides in alcohol-dioxane solutions *.

* Unpublished work carried out in this aboratory.

Table 3, giving the results of experiments in solutions containing various amounts of alcohol, shows that as the smaller alcohol concentrations are approached the rate continues to decrease steeply with no indication of attaining a limiting rate as is the case in the bimolecular alcoholysis of acid chlorides [9a].

In pure alcohol no catalysis by hydrogen chloride was detected up to concentrations of one mole per liter. Table 5 contains the first order rate constants of the alcoholysis in the various alcohols at four temperatures and the temperature coefficients calculated from them. The effect of the alkyl group shows the same order as that observed in the dioxane solutions. When the alcoholysis rates are high, as in methyl alcohol, the relative differences are smaller. The temperature coefficients are much higher for the reaction in the pure alcohols than in the dioxane solutions although the rates are of a much higher order of magnitude. The temperature coefficients of the more reactive ethyl chloromethyl ether are much smaller than those of the lower homolog, whereas in the dioxane solutions they are approximately the same. The rate of alcoholysis in 2-chloroethanol is considerably higher than in ethanol, which is the opposite of that observed in the dioxane solutions (table 2).

On the basis of these results, an approach to the mechanisms of the alcoholysis in the various media may be attempted. Although we are of the opinion that the alcoholysis and hydrolysis of 1-chloroethers in all probability follow a similar course, it is difficult to apply to the alcoholysis reaction in dioxane-alcohol solution the hydrion catalyzed mechanism proposed by Böhme [2c] for the hydrolysis in aqueous dioxane solution, as it would not lead to the production of formal. Böhme's mechanism does not conform with his views of the independence of the rate on the catalyst concentration since it requires that the rate be proportional to this concentration. The mechanism for the hydrolysis of 1-chloroethers given by Farren, Fife, Clark and Garland [2a] on the basis of their observations of the reverse reaction, the formation of 1-chloroethers, can neither be applied to the alcoholysis reaction as it would lead to alkyl exchange instead of the formation of the formal.

The results of our experiments in pure alcohol offer many points of comparison with those numerous kinetic investigations which deal with the reactions of certain organic halides [11]. It seems that some of the theoretical views based on the observations of these investigations may be advantageously considered in an effort to elucidate the mechanism of 1-chloroether alcoholysis. For the uncatalyzed reaction in pure alcohol the following two mechanisms are possible:

A. A nucleophilic attack of alcohol on carbon:

$$\begin{array}{l} ROCH_2Cl \longrightarrow ROCH_2OR + HCl \text{ bimolecular.} \qquad (II) \\ \nearrow \\ ROH \end{array}$$

B. Ionization of chloroether promoted by solvolysis of the chloride ion by alcohol:

$$ROCH_2Cl \longrightarrow ROCH_2^+ + Cl^- \text{ unimolecular,} \qquad (III)$$

followed by the fast reaction

$$ROCH_2^+ + ROH \longrightarrow ROCH_2OR + H^+$$

The results of our investigation seem to favour the latter of these two mechanisms, which are identical with those designated by Ingold and Hughes [11a] as S_N2 and S_N1, although very little conclusive evidence can be adduced to allow a final decision. The bimolecular mechanism is not supported by the fact that the rate of alcoholysis in 2-chloroethanol is faster than in ethanol since previous work has confirmed that the reactivity of the former alcohol in electrophilic displacements on oxygen is much weaker than that of the latter. Explanations based on the dielectric constants of the solvents can neither be adduced to support the bimolecular mechanism in this case since the dielectric constants of both alcohols have approximately the same value. We may then consider the alcoholysis reaction as a unimolecular solvolysis (III). It has been observed in other reactions which are classified under this name that solvents having a great tendency to solvate halide ion bring about a marked increase in the rate of reaction [11], and, in addition, that certain metal halides, which favour the ionization of triphenylmethyl chloride [12] by their ability to form complex halides, also accelerate this type of reaction [13]. This is paralleled by our observations of the effect of the different alcohols on the rate of chloroether alcoholysis and, especially, by the effect of addition of mercuric chloride, the rate of alcoholysis in ethanol at 15°C increasing from 3.86 (table 5) to 10.6 on addition of this salt to give a concentration of 0.017 *M*. The S_N1-mechanism is also given support by the observation that comparatively small additions of sodium alcoholate do not materially increase the rate. With larger additions of alcoholate, however, the reaction was bimolecular, being proportional to both the concentration of the chloroether and that of the alcoholate *. Similar changes in mechanism from the S_N1 to S_N2 as the concentration of the more active nucleophilic reagent is in-

* Experiments performed in ethanol solution containing 0.0733 moles per liter of sodium ethylate at 15°C gave with methyl chloromethyl ether a rate constant of 4.6 (3.86 in pure alcohol). With the larger additions the rate was too fast for accurate measurement but approximate results indicated the reaction to be bimolecular.

creased have been observed in the reactions of halogen acids [14]. The proposed unimolecular mechanism is in accord with the effect of the structure on the velocity. The change in electron releasing character of the group $ROCH_2$- when ethyl is introduced in place of methyl facilitates the ionization and causes an increase in velocity.

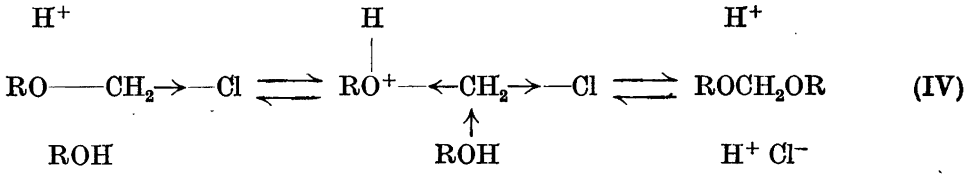
Ingold and Hughes [2b] have expressed the opinion that the S_N1-mechanism is a very plausible one for the hydrolysis of 1-chloroethers, but for obvious reasons it has not been possible to investigate the reaction in highly ionizing media. The mechanism is also in accord with the views of Cocker, Lapworth and Walton [1a].

The solvolysis by alcohol proposed above for the alcoholysis of chloroethers in pure alcohols may also be extended to alcohol-dioxane solutions, provided that the alcohol concentration is large. As this concentration decreases the part played by this reaction becomes negligible and the rate becomes very slow unless the solution contains hydrogen chloride. The acceleration effected by hydrogen chloride may also be of a solvolytic nature but there is an alternate possibility that a change in mechanism from S_N1 to S_N2 occurs as is the case in some reactions of alkyl chlorides as the ionizing power of the solvent decreases [11a]. The S_N2-mechanism as given by (II) is no longer valid, but if the reaction is formulated as following a mechanism analogous to that ascribed to the hydrolysis of acetals [15], the catalytic effect of the hydrogen chloride may be also accounted for.

For the hydrogen chloride catalyzed reaction in alcohol-dioxane solution, the following two mechanisms are thus left for consideration:

A A first order solvolysis by the undissociated hydrogen chloride, similar to (III).

B. A catalyzed reaction resembling the hydrolysis of acetals:



(IV)

Hydrogen chloride is only slightly ionized in dilute solutions of alcohols in dioxane [16] and undissociated hydrogen chloride has been found to induce the ionization of triphenylmethyl chloride and related compounds [17]. In pure alcohol solution hydrogen chloride is maintained to be fully ionized [18], which may account for the fact that it does not catalyze the alcoholysis reaction in this medium.

In the light of the solvolytic mechanism, it is easily understood that the effect of structure is the same in both the pure alcohol and the alcohol-dioxane solutions. This mechanism also conforms with the observations that the values of the rate constants continue to decrease as the molarity of the alcohol and the ionizing power of the solvent decreases. This mechanism is also able to account for the variations of the rate observed with varying hydrogen chloride and alcohol concentrations.

On the other hand, the solvolytic mechanism does not explain the slow rate of alcoholysis in 2-chloroethanol solution and the regularity shown by the equilibrium constants. These observations are, however, easily understood on the basis of mechanism (IV), which also accounts for the effect of structure on the rate. Here, as in acetal hydrolysis, the reaction is favored by increasing electron releasing character of the alkyl substituent. The gradual decrease in the rate as the alcohol concentration is decreased may also be explained according to this mechanism, but not the dependence of the rate on the concentration of hydrogen chloride when the proportion of alcohol in the solvent is varied. It should be noted, however, that these variations are very slight and may also be due to other complications [11b].

In view of these considerations it is quite possible that in the alcohol-dioxane solutions both the unimolecular solvolysis by undissociated hydrogen chloride and the solvated hydrion catalyzed reaction proceed concurrently, but it is not possible to bring forth at present quantitative, conclusive evidence in this respect.

EXPERIMENTAL

Materials. The 1-chloroethers were prepared according to Karvonen [19] and Farren, Fife, Clark and Garland [2a]. The boiling points were:

Methyl chloromethyl ether, b. $p._{762}$59.0 — 59.6°;

Ethyl chloromethyl ether, b. $p._{765}$82.4°;

Propyl chloromethyl ether, b. $p._{752}$108.5 — 109.0°;

Isopropyl chloromethyl ether, b. $p._{764}$96.8 — 97.6°.

Diethyl formal was obtained by Ghysels' [20] method. Its boiling point was 84 — 85° at 763 mm. pressure.

Methyl, ethyl and isopropyl alcohols were pure technical products and were purified with magnesium according to the methods described by Bjerrum and Zechmeister [21] and Lund and Bjerrum [22]. The 2-methoxyethanol and 2-chloroethanol were technical products and were purified by fractionation. The boiling points were:

2-Methoxyethanol, b. $p._{762}$124.5°;

2-Chloroethanol, b. $p._{760}$128.5°.

1,4-Dioxane of technical grade was purified by the method previously used [23,9].

Kinetic Measurements. The reactions were carried out in glass-stoppered flasks, from which samples were transferred with a five ml. automatic pipette into an erlenmeyer

flask containing sodium hydroxide solution. The liberated formaldehyde was titrated as described earlier [6].

In the experiments with dioxane solutions, dry hydrogen chloride was led into the solvent and its concentration was determined by titration with standard sodium hydroxide solution.

The rate constants were calculated from equations (6), (7), (11), (12) and (13) with time in minutes and concentrations in moles per liter. In the calculations 4-figure logarithms were used.

Zero time was the time of taking of the first sample. In the alcoholysis determinations carried out in dioxane solutions the amount of chloroether x_0 reacted at zero time was determined by extrapolation, the time between the addition of chloroether and zero time being known. In the fast reactions the initial sample was taken as soon as possible, while in the slower reactions it was taken after the reaction had proceeded for some time. The difference between the iodine consumption corresponding to the bisulphite solution added and the end value gave $b-\xi$.

In the reaction between ethylal and hydrogen chloride it was not necessary to determine x_0 by extrapolation but it was calculated from the difference between the amount of iodine solution corresponding to the added amount of sodium bisulphite and the initial value. The initial formal concentration d was determined by hydrolysing samples taken from the reaction mixture with approximately 2 M hydrochloric acid and titrating the liberated formaldehyde in the usual manner.

The procedure used in the fast reactions is the same as that used in the determination of the rates of alkaline hydrolysis of formic esters [14].

In the following are given examples of the rate determinations. The significance of the letters is the same as above. Time t' is in minutes, t'' in seconds; the rate constants have been calculated with time in minutes.

I. Alcoholysis in 2 M ethanol in dioxane ($a = 2$)

1. Methyl chloromethyl ether

15° C

$b = 0.1190$, $c = 0.1250$, $\xi = 0.1160$ ($= 11.85$ *). $K = 5.1$

t'	0	10	20	30	40	50	60	80	
$\xi - x$ *	11.57	8.79	6.50	4.93	3.34	2.20	1.69	0.87	
$c + x$ *	13.05	15.83	18.12	19.69	21.28	22.42	22.93	23.75	
k_1		0.095	93	87	89	91	86	83	av. 0.089

25° C

$b = 0.1171$, $c = 0.1250$, $\xi = 0.1137$ ($= 11.61$). $K = 4.2$

t'	0	6	12	18	24	30	37	45	
$\xi - x$	11.22	8.84	6.65	4.98	3.59	2.51	1.54	1.04	
$c + x$	13.16	15.54	17.73	19.40	20.79	21.87	22.84	23.34	
k_1		0.138	141	138	138	139	143	137	av. 0.139

* Expressed in ml of standard iodine solution.

35° C

$b = 0.1265$, $c = 0.1250$, $\xi = 0.1219$ (= 12.45). $K = 3.5$

t'	0	3	7	11	14	19	23	27	
$\xi - x$	11.75	9.65	7.32	5.34	4.28	2.60	2.12	1.38	
$c + x$	13.47	15.57	17.90	19.88	20.94	22.62	23.10	23.84	
k_1		0.224	214	212	206	213	196	201	av. 0.209

2. Ethyl chloromethyl ether

15° C

$b = 0.1560$, $c = 0.1135$, $\xi = 0.1380$ (=14.64). $K = 1.04$

t'	0	1.75	3.5	5.0	6.5	8.0	9.5	11.5	13.0	15.0	17.0	
$\xi - x$	13.14	11.72	10.22	8.87	7.95	6.66	5.62	4.92	4.12	3.53	2.83	
$c + x$	13.54	14.96	16.46	17.81	18.73	20.02	21.06	21.76	22.56	23.15	23.85	
k_1		0.215	224	235	224	235	239	222	226	217	217	av. 0.226

II. Alcoholysis in dioxane solutions of different alcohols

The alcoholysis of methyl chloromethyl ether in 1 M solutions of alcohol in dioxane (a = 1) at 15° C.

Methyl alcohol

$b = 0.1303$, $c = 0.1636$, $\xi = 0.1153$ (= 12.69). $K = 2.42$

t'	0	15	30	46	60	75	90	105	120	135	150	
$\xi - x$	12.61	11.38	9.99	8.71	7.87	6.94	6.03	5.28	4.68	4.12	3.53	
$c + x$	18.09	19.32	20.71	21.99	22.83	23.76	24.67	25.42	26.02	26.58	27.17	
k_1		0.0360	394	396	379	375	378	375	367	363	366	av. 0.0375

Ethyl alcohol

$b = 0.1306$, $c = 0.4918$, $\xi = 0.0839$ (= 9.23). $K = 1.13$

t'	0	5	15	20	25	35	45	
$\xi - x$	9.00	8.01	6.21	5.57	5.03	4.01	3.21	
$c + x$	54.37	55.36	57.16	57.80	58.34	59.36	60.16	
k_1		0.0300	313	302	291	286	281	av. 0.0296

III. Alcoholysis in dioxane solutions containing different concentrations of ethyl alcohol

The alcoholysis of methyl chloromethyl ether at 15° C

$a = 0.5$, $b = 0.1202$, $c = 0.1694$, $\xi = 0.0815$ (= 8.97). $K = 1.26$

t'	0	30	60	90	148	206	268	356	459	588	
$\xi - x$	8.97	8.47	7.93	7.42	6.57	5.75	5.17	4.24	3.36	2.53	
$c + x$	18.65	19.15	19.69	20.20	21.05	21.87	22.45	23.38	24.26	25.09	
k_1		0.0152	160	163	159	159	149	149	148	145	av. 0.0154

IV. Equilibrium constant determination

A. The alcoholysis of ethyl chloromethyl ether in 1 M solutions of ethanol in dioxane at 15° C ($a = 1$).

$b = 0.1805$, $c = 0.3246$, $\xi = 0.1247$ (= 14.22). $K = 1.15$

t'	0	7	12	18	24	30	38	45	
$\xi - x$	11.63	8.40	6.35	4.68	3.31	2.40	1.59	1.04	
$c + x$	39.59	42.82	44.87	46.54	47.91	48.82	49.63	50.18	
k_1		0.089	94	92	93	92	90	91	av. 0.092

B. The reaction between ethylal and hydrogen chloride in 1 M solution of ethanol in dioxane at 15° C ($a = 1$).

$d = 0.2044$, $c = 0.2547$, $\xi = 0.02938$ (=3.35). $K = 1.30$

t'	0	12	18	24	30	
$\xi - x$	2.94	1.94	1.56	1.26	1.01	
$c - x$	28.62	27.62	27.24	26.94	26.69	
k_2		0.079	81	82	83	av. 0.081

$d = 0.1969$, $c = 0.4101$, $\xi = 0.0481$ (= 4.88). $K = 1.27$

t'	0	10	15	20	25	30	35.5	
$\xi - x$	4.24	2.27	1.70	1.22	0.92	0.70	0.44	
$c - x$	46.11	44.14	43.57	43.09	42.79	42.57	42.31	
k_2		0.084	82	85	84	82	88	av. 0.084

V. Alcoholysis in pure alcohol

1. Alcoholysis in methyl alcohol

Methyl chloromethyl ether

5° C

0.0833 N iodine as standard solution.

t''	0	3.3	6.3	9.5	12.8	16.5	
$b - x$	4.77	2.70	1.62	0.98	0.54	0.35	
k		10.3	10.3	10.0	10.2	9.5	av. 10.1 Parallel values: 10.1, 10.1 and 10.3

15° C

0.0833 N iodine.

t''	0	3.7	7.0	
$b - x$	1.39	0.36	0.11	
k		21.9	21.7	av. 21.8 Parallel values: 23.1, 21.6, 21.7, 23.1 and 22.7

2. Alcoholysis in ethyl alcohol

Methyl chloromethyl ether

5° C

0.0862 *N* iodine.

t''	0	6.7	13.2	21.8	32.8	54.9	80.5	
$b - x$	6.97	5.84	4.97	3.99	2.99	1.79	0.90	
k		1.58	1.54	1.54	1.55	1.49	1.53	av. 1.54 Parallel values: 1.53, 1.49 and 1.54

15° C

0.0829 *N* iodine.

t''	0	4.4	8.9	14.4	20.5	26.5	33.3	43.1	
$b - x$	6.95	5.48	3.91	2.90	1.92	1.38	0.86	0.41	
k		(3.24)	3.88	3.64	3.77	3.66	3.77	3.94	av. 3.78 Parallel values: 3.83, 3.92,3.90, 3.87, 3.88, 3.93, 3.85 and 3.75

25° C

0.0833 *N* iodine.

t''	0	3.0	6.1	9.6	13.3	17.8	
$b - x$	6.73	4.42	2.72	1.62	0.98	0.50	
k		(8.4)	8.9	8.9	8.7	8.8	av. 8.8 Duplicate value: 9.2

35° C

0.0833 *N* iodine.

t''	0	2.8	5.7		
$b - x$	5.48	2.15	0.84		
k		20.1	19.7	av. 19.9	Duplicate value: 19.4

3. Alcoholysis with mercuric chloride as catalyst

The alcoholysis of methyl chloromethyl ether in ethyl alcohol solution containing 0.0170 moles/l mercuric chloride.

15° C

0.0862 *N* iodine.

t''	0	3.5	6.4	9.6	13.2	17.8	
$b - x$	5.54	2.95	1.71	0.96	0.63	0.27	
k		10.8	11.0	11.0	9.9	10.2	av. 10.6

SUMMARY

1. The rates of alcoholysis of several 1-chloroethers $ROCH_2Cl$ (R = methyl, ethyl, propyl and isopropyl) have been measured in alcohol and alcohol-dioxane solutions.

2. In pure alcohol the alcoholysis is rapid and follows the first order law.

3. In dilute solutions of alcohol in dioxane the reaction is reversible, slow and catalyzed by the hydrogen chloride formed. The rate of the alcoholysis is approximately directly proportional to the hydrogen chloride concentration. The reverse reaction is catalyzed by hydrogen chloride and the rate is proportional to the square of the hydrogen chloride concentration.

4. The rates of reaction of the 1-chloroethers in all the media investigated vary with the alkyl component R in the following order: methyl < ethyl < propyl < isopropyl.

5. In dioxane solutions of the different alcohols the rate decreases in the following order: methanol > ethanol > 2-chloroethanol. In the pure alcohols the order is: methanol > 2-chloroethanol > ethanol.

6. The activation energy of the reaction in dioxane solution is much smaller than that of the reaction in pure alcohol.

7. The results obtained indicate that the reaction in pure alcohol is a unimolecular solvolysis. In dilute solutions of alcohols in dioxane in which the reaction is catalyzed by hydrogen chloride the reaction is considered to follow, at least partly, a bimolecular mechanism.

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The Steric Relationships of Optically Active *a-iso*-Propylglutaric Acid, Fenchone and Camphor

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The steric relationships of *α-iso*-propylglutaric acid are of considerable interest inasmuch as this acid is formed on the degradation of several terpenoid compounds, *e. g.* fenchone. The pure optically active forms have recently been described by one of us [1], and the next step is then to connect them sterically with the active *α-iso*-propylsuccinic acid. This might be accomplished in a purely chemical way by degrading the glutaric acid without loss of activity.

Barbier and Loquin have given a practical method for degradation of carboxylic acids [2]. The ester of the acid is treated with organomagnesium compound and the resulting ketone or tertiary alcohol is oxidised, thus giving a new acid with one less carbon atom. Wieland has introduced the method into steroid chemistry [3], and Bouvet has utilized it for double-sided degradation of dicarboxylic acids [4]. For one-sided degradation of a dicarboxylic acid it is not directly applicable.

Komppa and Rohrmann have investigated the reaction of organomagnesium compound with glutaric anhydride and some of its derivatives [5, 6]. Compounds of the types (I—III)* were formed. By reaction of one mole of Grignard reagent, (I) is the chief product formed, and with two moles (II) is obtained for the most part. Still larger amounts of magnesium compound give principally (III). On oxidation, all these compounds may be supposed to yield succinic acid.

If the glutaric anhydride carries a substituent in one of the α-positions, the molecule is not symmetrical, and two sets of compounds (I—III) might be formed. The carbonyl group adjacent to the substituent will, however, probably react with a much lower velocity owing to sterical hindrance. The corresponding set of compounds will then appear in rather poor yield; on

* Formulas p. 377.

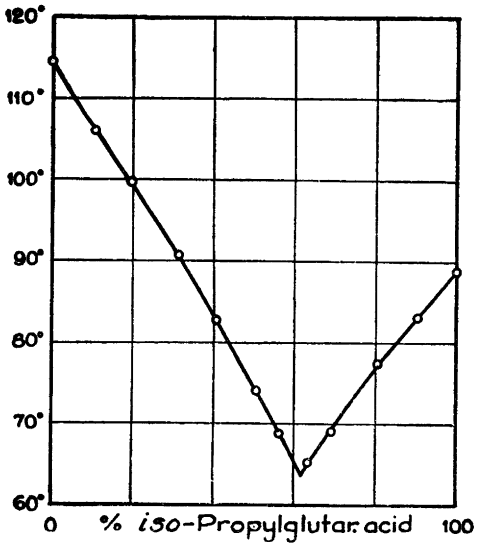


Fig. 1. (+)-*iso*-*Propylglutaric acid and* (+)-*methylglutaric acid.*

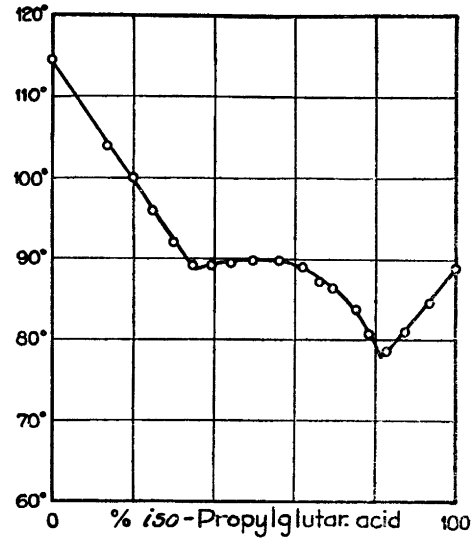


Fig. 2. (—)-*iso*-*Propylglutaric acid and* (+)-*methylglutaric acid.*

oxidation, α-substituted succinic acid and a minor quantity of succinic acid might be formed.

On treating the anhydride of α-*iso*-propylglutaric acid with methyl magnesium iodide and subsequent oxidation of the reaction products with chromic acid, α-*iso*-propylsuccinic acid was obtained. As no succinic acid was detected, the reaction can in this case be utilized for one-sided degradation of the substituted glutaric acid.

When the experiment was repeated with the anhydride of *dextro*-rotatory α-*iso*-propylglutaric acid, a *levo*-rotatory *iso*-propylsuccinic acid was obtained. Only slight racemisation had taken place. As the asymmetric carbon atom is not involved in the reactions, it is thereby surely established that the acids with the same configuration have opposite directions of rotation.

The result was not quite unexpected. Recently, one of us has found by another method that α-methylglutaric acid and methylsuccinic acid with opposite directions of rotation must be sterically related [7]. There was of course a certain probability that the same would be true for the *iso*-propyl derivatives. On the other hand, the new results afford additional support for the conclusions regarding the methyl-substituted acids.

As has been stated earlier, methylsuccinic acid and *iso*-propylsuccinic acid with the same mode of optical rotation must also be similar in configurative respect, both giving quasi-racemic compounds with ethylsuccinic acid

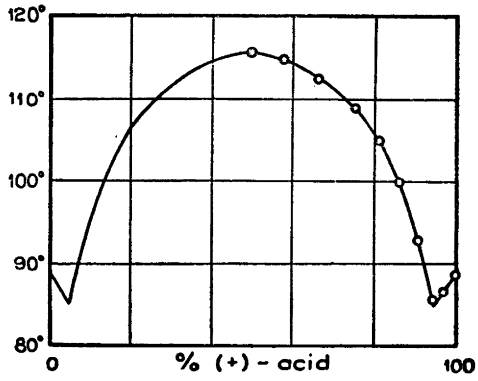


Fig. 3. (+)-*and* (—)-*iso-Propylglutaric acid.*

of opposite mode of rotation [8]. The question can, however, also be settled without the aid of the latter acid. (+)-Methylsuccinic acid and (—)-*iso*-propylsuccinic acid give a melting point curve clearly indicating the existence of a molecular compound 1 : 1, while the acids with the same mode of rotation give a diagram of the common eutectic type (figs. 1 and 2). The melting point curve for the two antipodes of *iso*-propylsuccinic acid (fig. 3) reveals a strong tendency to form a true racemic compound, and obviously the same tendency manifests itself not only against the next lower homologue, but also against the methylsuccinic acid.

In previous communications, the methylsuccinic acid could be sterically joined to the malic acid [8, 9, 10], and the *α-iso*-propylglutaric acid is thus sterically connected to the system of the glyceraldehyde. According to the convention of Fischer [11], the (+)-malic acid should be written (IV), and for the configuratively related forms of the other acids mentioned above we have the projection formulas (V—VII).

Some years ago, Hückel gave a survey of the steric relationships of the terpenes and related substances [12]. He pointed out, that these compounds had not yet been connected sterically to the system of the glucose and the glyceraldehyde. For the terpenoid compounds, he therefore proposed an independent sterical system, referring to the (+)-camphor as standard substance. According to his convention, the part of the molecule acting as the bridge of a bicyclic system is always imagined to lie in front of (above) the plane of the paper. The wellknown formula (VIII) is then considered as a stereoformula and arbitrarily ascribed to the (+)-camphor. The formulas of all compounds sterically related to the camphor can then be written in an unambigous way; for (+)-α-pinene we have for instance the formula (IX) and for (+)-fenchone (X).

By stepwise degradation of (+)-fenchone, Wallach [13, 14] obtained, among other products, *α-iso*-propylglutaric acid (XIII). The intermediate steps are illustrated by the formulas (XI—XII). One of the asymmetric carbon atoms of the fenchone is not involved in the reactions, and the acid obtained was levo-rotatory, having $[\alpha]_D = -15.82°$. It is thus represented by projection formula (VII), and it is now possible to connect the hitherto independent sterical systems of Fischer and Hückel. If (VII) is interpreted in accordance with the Fischer convention, we find on comparing (X) and (XIII) that the bridge in (X) must be situated *behind* the plane of the paper. The same must then of course hold good for the formulas of (+)-camphor (VIII) and (+)-α-pinene (IX).

The two sterical systems are thus by accident not consistent with each other. In this case is seems natural to give precedence to the much older system of Fischer and to change the notation of Hückel. It must be remembered, however, that the original convention of Fischer is not always observed. Several modern textbooks interpret the common projection formulas in the opposite way. It would be very fortunate if unanimity could be reached on this point.

As pointed out by Hückel [12], the so-called (—)-*trans*-menthone (XIV) having two asymmetric carbon atoms is sterically related on one side to the (+)-camphor (same configuration at C_4) and on the other to (+)-pulegon (XV) (same configuration at C_1). In (XIV) the *iso*-propyl group must consequently be situated behind the plane of the paper. As has been stated earlier [8], the methyl group of (+)-pulegon must be situated in front of (above) the plane of the paper. We have here an additional proof of the *trans*-configuration of the menthone.

EXPERIMENTAL

Racemic and dextrogyric iso-*propylglutaric* acid and the corresponding anhydrides were prepared in the manner earlier described by one of us [1].

Degradation of racemic acid. 6.9 g (0.044 moles) of racemic anhydride were dissolved in 150 ml of carefully dried benzene. The Grignard reagent, prepared from 19.1 g (0.135 moles) methyl iodide and 3.15 g magnesium in 40 ml ether, was gradually added. After standing for 20 hours, the solution was heated to the boiling point of the benzene for 15 minutes and then poured out into 500 ml of water, acidified with 10 ml conc. sulphuric acid. The reaction products were extracted with ether. The ether was evaporated and the residue refluxed for 1 hour with a solution of 6 g sodium hydroxide in 60 ml of water. Finally the benzene was driven off with steam.

The different products were separated according to Komppa and Rohrmann [5]. The alkaline solution was acidified with sulphuric acid and distilled with steam; from the remaining solution the keto acid was extracted with ether. The distillate contained the lactone and the unsaturated acid; they were taken up in ether from which the unsaturated acid was extracted with dilute sodium carbonate solution.

Following these directions 4.2 g of »keto acid», 1.7 g of »lactone», and 0.6 g of »unsaturated acid» were obtained. The products were not analysed or further purified. 2.6 g of the »keto acid» were dissolved in a mixture of 95 ml water, 7.7 g conc. sulphuric acid and 4.15 g chromic acid. The mixture was heated on a water bath for 5 hours and refluxed for another 5 hours. About 2/3 of the chromic acid were reduced. After filtration, the solution was extracted with ether and the ether evaporated. The residue, which had a strong odour of acetic acid, was dissolved in water and heated on a steam bath in order to remove volatile acids. The remaining viscous liquid (1.5 g) solidified after standing for some days in a dessicator. It was dried on a porous plate and recrystallised three times from a little water. The remaining product (0.1 g) had m. p. 115.7—116.8° and was identified as racemic *iso*-propylsuccinic acid. An authentic specimen of this acid had m. p. 115.5—116.7°, a mixed sample 115.5—116.8°.

0.0708 g acid: 6.93 ml 0.1263 *N* NaOH.
$C_7H_{12}O_4$ Equiv. wt. calc. 80.1, found 80.9.

Isolation of the acid through the sparingly soluble calcium salt might probably have given a better yield.

Oxidation experiments with the »unsaturated acid» met with no success.

Degradation of (+)-iso-propylglutaric acid. 7.0 g of the corresponding anhydride[1] were treated with methyl magnesium iodide in the manner described above; the yield of »keto acid» amounted to 3.4 g. It was dissolved in a mixture of 125 ml water, 5.4 g of chromic acid and 10 g conc. sulphuric acid, heated on a water bath for 9 hours and then refluxed for 7 hours. The reaction mixture was worked up as described above, yielding 1.45 g of a viscous liquid which partly crystallised on standing. The crystals were separated and recrystallised twice from benzene. As the acid was still rather impure, it was dissolved in a little water and neutralised with ammonia. On addition of calcium chloride solution, the calcium salt was gradually deposited as a crystalline precipitate which was collected after two days. Yield 0.40 g (fraction 1).

The uncrystallisable part of the reaction product and the acid contained

in the benzene mother liquors were combined and converted to calcium salt in the same manner. This salt was purified by reprecipitation. Yield 0.28 g (fraction 2).

The salt fractions were treated with hydrochloric acid, the organic acid was extracted with ether, and recrystallised from a little benzene. It was identified as levogyric *iso*-propylsuccinic acid.

Fraction 1 yielded 0.28 g acid, m. p. 85—97°.

0.0989 g acid: 9.70 ml 0.1263 *N* NaOH.
$C_7H_{12}O_4$ Equiv. wt. calc. 80.1 found 80.7
0.1016 g acid dissolved in water to 4.87 ml: $\alpha_D = -0.37°$: $[\alpha]_D = -17.7°$.

The activity indicates an *iso*-propylsuccinic acid containing 89 % of (—)-form and 11 % of (+)-form. The melting point found fits well in the diagram fig. 3. 0.0162 g of the acid was mixed with 0.0127 g of an authentic specimen of (+)-*iso*-propylsuccinic acid (m. p. 88—89°). The mixture, which ought to contain 50 % of each antipode, had m. p. 115.8—116.8° and gave no depression with an authentic specimen of racemic *iso*-propylsuccinic acid.

Fraction 2 of the calcium salt yielded 0.18 g of an acid with m. p. 84—87°.

0.1055 g acid dissolved in water to 4.87 ml: $\alpha_D = -0.465°$; $[\alpha]_D = -21.5°$.

The activity indicates an acid containing 96 % of the (—)-form and 4 % of the (+)-form. On mixing with a calculated amount of (+)-acid, racemic *iso*-propylsuccinic acid was obtained (no melting point depression with an authentic specimen).

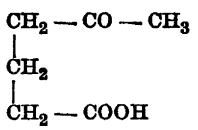
The *melting point diagrams* were determined by Rheinboldt's method [15]. In the figures 1—3 the composition is given in mole-%.

SUMMARY

Dextro-rotatory *α-iso*-propylglutaric acid was by oxidative degradation converted to levo-rotatory *iso*-propylsuccinic acid. The steric relationships of these acids are thus established.

As (—)-*iso*-propylglutaric acid can be obtained on degradation of (+)-fenchone, a number of terpenoid compounds are in this way sterically connected to the system of glyceraldehyde.

If the projection formula of (+)-glyceraldehyde is interpreted in accordance with the original Fischer convention [11], the bridge in the formulas of (+)-camphor, (+)-α-pinene and (+)-fenchone (VIII—X) must be imagined to lie behind the plane of the paper.



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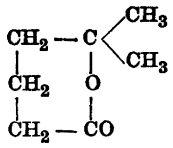

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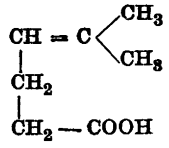

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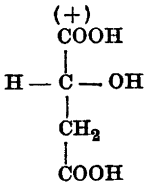

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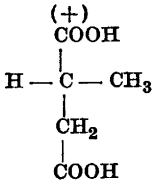

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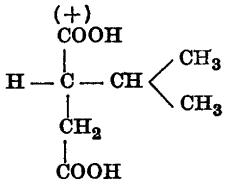

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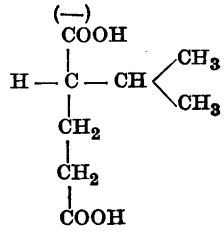

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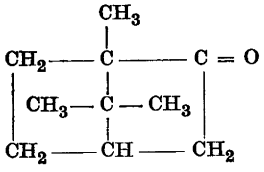

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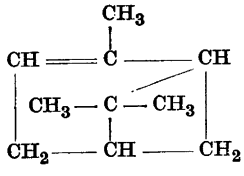

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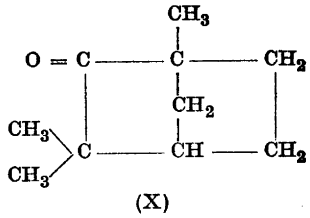

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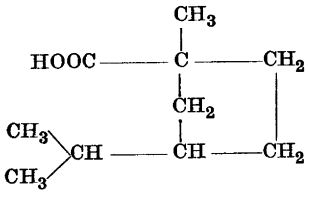

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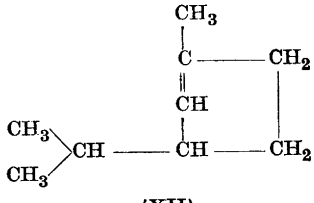

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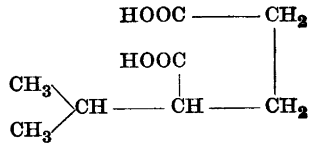

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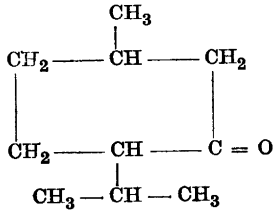

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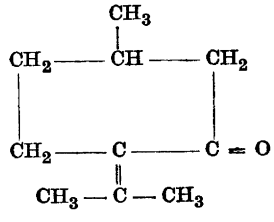

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The Action of Bromine on Furans

A New Synthesis of 2,5-Diacetoxy-2,5-dihydrofuran

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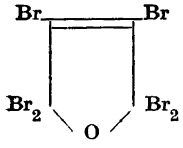
The reaction between bromine and simple furans is believed to be initiated by a 1,4-addition of bromine to the double bonds of the furan nucleus [1], thus yielding a 2,5-dibromo-2,5-dihydrofuran. However, so far only three such preliminarily formed addition compounds have actually been isolated and analyzed. Hill and Hartshorn [2] obtained a hexabromo-dihydrofuran from the interaction of tetrabromofuran and bromine. Moureu, Dufraisse and Johnson [3] prepared a tetrabromo addition compound of furylacrylic acid by the action of a solution of bromine in chloroform at —15°; and Gilman and Wright [4] isolated a dibromo addition product of ethyl furylacrylate by a similar synthesis. In consistency with the conception of 1,4-addition the formulas I—III are to be ascribed to these substances.



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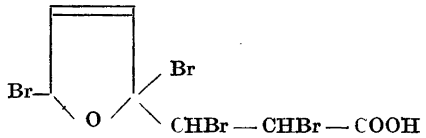

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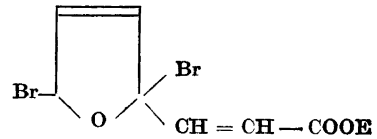


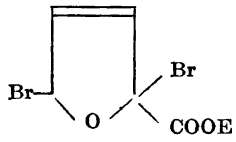
III

Hill and Hartshorn did not propose any formula for their compound, but formula I is in agreement with the fact, that the bromide was hydrolyzed by water to dibromomaleic acid.

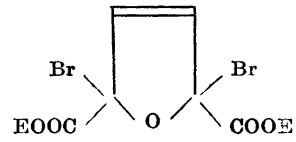
Moureu, Dufraisse, and Johnson suggested that a 1,1-addition of bromine to the oxygen of the furan nucleus had taken place, but in view of the dicussion given earlier, this is not likely (see Clauson-Kaas [1]).

Gilman and Wright took no final decision as to the formula of their dibromo ester, but are inclined to formula III.

The formation of a dibromo compound of ethyl furoate has been claimed by Schiff and Tassinari [5], but the existence of this substance was later questioned by Hill and Sanger [6]. Yet it is not impossible, that Schiff and Tassinari's substance actually has been ethyl 2,5-dibromo-2,5-dihydrofuroate (IV). In this connection it should be mentioned, that the oil obtained by Klinkhardt [7] when treating 2,5-dicarbethoxyfuran with bromine might have a similar structure (V). Still neither of these addition products were analyzed or subjected to a closer study.



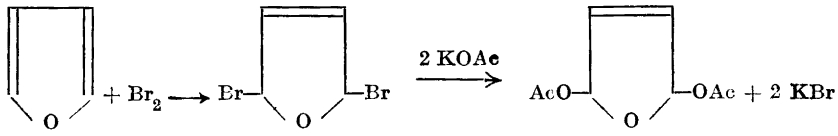
IV



V

The above experiments have not been repeated here; but the action of bromine on furan itself has been investigated and proof of the formation of a 1,4-addition compound, namely 2,5-dibromo-2,5-dihydrofuran, is given.

It was to be expected, that 2,5-dibromo-2,5-dihydrofuran (VI) would be very unstable and no attempt was made to isolate it in a pure state. From earlier experiments on the alkoxylation of furan with methanolic bromine [1] there was some reason to believe that the bromine atoms of dibromodihydrofuran might be sufficiently active to react with metal salts of organic acids to yield acyloxy compounds and metal bromide. Therefore the bromination of furan was undertaken in a solution of acetic acid containing two moles of potassium acetate. In fact the reaction took the expected course and 2,5-diacetoxy-2,5-dihydrofuran (VII) was obtained in a good yield.



VI VII

This synthesis proves that the initial reaction between furan and bromine is a 1,4-addition of bromine to the double bonds of the furan nucleus.

The diacetoxydihydrofuran has earlier been prepared by the action of lead tetraacetate on furan [1] and the identity of the two products was easily established by comparison of the physical properties and by identification of the malealdehyde formed by hydrolysis.

EXPERIMENTAL

20 g of anhydrous potassium acetate (0.2 mole) and 7.25 ml of freshly distilled furan (0.1 mole) are dissolved in 100 ml of perfectly dry acetic acid and the mixture cooled to 10°. A solution of 5 ml of bromine (0.1 mole) in 100 ml of acetic acid is added under efficient stirring during 5—10 minutes. The temperature is kept at 10°. The potassium bromide formed by the reaction is filtered off by suction and the filtrate evaporated in vacuum. When the distillation of acetic acid has ceased, 100 ml of ether is added to the residue. A small precipitate of potassium bromide and potassium acetate is filtered off and washed with ether. The total ether fraction is evaporated on the water bath at ordinary pressure and the residue distilled in vacuum. There is a small fore-run of acetic acid, then the diacetoxy compound distils at 129—132°/9 mm as a perfectly colourless, very viscuous oil (previously found[1] 128—129°/10 mm). Towards the end of the distillation the temperature is allowed to rise to 140°. Yield 13 g = 70 %.

$C_8H_{10}O_5$ (186)	Calc.	C 51.60	H 5.42
	Found	C 51.31	H 5.34

Hydrolysis and identification of malealdehyde as bis-phenylhydrazone was performed as described earlier [1]. Yield of crude product 72 %. Yield after recrystallization 62 %. M. p. 170—172° (Kofler stage, corr. Clauson-Kaas and Fakstorp [8] 171°).

$C_{16}H_{16}N_4$ (264) Calc. N 21.21 Found N 21.32

SUMMARY

The action of bromine on the furan nucleus is discussed and the conception of an initial 1,4-addition is confirmed by the synthesis of 2,5-diacetoxy-2,5-dihydrofuran from furan and bromine in a solution of acetic acid containing potassium acetate.

The analyses have been performed in the most careful way by my colleague Mr. O. Rosenlund Hansen.

During the investigation I have received financial aid from *Tuborg Fondet.*

I am grateful to the Director of *Universitetets kemiske Laboratorium,* Copenhagen, Prof. Dr. A. Langseth, for his kind interest in my work.

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ACTA CHEMICA SCANDINAVICA 1 (1947) 382—387

On the Determination of Reducing Sugars by Titration with the Sugar Solution

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The accuracy with which it is possible to carry out a quantitative determination of free aldoses and ketoses depends on the ratio between the free reducing groups and the glucoside bonds. The reason is that during the analysis itself there occurs a minimal rupture of glucoside bonds whereby new reducing groups are liberated.

The hydrolysis of the glucoside bonds is — other conditions being identical — dependent on the [OH^-]. This was already shown by Kjeldahl[1] with the disaccharides maltose and lactose. The inversion by OH^- of sucrose, which is strictly speaking a non reducing sugar, causes an increase of the »reduction power» with increasing pH, (Spengler, Tödt and Scheurer[2].) The same hydrolysis no doubt occurs to a greater or less extent with all oligo-and polysaccharides upon boiling in an alkaline solution.

The hydrolysis of glucoside bonds also depends on the oxidation-reduction-potential of the oxidizing agent. This was shown with partially broken down starch in our earlier paper »On the Determination of Reducing Sugars».[3]

Especially when the ratio between reducing groups and glucoside bonds is very small — as in the case of small amounts of invert sugar in cane sugar or in slightly broken down starch — the result of reductometric determinations may become problematic without proper corrections. It is possible to correct for the reducing effect of sucrose, but it is impossible to introduce corrections for the cleavage of glucoside bonds in a mixture of oligo- and polysaccharides such as they are formed by degradation of starch with α-amylases. The method involving an oxidizing agent with the lowest oxidation-reduction-potential in

* We wish to express our thanks to the Management of the Tuborg Breweries for good working conditions.

a medium with the lowest pH must in principle be preferable since it will give the most correct — although not the correct — result.

Of the oxidizing agents most generally employed in the determination of reducing sugars, the $Cu^{2+} \rightleftharpoons Cu^{+}$-system has by far the lowest oxidation-reduction-potential [3], and the use of copper methods must therefore, under otherwise identical conditions, cause the least oxidative cleavage of glucoside bonds. Of the different copper methods the preferable one must be the method in which the reaction mixture has the lowest pH and therefore causes the least hydrolytic cleavage of glucoside bonds.

Since copper methods for the determination of reducing sugars by direct titration with the sugar solution have gained a very widespread use, being so rapid, we have, bearing the mentioned points in mind, tried to improve the procedure. The reaction between reducing sugars and alkaline copper solutions is extremely slow at room temperature, and boiling is required to attain a reasonable velocity. But it is impossible to titrate a boiling sugar solution with alkaline copper solution. As there is a deficit of oxidizing agent, the alkali will alter the sugar before the oxidation has taken place. A direct determination is possible, however, by titrating the boiling copper solution with the sugar solution. In the determination of reducing sugars by direct titration a great advance was made by Lane and Eynon [4] when they discovered that methylene blue can be used as an indicator of the end point. Its use is based on the fact that it is reduced and decolorized by minute amounts of reducing sugars, but not so long as any cupric salt is present. Since, however, the reaction is accompanied by the precipitation of red Cu_2O, which remains suspended in the boiling medium, it may prove difficult, especially in poor light, to observe the decoloration of the methylene blue.

A method employing a reagent which contains complexly bound Cu^{2+} and $Fe(CN)_6^{4-}$ in a carbonate buffer solution has the following advantages over methods relying on Fehling's solution, which contains NaOH:

the hydrolytic cleavage of glucoside bonds in oligo- and polysaccharides becomes less,

the Cu^{+} is precipitated as almost white and very slightly soluble cuprous-ferrocyanide which does not interfere with the observation of the decoloration of methylene blue.

We have made no detailed investigation of the precipitate, cuprous-ferrocyanide, but probably it has the same composition as a potassium-cuprous-ferrocyanide investigated first by Messner [5], later by Bhaduri and Sarkkar [6] and lastly by Reihlen and Zimmermann [7], namely $K_2Cu_2Fe(CN)_6$, $7H_2O$.

Table 1. Reagents.

	g/l	moles/l	ml/ analysis	mmoles/ analysis
I $CuSO_4$, $5H_2O$	24.97	0.1	10.00	1.00
II K-Na-tartrate, $4H_2O$	141	0.5	10.0	5.0
II K_2CO_3	138	1.0	10.0	10.0
III $K_4Fe(CN)_6$, $3H_2O$	51	0.12	5.0	0.6
IV Methylene blue in water	2.0	—	—	—

Owing to the acid action of the Cu^{2+}ion, the mixing of the reagents in the prescribed quantities is accompanied by a conversion of 1/5 of the K_2CO_3 into $KHCO_3$, so that the ratio K_2CO_3:$KHCO_3$ becomes 4 : 1.

$$Cu^{2+} + 10\ CO_3^{2-} + 2\ H_2O \rightleftharpoons Cu(OH)_2 + 8\ CO_3^{2-} + 2\ HCO_3^-$$

The pH of such a buffer solution is about 10.4. The $CuSO_4$ and the tartrate solutions must be carefully mixed in order to prevent any precipitation of cupric-ferrocyanide on adding the ferrocyanide solution. If the prescribed ratio $Fe(CN)_6^{4-}$:$Cu^{2+} = 0.6 : 1$ is substantially reduced, Cu^+ will partly be precipitated as Cu_2O; if the ratio is increased no advantage is gained. In Fehlings solution the ratio Cu^{2+}:tartrate is 1 : 4.4. The ratio 1 : 5 is found to be very suitable for our purpose. K_2CO_3 is found to be preferable to Na_2CO_3. To replace copper tartrate with potassium-copper-carbonate in order to eliminate any organic substance [3] proves impossible, since Cu^{2+} is so loosely bound in the potassium-copper-carbonate that cupric-ferrocyanide is immediately precipitated. Cupric-ferrocyanide does not precipitate directly upon mixing of the reagents I, II and III since Cu^{2+} is complexly bound by tartrate. But the complex is not stable enough to prevent a slow precipitation. It is therefore preferable, before starting a series of analysis to mix I and II and first to add III immediately before heating. Any cupric-ferrocyanide precipitated will however dissolve during the heating.

STANDARDIZATION AND METHOD OF TITRATION

For accurate work one should standardize against the same pure sugar under exactly the same conditions as those under which one is working. In the case of glucose one should either use glucose which has been purified by recrystallization from absolute ethanol or anhydrous glucose C. P. All preparations must be dried at 100° C for 1—2 hours. Standard invert sugar solution is prepared by acid hydrolysis of sucrose C. P.[8, 9]. Since it undoubtedly will be impossible for many analysts to prepare maltose of the necessary purity, and

since even the best commercial preparations are not of the desired purity we have in the following table listed relative values for maltose, glucose being put at 100.

The standard solutions shall contain 1.200 g anhydrous glucose or invert sugar, or 2.000 g maltose hydrate per 1000 ml. The standard solution is poured into a 50 ml burette the tip of which is bent twice at right angles, to prevent too much heating of the burette. 10.00 ml of I (automatic pipette!) and 10 ml of II are pipetted into a 100 ml flat bottom flask. After shaking, 5 ml of III are added. Only the $CuSO_4$ solution has to be pipetted accurately. The flask is placed on a wire gauze over a Bunsen burner. The burette is arranged so that the tip of the stopcock extends a few mm into the neck of the flask. 24 ml of glucose solution are added. After shaking, the contents are heated to boiling. Glass bead! When the solution boils, a stopwatch is started. In order to exclude atmospheric oxygen the reaction mixture must at no time cease boiling. After boiling for 2 minutes, 2—3 drops of methylene blue are added, and at intervals of about 15 seconds 3—4 drops of the sugar solution. When all Cu^{2+} has been reduced to Cu^{+}, the methylene blue is reduced whereby the blue colour disappears. The less the overtitration, the slower the colour change will take place. The decoloration is so distinct that the titration can be repeated with but a few drops' deviation. The titration must be completed within a boiling time of 3 minutes $\pm$ 15 seconds. The determination is repeated another 3 times, adding immediately 0.2 ml less sugar solution than the total consumption. The number of milligrams corresponding to 10.00 ml 0.1 *M* $CuSO_4$ is calculated from the mean of the last three determinations. The corresponding values for other sugars may be obtained by multiplying with the factors given in the following table. (The sugars used are the same as described in our previous paper [3]). If great accuracy is desired, the standardization must, as already mentioned, be made against the same sugar as the one which it is desired to determine, and must be repeated each time one of the components of the reagent is renewed. Ordinarily, though, it will only be necessary to standardize against glucose when the $CuSO_4$ solution is renewed.

Table 2. The reduction equivalent (RE) of various sugars.

	RE	Glucose = 100
Glucose, anhydrous	6.0	100
Invert sugar	5.9	101
Galactose	5.2	117
Maltose, anhydrous	7.3	159
Lactose, hydrate	7.9	152

DETERMINATION

To avoid any appreciable change of pH in the reaction mixture, it is absolutely necessary to neutralize acid or alcaline sugar solutions. If the approximate concentration of the sugar in the sample is unknown, one should proceed by the incremental method of titration. 10 ml of the sugar solution are added immediately and the mixture is heated to boiling. Glass bead! After boiling for 1 minute, the sugar solution is added in portions of several milliliters at intervals of 15 seconds. When the blue colour of the copper has almost disappeared, 2—3 drops of methylene blue are added, and the addition of sugar solution continues drop by drop until the blue colour of the indicator disappears. The actual analysis is now arranged on the basis of one or several such preliminary experiments. Since the oxidation of the sugar — though to a slight degree — is dependent on the reaction volume (see later), the final volume must be the same as in the standardization. For higher precision the sugar solution in question must either be suitably diluted or one must add enough water before boiling to make the final volume of the reaction mixture total 50 ml. Finally, care must be taken to have the definitive determination completed within a total boiling time of 3 minutes $\pm$ 15 seconds, and otherwise the analysis has in all details to follow the same procedure as that of the standardization.

The standard error of the titration is about 0.2 ml, corresponding to a relative error of about 0.8 %.

DISCUSSION

The effect of the reaction volume has only been investigated in the case of glucose, and is evident from the following figures:

Total reaction volume in ml	Titration with ml	RE
45	20	6.08
50	25	6.05
55	30	6.03

The reduction equivalent, RE, decreases with increasing reaction volume. If the total reaction volume is kept between 45 and 55 ml, one may, without committing too great errors, reckon with a constant RE. If particular accuracy is required, the final volume should be as close to 50 ml as possible.

The oxidation of the sugars does not proceed stoichiometrically. As in other methods employing Cu^{2+} or $Fe(CN)_6^{3-}$, galactose is oxidized less than glucose. Glucose and invert sugar reduce 6 equivalents Cu^{2+} per molecule.

This corresponds, theoretically, to a break-down to a pentonic acid. The disaccharides maltose and lactose show a larger consumption of Cu^{2+}, owing to hydrolysis of the glucoside bonds.

During the last year the method has been employed in hundreds of routine analyses at the Tuborg Laboratory and at the Laboratory of Løvens kemiske Fabrik, Copenhagen.

SUMMARY

A rapid method of determination of reducing sugars by titration with the sugar solution is described. A reaction mixture is chosen which gives the lowest hydrolytic and oxidative cleavage of glucoside bonds in oligo- and polysaccharides. The reaction mixture contains Cu^{2+}, tartrate and $Fe(CN)_6^{4-}$ in a carbonate buffer; pH about 10.4. Cu^{+}, formed by reaction of Cu^{2+} with the sugar, is under these conditions precipitated as almost white potassium-cupro-ferrocyanide, which does not interfere with the observation of the decoloration of methylene blue indicator.

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The Number Average of Diffusion Constants

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The diffusion curve of a normally diffusing substance with diffusion constant D_i and concentration c_i is obtained from the equation

$$y_i = \frac{c_i}{2\sqrt{\pi D_i t}} \cdot e^{-\frac{x^2}{4 D_i t}} = \psi(c_i, D_i) \qquad (1)$$

where x is the distance from the original, sharp boundary, y_i is the concentration gradient $\frac{dc_i}{dx}$ and t is the time. If several substances with different diffusion constants diffuse with a common original boundary we get a sum of diffusion curves:

$$y = \sum_i \psi\,(c_i, D_i) \qquad (2)$$

It has been shown [1, 2], that the formula

$$D_m = \frac{1}{2t} \cdot \frac{m_2}{m_0} \qquad (3)$$

gives a weight average of the diffusion constants:

$$D_m = \frac{\Sigma\, c_i D_i}{\Sigma\, c_i} \qquad (4)$$

Here, m_2 is the second moment of the curve, defined by the integral

$$m_2 = \int_{-\infty}^{+\infty} x^2\, y\, dx \qquad (5)$$

and m_0 is the "zero'th" moment, which is equal to the area A between the curve and the x-axis. This area is proportional to the concentration:

$$m_0 = A = \int_{-\infty}^{+\infty} y \, dx = k \, c = k \int dc \tag{6}$$

The same average (equation (4)) is obtained by using Boltzmann's equation

$$D = \frac{1}{2\,ty} \cdot \int_{x}^{\infty} x \, y \, dx \tag{7}$$

and forming the quotient $\frac{\int D \, dc}{\int dc}$, the integrations extended over the whole curve.

The formula

$$D_A = \frac{A^2}{4\pi t \, H^2} \tag{8}$$

where H is the maximum ordinate, gives the average

$$D_A = \left(\frac{\Sigma \, c_i}{\Sigma \, \frac{c_i}{\sqrt{D_i}}} \right)^2 \tag{9}$$

On differentiating (1) we get

$$\frac{dy_i}{dx} = - \frac{x \, y_i}{2 \, D_i \, t} \tag{10}$$

By summation of (10) for a constant x we get

$$\frac{\Sigma \, dy_i}{dx} = \frac{dy}{dx} = - \frac{x}{2t} \cdot \Sigma \, \frac{y_i}{D_i} \tag{11}$$

If D_i is a constant ($= D$) for all the components in the mixture this equation gives

$$D = -\frac{x\ \Sigma\ y_i}{2t} \cdot \frac{dx}{dy} = -\frac{x \cdot y \cdot dx}{2t \cdot dy} \tag{12}$$

If D_i varies and the formula (12) is applied at the point x, we get the average

$$D_x = \frac{\Sigma\ y_i \cdot dx}{\Sigma \frac{y_i}{D_i} \cdot dx}$$

or, as $y_i\ dx = k\ dc_i$

$$D_x = \frac{\Sigma\ dc_i}{\Sigma \frac{dc_i}{D_i}} \tag{13}$$

The number average is defined as

$$D_n = \frac{\Sigma\ c_i}{\Sigma \frac{c_i}{D_i}} = \frac{\int dc}{\int \frac{dc}{D}} \tag{14}$$

and thus D_x is a number average at the point x. As D_i is independent of x, we can perform an integration over the whole curve and get

$$k \cdot \int \frac{dc}{D} = \int \Sigma \frac{y_i}{D_i}\ dx = \int \frac{\Sigma\ y_i\ dx}{D_x} = \int \frac{y\ dx}{D_x} \tag{15}$$

This equation can be used for calculating a number average D_n of the diffusion constants.

It is also possible to get a formula for the direct calculation of D_n from the curve. The introduction of D_x from equation (12) into (14) and (15) gives

$$D_n = \frac{\int y\ dx}{\int \frac{y\ dx}{D_x}} = -\frac{\int y \cdot dx}{2t \int \frac{y\ dx \frac{dy}{dx}}{x\ y}} = -\frac{1}{2\ t} \cdot \frac{\int y \cdot dx}{\int \frac{\frac{dy}{dx} \cdot dx}{x}} \tag{16}$$

In a still simpler form this can be written

$$D_n = -\frac{1}{2t} \cdot \frac{A}{\int \frac{dy}{x}} \tag{17}$$

where the integration should be extended over the whole curve. The equations (16) and (17) thus make it possible to calculate the number average D_n. Jullander [3] and, later, Singer [4] have shown that D_n should be especially valuable for the calculation of the weight average of molecular weights by using Svedberg's formula. As no method for the calculation of D_n has been available, the formulas derived here should fill a gap and give further possibilities of characterizing the polydispersity of a substance.

SUMMARY

The formula

$$D_n = -\frac{1}{2t} \cdot \frac{A}{\int \frac{dy}{x}}$$

gives the number average, $\frac{\int dc}{\int \frac{dc}{D}}$, of the diffusion constants of a mixture. Here x is the distance from the original, sharp boundary, y is the concentration gradient, and t is the time. A is the area $\int y dx$, and the integrations shall be extended over the whole curve.

The formula derived gives new possibilities of characterizing the polydispersity of a substance.

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Adsorption Analysis of Alkaloid Salts of Polyvalent Acids

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Adsorption methods have been used before for the analysis of alkaloids. Merz and Franck [1] and later Björling [2] and Christiansen [3] sucked alcoholic solutions of extracts containing alkaloids through aluminium oxide and eluted with alcohol. In the elute they obtained mainly the free alkaloid bases which could then be titrated. Reimers and Gottlieb [4] and Reimers [5] applied the same principle to pure alkaloid salts in alcoholic solution, and were able, in many cases, to resolve the salt quantitatively into the free base, which was eluted, and the acid, which was adsorbed. The methods of analysis thus worked out were simple and exact. On some alkaloid salts, however, the method failed: the base was eluted slowly or incompletly or else the alkaloid salt passed through the column. Björling and Ehrlén [6] tried to explain these irregularities, and found that a necessary condition for success was apparently that the base should not be too strong. Another condition, naturally, was that the base, once liberated, could be eluted easily. The process was believed to imply an exchange of ions in accordance with the equation:

$$\text{Oxide-O-Na} + \text{BH}^+ = \text{Oxide-O-H} + \text{B} + \text{Na}^+$$

where Oxide-O-Na = hypothetical aluminate in the oxide
B = base.

Ungerer [7] came to similar conclusions in his work on permutite and alkaloid salts.

Most of the experiments had been carried out on hydrochlorides of the bases. Now, even if the base was weak and easily eluble, it could not always be eluted readily from its salts with polyvalent acids. Thus, from codeine hydrochloride codeine was eluted to 100 % with a certain amount of alcohol, but from codeine phosphate this number decreased to 60—70. Similar be-

haviour was observed in the pair of substances atropine hydrochloride — atropine sulphate.

The object of this paper is to attempt to to throw some light on these problems and to work out a method of analysis for salts of bases and polyvalent acids.

There are relatively few earlier investigations on this subject.

Synthetic resins. Bhatnagar, Kapar and Puri [8] found that sulphuric acid was adsorbed more on basic synthetic resins than nitric and hydrochloric acids. Myers [9] and Myers, Eastes and Urquhart [10] showed that an alkylene polyamine resin bound phosphoric, sulphuric, and hydrochloric acids in quantities corresponding to 8.0, 4.1 and 2.46 millimoles per gram adsorbent, quite an important difference.

Coal. Phelps and Peters [11] found that some organic acids were adsorbed on coal only as molecules, not as ions. The adsorption was greatest at the pH of the free acid. Régnier *et al.* [12, 13, 14, 15] showed that various salts of the novocaine base were adsorbed on coal to a different degree. Dibasic citrate was adsorbed best, followed in order by monobasic citrate, phenyl propionate, hydrochloride, and isobutyrate. Base and acid were adsorbed to approximately the same extent, and it seemed probable that the salts were adsorbed as molecules, not as ions. In addition, the sodium salts of the same acids behaved similarly. In mixtures of novocaine salts and sodium salts it was evident that one of the components influenced the adsorption of the other.

Aluminium oxide. Lottermoser and Edelmann [16] revealed considerably different degrees of adsorption for different ammonium salts on aluminium oxide in aqueous solution. Schwab and Dattler [17] were able to separate some inorganic an-ions in aqueous solution by means of »acid» aluminium oxide. The sulphate was adsorbed more than the chloride. Kuhn and Wieland [18] were able to elute a »Wuchsstoff» out of acid aluminium oxide with sodium sulphate, but not with sodium chloride. The sulphate was bound more firmly than the »Wuchsstoff», which was in turn bound better than the chloride.

Grettie and Williams [19] and Whitehorn [20] adsorbed alkaloids, amino compounds, etc., on various adsorbents and found some rules regulating the adsorption.

EXPERIMENTS

As adsorbent was chosen aluminium oxide, standardised by Brockmanns method [21], which has constant properties and is now produced by several factories. It was tested in the following way.

In most of the experiments 10 g of the oxide was placed in a glass-tube, 1 cm wide, fitted at one end with a perforated cork, covered with a plug of cotton-wool.

Blind test. Alcohol of different concentrations was sucked through the column, the filtrate was diluted with an equal volume of water, and titrated with one tenth normal hydrochloric acid using bromphenol blue as an indicator. The amount of acid used up did not exceed 0.01—0.02 ml for every 25 ml of 95 or 85 % (vol.) alcohol. With 75 % alcohol the consumption increased to 0.6 ml.

A low consumption of acid was also obtained when 5 ml of the 95 or 85 % alcohol was mixed with 2 drops of 2 *N* hydrochloric acid or sulphuric acid or with the same amount of sodium hydroxide, and the mixture eluted with the alcohol in question.

The blind consumption was, thus, very small and independent of the presence of moderate quantities of acid or alkali. Too much water in the alcohol must, however, be avoided.

Adsorption test. About 0.1200 g of procaine hydrochloride was dissolved in 5 ml of alcohol, the solution was transferred to the top of the column by small amounts of alcohol, and was sucked into it. The column was then washed with successive amounts of alcohol of 5 ml each. When 10 ml of the elute had been collected it was titrated with one tenth normal hydrochloric acid and the alcohol concentration was adjusted to 50 % at the end of the titration (bromphenol blue, green colour, see Baggesgaard-Rasmussen and Reimers [22]). The next 5 ml of the elute was collected in the same flask and titrated in the same manner, and so on until the consumption of acid decreased to zero. This happened after one or two elutions, when the total quantity of acid corresponded to 100 % of the procaine base contained in the hydrochloride.

As a check the total filtrate was evaporated to 5—10 ml on a water-bath and extracted exhaustively with chloroform, which was drawn off, filtered, and distilled off almost completely. The residue was mixed with 10.00 ml of one tenth normal hydrochloric acid, the rest of the chloroform was evaporated, and the solution was titrated back with one tenth normal sodium hydroxide (methyl red). Again, the results corresponded to 100 % of the procaine base (see *e. g.* table 1).

For the main experiments procaine was chosen as a test substance. Alcoholic solutions of different salts of the base were prepared in the following manner.

A procaine hydrochloride solution was mixed with excess of sodium carbonate and extracted with chloroform which was then dried and distilled off. The resultant procaine base was dissolved in ethanol (99.5 vol. %) and the strength of the solution was adjusted to approximatively 0.12 mol (bromphenol blue). 20.00 ml of this base solution was pipetted into a 25 ml volumetric flask and neutralized with a calculated amount of an acid, 2-normal aqueous solution (about 1.2 ml). The flask was filled with alcohol (95 vol. %) up to the mark. The solution contained procaine in about one tenth molar concentration in 94 vol. % alcohol.

Above all, it seemed necessary to find out what substances were carried down into the elute. For this purpose 5.00 ml of a procaine salt solution was pipetted on the top of the oxide column and sucked into it. Elution was performed with 20 ml of alcohol, the elute was then diluted with 15 ml of water and titrated potentiometrically with one tenth normal hydrochloric

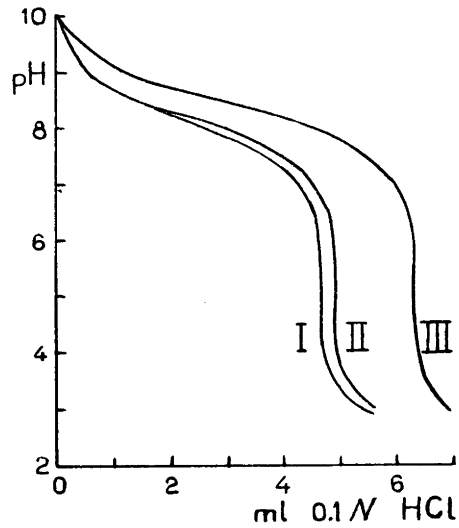

Fig. 1. Potentiometric titration curves.
I. Procaine tartrate eluted with alcohol.
II. Procaine hydrochloride, acetate, or borate eluted with alcohol.
III. Procaine base eluted with alcohol and procaine base titrated directly.
The initial concentrations of the solutions are the same in I and II and somewhat greater in III.

acid (glass electrode-calomel electrode). The titration curves were always congruent, no matter what acid formed the an-ion of the procaine salt. They also ran parallel to the curves obtained on titration of a procaine base solution under the same conditions (fig. 1). Appreciable amounts of interfering substances — *e. g.* aluminium ions or weak acids — could not, therefore, have been eluted.

Qualitatively, no negative ion from the salt could be detected in the elutes. On the contrary, they were all found in the upper parts of the column not in the lower parts. The same was later found to be the case with other alkaloid salts.

After evaporation of the alcohol from the elutes, their contents of procaine base were determined as before by extraction with chloroform. The same values were obtained as with direct titration of the elutes (see table 1).

Thus, it seemed to be fully established that the oxide retains all the acid contained in the procaine salts and that all the alkali titrated in the elutes is procaine base and nothing else.

The next question to be answered was: is the base eluted differently from the various procaine salts? Figure 2 shows that this is the case. The percentage of the base found in 10—20 ml of the elute was 100 % for procaine hydrochloride, nitrate, acetate, borate *, benzoate, and monochloro-acetate. On

* 1 mole of boric acid + 1 mole of the base.

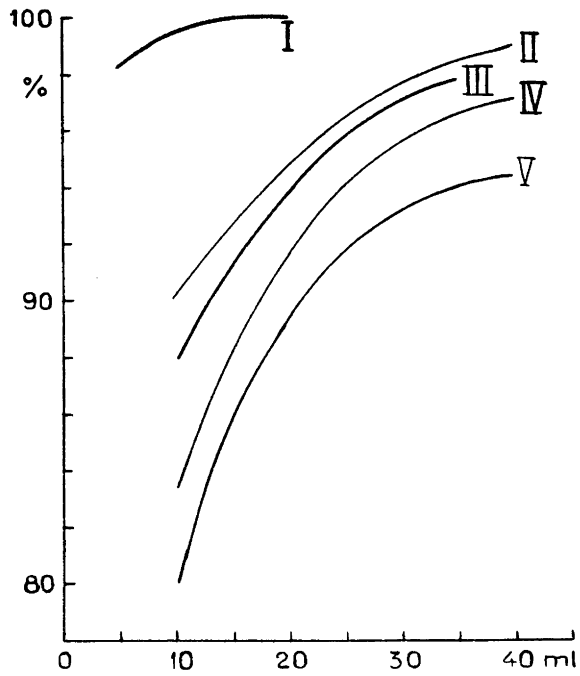

Fig. 2. Procaine base eluted from different salts. Abscissa: Volume of alcohol used for the elution. Ordinata: Yield of procaine base in per cent of the calculated value.
Curve I from procaine hydrochloride, nitrate, acetate, monochloro-acetate, borate, and benzoate.
Curve II from procaine citrate. Curve IV from procaine oxalate.
» III » » sulphate. » V » » tartrate.
The values on which the curves are based are the averages of several experiments.

Table 1. Procaine base eluted from different salts.

Procaine salt investigated	Per cent of the calculated value found		Eluted with ml alcohol
	on direct titration of the elute	after extraction of the elute	
Hydrochloride	100.0	100.0	10
	100.2	100.4	10
Tartrate	94.0	93.6	35
	95.8	95.2	40
Acetate	99.7	99.2	15
Citrate	98.4	98.4	35
Benzoate	100.6	101.0	15
Monochloro-acetate	99.4	98.8	15
Oxalate	97.1	97.1	40
	96.8	97.0	35

the other hand, the neutral procaine salts of sulphuric, tartaric, citric, and oxalic acid behaved differently. The elution of the base was slower, and a 100 % recovery was never attained, or at least only after a very long time. A simple explanation is that these procaine salts are less soluble in alcohol than the salts that behave »normally». This is, indeed, the case with procaine sulphate and procaine oxalate, which formed supersaturated solutions when prepared as described above. The solubilities of some of the salts were determined by shaking for 8 hours and evaporating the saturated solution (for results see table 2).

Table 2. Approximate solubilities of some procaine salts.

Solvent: 95 vol. % alcohol. Temperature: 17—19°.

	Grams of salt in 100 ml solution
Oxalate	0.6
Sulphate	0.8
Hydrochloride	4.4
Tartrate	7.5
Monochloro-acetate	11.8
Benzoate	> 50
Acetate	> 50
Citrate	> 50

It is evident that the solubility of procaine tartrate or citrate has no connexion with their abnormal behaviour. The explanation must be that the salts of these poly-basic acids are bound more firmly to the oxide, depending on the nature of the acid (*cf.* 9—15), so that the exchange of ions cannot take place as easily as with the salts of mono-basic acids.

This theory is supported by the following experiment. In a column used for the analysis of procaine tartrate eluted with 15 ml of alcohol, the procaine and the tartaric acid were found together in the uppermost parts of the oxide. No acid and scarcely any base could be detected in the lower parts of the column.

An important part of the task of solving the problem is obviously to find out some means of eluting the base totally out of the »abnormal» salts. It is impossible to increase the elutive power of the alcohol by diluting it with water. Apart from the fact that the blind consumption of acid will be too great, the diluted alcohol will elute the procaine partly as salt. Addition of the strongly polar base pyridine proved to be of no value as it was eluted together with the procaine, thus causing a considerable error on titrating.

However, the addition of other compounds was more successful. If the procaine tartrate solution was mixed with 2 drops of 2 *M* hydrochloric acid,

Table 3. Elution of procaine base from the tartrate. The influence of certain additions.

To 5 ml of procaine tartrate solution is added			The yield of procaine base in per cent of the calculated value, when eluted with alcohol ml							Per cent found on extraxtion
Compound	Amount	Solvent	10	15	20	25	30	35		
Nil			80.7	85.9	89.6	91.6	91.6			
HCl	0.10 ml 2 *M*	water	83.6	88.3	90.3	92.3	93.7	93.7		
			84.4	90.5	92.3	94.1	96.3	96.3		
HCl	0.25 ml 2 *M*	water	98.9	100.2	100.4	100.4				
			98.0	99.4	100.6	100.6				100.6
HNO_3	0.25 ml 2 *M*	water	92.7	96.7	98.6	99.2	99.2			
			90.9	95.7	98.8	99.4	99.4			
HAc [3])	0.25 ml 2 *M*	water	84.3	89.0	91.1	93.1	94.9	96.1		
HBz [4])	0.25 ml 2 *M*	alcoh.	83.1	87.6	90.9	92.9	96.3			
H_2Ta [5])	0.25 ml 1 *M*	water	66.5	75.0	79.7	83.5				
			65.0	72.4	77.8	80.2	82.1	83.1	89.7 [2])	88.0
NaOH	0.25 ml 2 *M*	water	98.8	99.0						
KOH	1.00 ml *M*/2	alcoh.	95.7	97.6	99.0					
NH_3	0.25 ml 2 *M*	water	96.4	97.4						
LiCl	0.25 ml 2 *M*	water [1])	96.7	98.2	100.6	100.6				100.6
			94.9	97.8	99.8	99.8				98.6
LiCl	0.10 ml 1 *M*	alcoh.	85.0	89.8	91.7	93.7	95.3	97.2		
			81.1	87.0	90.9					
NaJ	1.00 ml 1.3 *M*	alcoh.	100.0	100.0						100.4
			100.0	100.0						
KJ	1.00 ml *M*/2	alcoh.	90.6	100.5	100.5					
			99.8	99.8						
NH_4Br	1.0 ml 0.6 *M*	alcoh.		100.0	100.0					
				100.5	100.5					
$MgCl_2$	1.0 ml 0.7 *M*	alcoh.	92.5	97.2	98.6	99.8	99.8			
			98.4	98.4						
$CaCl_2$	0.5 ml 0.2 *M*	alcoh.	82.9	88.6	92.2	93.7	94.9	96.0		
	1.2 ml		84.8	90.5	92.8	93.5	94.9	96.0		

[1]) + 0.1 ml of water, otherwise precipitate.
[2]) Eluted with 65 ml of alcohol.
[3]) HAc = acetic acid.
[4]) HBz = benzoic acid.
[5]) H_2Ta = tartaric acid.

equivalent to 40 % of the base, the elution of this solution still gave the same result as before, *i. e.* incomplete recovery of the base. But when 5 drops of the acid, corresponding to 100 % of the base, the process was carried out just as easy as with pure procaine hydrochloride. Easy elution was brought about, too, by adding an equal amount of nitric acid, but not by acetic, benzoic, or tartaric acids. Indeed, the latter acid made the elution still more incomplete (see table 3).

Likewise, it was possible to facilitate the elution of the base by adding equivalent quantities of aqueous sodium hydroxide, or of ammonium hydroxide, or of an alcoholic potassium hydroxide solution. In these last cases, however, the time of elution was seriously prolonged, for precipitates were formed which obstructed the passage through the oxide. The precipitates apparently consisted of alkali tartrates, which are little soluble in alcohol.

Good results were also obtained if the procaine tartrate solution was mixed with equivalent amounts of alcoholic solutions of certain salts before entering the column, or if the elution was performed with these salt solutions instead of alcohol. Thus, 100 % of the base was found in elutes of 15 to 20 ml volume with LiCl, $LiNO_3$, NaJ, NaBr, KJ, NH_4Br, and $MgCl_2$. With $CaCl_2$, $Ca(NO_3)_2$, and $SrCl_2$ complete elution failed.

Table 4. Elution of procaine base from the tartrate with alcoholic solutions of certain salts.

Composition of salt solution	The yield of procaine base in per cent of the calculated value, when eluted with salt solution ml						Per cent found on extraction
	10	15	20	25	30	35	
LiCl 0.1 *M*	94.9	99.4	100.6				99.4
	92.3	99.2	100.6				
LiCl 1 *M*	99.6	101.4					
	98.6	100.5	101.6				99.6
$LiNO_3$ 0.06 *M*	99.2	100.2					
	100.2	100.2					
NaBr 0.8 *M*	99.2	100.6	100.6				
	97.2	99.4	100.8	100.8			
$CaCl_2$ 0.2 *M*	73.8	75.3	76.6	77.3			76.1
	73.2	74.1	75.5	76.4	77.6	77.6	78.3
$Ca(NO_3)_2$ 0.05 *M*	79.3	81.3	83.5	85.8	85.8		
	80.3	80.3					
$SrCl_2$ 0.075 *M*	75.0	75.0					
	77.0	77.8	79.9	79.9			

. Nitric acid seems to act more slowly than hydrochloric acid, but the phenomenon has not been investigated further.

Ammonia does not appear to have a full effect.

The addition of magnesium chloride apparently caused precipitates in the strongly basic column, for the surface of the oxide became hard and almost impermeable to the solution. Hard surfaces were also obtained with the alkali hydroxides.

Additions of less than the equivalent of the procaine present (0.5 milliequivalents) did not have the desired effect, *e. g.* hydrochloric acid 0.2 milliequivalents and lithium chloride 0.1 millieq.

Experiments with procaine citrate gave the same results as with the tartrate (*cf.* tables 3 and 4). The addition of hydrochloric acid or sodium iodide gave a full elution of the base with alcohol, as did elution with lithium nitrate. Magnesium chloride had a doubtful effect, calcium chloride acted in a positive manner but »poisoned» the oxide. The figures resemble those obtained with procaine tartrate and are omitted here.

The experience gained from experiments with procaine was applied to other alkaloid salts, some of which have formerly resisted attempts at analysis by means of adsorption. The salts tested hitherto are: codeine phosphate, atropine sulphate, dicodid bitartrate, ephedrine sulphate, benzedrine sulphate, and oxedrine tartrate. Since it is the simplest method, elution with alcoholic salt solutions only has been tested. The results are shown in table 5.

Sodium bromide in alcohol is evidently an excellent eluent. In the case of oxedrine tartrate it failed, owing to the fact that the base itself could not be eluted quantitatively. Addition of salt, however, had a positive effect.

The mechanism of the processes involved in the experiments described on the preceding pages has not yet been fully established. The investigation has been continued on other compounds and under different conditions. Separation analyses will be tried.

SUMMARY

Several organic bases are eluted with alcohol from salts of mono-valent acids by adsorption analysis in an alcoholic medium using aluminium oxide, standardised by Brockmanns method, easily and quantitatively. A volumetric assay has previously been based on this fact. From salts of poly-valent acids the elution of base is slow and incomplete, but can be increased by the addition of certain acids, bases, or salts, or by performing the elution with an alcoholic salt solution. A simple mode of quantitative determination of a number of alkaloid salts is described.

Table 5. Elution of free base from various alkaloid salts by means of salt solutions.

	The yield of free base in per cent of the calculated value after elution with ml							Per cent found on extraction
	5	10	15	20	25	30	45	
0.2 g *codeine phosphate* dissolved in 1 ml H_2O + 10 ml alcohol. Eluted with:								
Alcohol	25.1	53.5	66.0	68.3				
Continued with $LiNO_3$[1])	71.8	82.2	97.2	99.1	100.2			100.2
Alcoh. NaBr 0.1 *M*		89.1	99.6	100.2	100.2			100.4
		96.4	99.8	100.2	100.2			99.5
0.2 g *atropine sulphate* dissolved in 5 ml alcohol. Eluted with:								
Alcohol	72.5	77.3	83.1	89.2	91.1	92.2	94.3	95.1
Alcohol	70.2	80.3	84.8					
Continued with $LiNO_3$[1])	88.5	101.4						
Alcoh. $LiNO_3$ 0.3 *M*	87.1	99.0	100.0	100.9				
Alcoh. NaBr 0.1 *M*	87.0	99.0	99.7	99.7				99.9
	94.0	99.4	99.4					99.2
0.25 g *dicodid bitartrate* dissolved in 1 ml H_2O + 5 ml alcohol. Eluted with:								
Alcohol	29.9	51.6	58.3					
Continued with $LiNO_3$[1])	59.7	81.9	96.8	98.5	100.1			
Alcoh. NaBr 0.1 *M*		90.4	99.8	100.2	100.2			99.1
		98.7	99.3	100.6	100.6			100.8
0.1 g *ephedrine sulphate* dissolved in 1 ml H_2O + 5 ml alcohol. Eluted with:								
Alcohol	47.9	59.8	65.3	71.5	74.5	76.5	84.7[2])	83.6
Alcoh. KI 0.1 *M*	64.5	98.4	100.4	100.4				100.3
Alcoh. NaBr 0.1 *M*	79.2	94.2	99.6	100.7				100.9
0.1 g *benzedrine sulphate* dissolved in 1 ml H_2O + 5 ml alcohol. Eluted with:								
Alcohol	39.1	47.9	53.6	58.9	63.0	64.9	67.7	
Alcoh. NaBr 0.1 *M*	41.9	78.4	94.5	98.1	100.6	100.6		100.6
	39.5	72.4	95.0	100.8	100.8			99.1

[1]) 0.3 *M* alcoholic solution.
[2]) Eluted with 50 ml of alcohol.

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The Inactivation Velocity of Penicillin G by Acids as a Function of Temperature

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In a previous paper [1], the inactivation velocity of penicillin G in acid solution was investigated at 30° C and at varying hydrogen ion concentrations. In the present work, the effect of a variation in temperature on this process is treated.

THEORY

In the paper mentioned above, the following equation is given for the relation between the rate of inactivation and the hydrogen ion concentration at constant temperature and salt concentration:

$$k = \left(\frac{k_+}{K_+} \cdot c_{H^+} + \frac{k_\pm}{K_\pm} \cdot K_0\right) \frac{c_{H^+}}{K_0 + c_{H^+}} \qquad (1)$$

At a constant hydrogen ion concentration, the terms on the right side of this formula, k_+, $k_\pm$, K_+, and $K_\pm$, K_0 are temperature dependent.

The dissociation constants vary with the temperature in the following way:

$$\frac{dK_+}{dT} = K_+ \frac{Q_+}{RT^2}\,; \quad \frac{dK_\pm}{dT} = K_\pm \frac{Q_\pm}{RT^2}\,; \quad \frac{dK_0}{dT} = K_0 \frac{Q_0}{RT^2} \qquad (2)$$

where Q_+, $Q_\pm$, and Q_0 are the molar heat absorptions at the corresponding dissociation processes:

$$\begin{aligned} HPnH^+ &\rightleftharpoons HPn + H^+ \\ Pn^-H^+ &\rightleftharpoons Pn^- + H^+ \\ HPn &\rightleftharpoons Pn^- + H^+ \end{aligned} \qquad (3)$$

For the velocity constants k_+ and $k_\pm$ the variation with the temperature is given by

$$k_+ = Z \cdot e^{-\frac{E+}{RT}} \quad \text{and} \quad k_\pm = Z \cdot e^{-\frac{E\pm}{RT}} \tag{4}$$

Each of these two equations refers to one of the two velocity determining processes, *viz.*

$$HPnH^+ \longrightarrow Pn' \quad \text{and} \quad PnH^+ \longrightarrow Pn' \tag{5}$$

In (4), Z denotes the number of collisions between one penicillin molecule and water molecules per unit of time; E_+ and $E_\pm$ denote the activation energies. This holds under the following suppositions:

The activation of the reacting penicillin molecules occurs exclusively by collision with water molecules, and the activation energy is not gathered from degrees of freedom other than the translatory one.

All collisions the energy of which surpasses the activation energy cause activation, irrespective of the orientation of the penicillin molecule at the moment of the collision.

All activated molecules are subjected to the irreversible process (5) before the activation energy has time to spread by new collisions with water molecules.

If these conditions are not fulfilled — and we can scarcely assume that they are — we obtain equations conformable to (4), where Z, E_+, and $E_\pm$ only should be attributed other values than the collision number and the activation energies.

Assuming that Z is independent of the temperature, we obtain

$$\frac{dk_+}{dT} = \frac{k_+}{T^2} \cdot \frac{E_+}{R} \qquad \frac{dk_\pm}{dT} = \frac{k_\pm}{T^2} \cdot \frac{E_\pm}{R} \tag{6}$$

The equations (2) and (6) indicate the temperature dependence of the variable quantities of equation (1). From this, we find as an expression for the variation of the total decomposition velocity as a function of the temperature

$$E_A = -R\frac{d\ln k}{d\frac{1}{T}} = \frac{1}{k} \cdot \frac{k_+}{K_+} \cdot c_{H^+} \left[(E_+ - Q_+) \frac{c_{H^+}}{c_{H^+} + K_0} - Q_0 \frac{c_{H^+} \cdot K_0}{(c_{H^+} + K_0)^2} \right]$$

$$+ \frac{1}{k} \cdot \frac{k_\pm}{K_\pm} \cdot c_{H^+} \left[(E_\pm - Q_\pm + Q_0) \frac{K_0}{c_{H^+} + K_0} - Q_0 \frac{K_0^2}{(c_{H^+} + K_0)^2} \right] \tag{7}$$

where E_A denotes the Arrhenius critical increment.

In order to arrive at expressions which are less complicated and better suited for experimental checking, we shall examine especially the conditions prevailing at very low and very high hydrogen ion concentrations. For this purpose, equations (10) and (11) from the previously mentioned paper [1] will be used:

$$k = \frac{k_+}{K_+} \cdot c_{H^+} \quad \text{for } c_{H^+} \gg K_0 \tag{8}$$

$$k = \frac{k_\pm}{K_\pm} \cdot c_{H^+} \quad \text{for } c_{H^+} \ll K_0 \tag{9}$$

By inserting these conditions into (7) we obtain

$$-R \frac{d\ln k}{d\frac{1}{T}} = E_+ - Q_+ = E_{A_+} \quad \text{for } c_{H^+} \gg K_0 \tag{10}$$

$$-R \frac{d\ln k}{d\frac{1}{T}} = E_\pm - Q_\pm = E_{A_\pm} \quad \text{for } c_{H^+} \ll K_0 \tag{11}$$

where E_{A_+} and $E_{A_\pm}$ denote Arrhenius' critical increments for the two processes.

In other words, for low and high hydrogen ion concentrations we should find a linear relation between the logarithm of the velocity constant and the reciprocal of the absolute temperature, in agreement with Arrhenius' law, on the supposition that E_+, $E_\pm$, Q_+ and $Q_\pm$ are constant.

For hydrogen ion concentrations of the same order of magnitude as K_0 we obtain a curvilinear interdependence between the logarithm of the velocity constant and the reciprocal temperature. Here, — however, not at low and high hydrogen ion concentrations — Q_0 enters the expression for the temperature dependence. This is due to the fact that, in this case, the process consists of two parallel processes, viz. the inactivation of penicillin molecules with undissociated carboxyl groups and, on the other hand, the inactivation of penicillin ions where the hydrogen ion of the carboxyl groups is dissociated off. The two processes occur at different rates and, consequently, the total rate is changed when the ratio between the two reacting substances (*i. e.* their relative concentration) is changed. This ratio is determined by the dissociation constant K_0 which is temperature dependent according to equation (2) to a degree given by the magnitude of Q_0. If Q_0 is 0, which cannot be excluded since we deal with a carboxyl group, K_0 will be independent of the temperature.

In this case, the velocity variation of the total process as a function of temperature will be due to the change in velocity of the single processes. Even if the single processes follow Arrhenius' law, the total process will not do so, unless the two quantities E_{A+} and $E_{A\pm}$ in equations (10) and (11) are equally large. Since, however, this condition is not fulfilled, as it will be seen in the experimental section of this work, the experimental checking of the validity of equation (7) at hydrogen ion concentrations of the same order of magnitude as K_0 cannot be performed on the basis of Arrhenius' law.

E_{A+} and $E_{A\pm}$ are accessible to experimental determination by means of equations (10) and (11). From (8) and (9) we can, by means of equations (2) and (6) find the terms k_+/K_+ and $k_\pm/K_\pm$ at different temperatures. By experimental determinations of a series of corresponding values of T and k we may find E_A for different values of T. If the hydrogen ion concentrations are known in the single experiments, it will be possible in this way for each value of T to determine a relation between possible values of the two remaining terms of (7), *viz.* K_0 and Q_0. If Q_0 is assumed to be independent of the temperature, the numerical value of Q_0 and, moreover, K_0 as a function of the temperature, can be determined. By inserting the values obtained into equation (12)

$$-R\frac{d\ln K_0}{d\frac{1}{T}} = Q_0 \tag{12}$$

the validity of (7) can be checked.

In practice, however, this procedure is not convenient, since it involves too much calculation work.

It is much more convenient to determine the value of K_0 at different temperatures directly from equation (1) which, for this purpose, has to be converted into

$$K_0 = -c_{H^+}\frac{\frac{k_+}{K_+}c_{H^+} - k}{\frac{k_\pm}{K_\pm}c_{H^+} - k} \tag{13}$$

c_{H^+} and k are determined at different temperatures and k_+, K_+ and $k_\pm$ $K_\pm$ are found at the corresponding temperatures from the formulae

$$\log\left(\frac{k_+}{K_+}\right)_{T_1} = \log\left(\frac{k_+}{K_+}\right)_{T_2} + \frac{E_{A+}}{R}\left(\frac{1}{T_1} - \frac{1}{T_2}\right) \tag{14}$$

$$\log\left(\frac{k_\pm}{K_\pm}\right)_{T_1} = \log\left(\frac{k_\pm}{K_\pm}\right)_{T_2} + \frac{E_{A\pm}}{R}\left(\frac{1}{T_1} - \frac{1}{T_2}\right) \tag{15}$$

Here, E_{A+} and $E_{A\pm}$ are determined experimentally by means of (10) and (11). R has the numerical value 4.571 cal, if log denotes Briggian logarithms. If we put $T_2 = 303.1$,

$$\log\left(\frac{k_+}{K_+}\right)_{T_2} = 0.80 \qquad \log\left(\frac{k_\pm}{K_\pm}\right)_{T_2} = 1.32$$

From the previous work [1], the values 0.74 and 1.35 are obtained. The figures given here are the results of a more accurate determination [6].

The values for K_0 found in this way may be applied to the determination of Q_0 by means of equation (12). The accuracy cannot be expected to be very high, since the differences in (13) can only be determined very roughly.

EXPERIMENTAL

The rate of inactivation of penicillin G at different temperatures wa investigated by means of the method described previously.

Three series of experiments were performed, *viz.* one for $c_{H^+} \gg K_0$, one for $c_{H^+} \ll K_0$, and, finally, one where the hydrogen ion concentrations were of the same order of magnitude as K_0. Since K_0 has the numerical value of ca. 10^{-3}, dilute hydrochloric acid, Sørensen's acetate-acetic acid, and Sørensen's glycine hydrochloric acid, respectively, were chosen as buffer solutions. To all buffers was added sodium chloride to a total molarity of 0.5. When choosing the hydrogen ion concentrations, not only the magnitude of K_0 had to be taken into consideration, but moreover the fact that the suitable range of temperature is limited, and that the velocity constants must lie within certain limits. Experiments below 0° C or above 100° C are unfeasible, while the limits for the measurable velocity constants are given by the still possible degrees of decomposition and the duration of the experiment. In practice, the degree of decomposition cannot be changed very much from experiment to experiment: a reduction of the degree of composition applied here would involve a lower accuracy of the measurements while, on the other hand, an essential increase was found impossible, because the quantities of penicillin available for the experiments were too small. The duration of the experiment, however, can be varied within rather wide limits; it is difficult to reduce the total duration of the experiment to less than 4 minutes since, in that case, the time and the temperature cannot be determined with sufficient accuracy. The upper limit for the duration of the experiments is given by the demand of sterility; it is difficult for many days to maintain sterility in a glass from which samples are drawn from time to time, especially at temperatures where micro-organisms

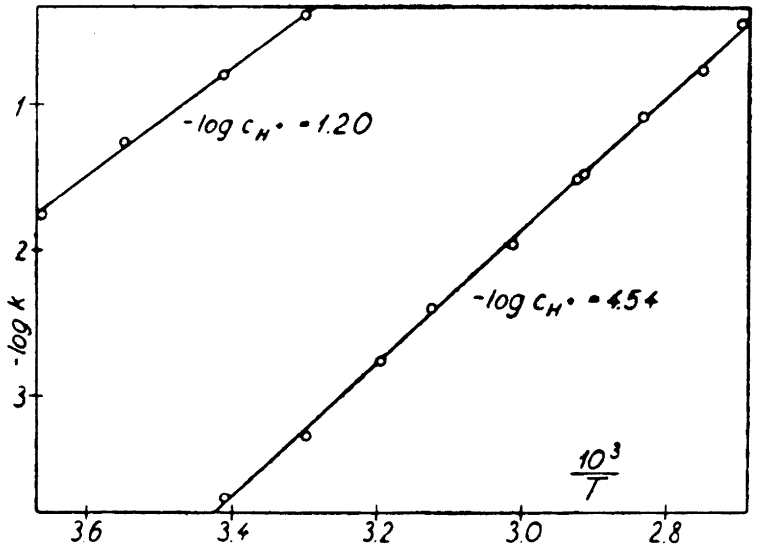

Fig. 1. Inactivation velocity at constant hydrogen ion concentrations as a function of temperature.

grow easily; the longest time applied was 6 days. The results obtained in two series of experiments are shown in fig. 1, where the logarithm of the velocity constants is plotted as a function of the reciprocal absolute temperature. The four straight lines which border the figure symbolize approximately the limitations mentioned above.

In order to check the validity of equation (7) the values for Q_0 and K_0 can now be inserted and the values of the left side of the equation can be calculated for different temperatures. This is, however, superfluous, since in the deduction of (7) no other theoretical suppositions were made than those already applied in the determination of K_0 and Q_0. Thus, the test for the accuracy of the theory will consist only in an investigation of whether the values found for K_0 satisfy equation (12) within the limits of the experimental accuracy. In this connection, Q_0 can be considered independent of the temperature, because the values known for the heat of dissociation of carbonic acids do not vary more than what falls within the limits of the experimental accuracy in the range of temperature used for the velocity measurement of the present process.

The experiments were performed in water- or oil thermostats. At higher temperatures (70—100° C) it was found difficult to maintain the same temperature in the reaction mixture and in the thermostat; this might be due to the circumstance that it was un-

feasible to apply stirring of the reaction mixture which had to be kept sterile with regard to the penicillin measurements. For the same reason, the thermometer could not be placed into the reaction mixture, but it was put into a glass containing the same amount of liquid, which was treated in the same way as the experimental glass. The temperature used in the calculations was an estimated mean of the temperature readings made in the course of the experiment. The uncertainty of the temperature determination was found to be less than 0.1°; as it will be shown later, this is a sufficient accuracy in view of the fact that the uncertainty of the determination of the velocity constants is rather high.

CALCULATION

The velocity constants were calculated according to the method described previously [1]; therefore, this point will not be treated here. However, it is necessary to deal with the calculation of the hydrogen ion concentrations in the applied buffers as a function of the temperature.

In buffers of hydrochloric acid, the hydrogen ion concentration is equal to the hydrochloric acid concentration and is independent of the temperature. However, the hydrogen ion concentrations of glycine- and acetate buffers depend on the temperature, because the dissociation constants of the buffer substances are temperature dependent. In view of the fact that a direct measurement of the hydrogen ion concentration at the experimental temperature is encumbered with great difficulties, all reaction mixtures were cooled (heated) after the conclusion of the experiments to 20° C and the hydrogen ion concentration was determined at this temperature by means of the method described previously. The hydrogen ion concentration of glycine buffers at the experimental temperature can be calculated on the basis of Owen's [2] measurements of the dissociation constant of glycine at various temperatures. Owen's measurements comprise salt concentrations from 0.005 *M* to 0.04 *M*. Within this range the heat of dissociation seems to be independent of the salt concentration and, consequently, it can be assumed with the accuracy necessary here that the heat of dissociation also will have the same value for 0.5 *M* NaCl. The heat of dissociation however, varies considerably with the temperature, a fact which must be taken into account in the calculations. The highest temperature applied by Owen is 45° C. The hydrogen ion concentration in a glycine buffer at 50° C, was determined by extrapolation.

In the case of acetic acid, more detailed measurements are available; here, even the dependence of the heat of dissociation on the salt concentration over large concentration ranges is taken into consideration. Harned and Hickey [3] have determined the dissociation constant of acetic acid in sodium chloride solutions at 0—40° C and found that the following equation holds:

$$-\log K_T = -\log K_{max} + 5 \cdot 10^{-5} (T - T_{K_{max}})^2 \qquad (16)$$

Harned and Embree [4] have shown that this equation is also valid over a somewhat larger range of temperature, at any rate if the salt concentration is zero. Since, however, Harned and Hickey found the constant $5 \cdot 10^{-5}$ to be independent of the salt concentration, it seems permissible to apply equation (16) in the temperature range 20—100° C. On the basis of the examples available in the literature concerning the application of this equation, it must be assumed that the error in this calculation of log K_T is less than 0.03. Harned and Hickey found for 0.5 M NaCl — log $K_{max} = 4.476$. The temperature, $T_{K_{max}}$ where this maximum value is found, was determined by them to be 305.5° abs.

In the author's experiments, the concentrations of acetic acid and o sodium acetate are the same, *viz.* 0.05 M. Since the hydrogen ion concentration at all the temperatures employed is low relative to these concentrations we obtain

$$-\log\ (c_{H^+})_T = -\log\ (c_{H^+})_{T_0} + 5 \cdot 10^{-5} \left[(T - 305.5)^2 - (T_0 - 305.5)^2\right] \qquad (17)$$

from which the hydrogen ion concentrations may be calculated from values determined at 20° C, by inserting the value of T.

The velocity constants found in acetate buffer at different temperatures and hydrogen ion concentrations are converted to one and the same hydrogen ion concentration by means of equation (9), the supposition $c_{H^+} \ll K_0$ holding in these experiments. A corresponding conversion of the velocity constants to one and the same hydrogen ion concentration for the decomposition experiments performed in glycine buffer cannot be carried out directly, since here the hydrogen ion concentration is of the same order of magnitude as K_0. On the other hand, the application of these latter figures does not require such a conversion.

Table 1. Inactivation velocity in dilute hydrochloric acid. 0.5 M NaCl.

$\frac{10^3}{T}$	-log c_{H^+}	-log k 1/min.
3,661	1.20	1.76
3,549	1.20	1.36
3,412	1.20	0.80
3,299	1.20	0.39

Table 2. Inactivation velocity in acetate - acetic acid. 0.5 M NaCl.

$\frac{10^3}{T}$	-log c_{H^+} calc.	-log k experimental	-log k calc. for -log c_{H^+} = 4,54
3,410	4.54	3.70	3.70
3,298	4.54	3.28	3.28
3,195	4.54	2,76	2.76
3,125	4.55	2.41	2.40
3,012	4.57	1.99	1.96
2,924	4.60	1.58	1.52
2,915	4.61	1.55	1.48
2,833	4.65	1.19	1.08
2,755	4.70	0.92	0.76
2,698	4.76	0.66	0.44

Table 3. Inactivation velocity in glycine-hydrochloric acid. 0.5 M NaCl.

$\frac{10^3}{T}$	-log c_{H^+} 20° C exp.	-log c_{H^+} T° calc.	-log k	-log $\frac{k_+}{K_+} c_{H^+}$	-log $\frac{k_+}{K_+}$	-log K	-log k calc.
3,531	2.90	2.94	2.77	3.04	2.69	2.60	2.80
3,410	2.97	2.97	2.30	2.60	2.16	2.85	2.29
3,298	2.98	2.95	1.79	2.15	1.63	2.80	1.77
3,198	2.98	2.93	1.30	1.73	1.13	2.80	1.28
3,094	2.97	2.91	0.89	1.33	0.65	3.00	0.83

RESULTS AND DISCUSSION

In tables 1 and 2 the results are shown of $c_{H^+} \ll K_0$ and of $c_{H^+} \gg K_0$, respectively. The converted velocity constants are plotted in fig. 1 which shows -log k as a function of $1/T$. The points fall with good approximation on straight lines. As it results from table 2, however, a curvilinear dependence is found between the logarithms to the non-converted velocity constants and $1/T$.

From the coordinates of the plotted points and by means of the equations (10) and (11) we obtain (using the method of least squares),

$$E_{A+} = 17\ 570 \pm 340 \text{ cal/mole} \tag{18}$$

$$E_{A\pm} = 20\ 980 \pm 220 \text{ cal/mole} \tag{19}$$

Since the mean errors of these two figures are so small relative to their difference, we may state with great certainty that the true values must be different.

$$\text{At } 30^\circ\text{ C } -\log k = 0.38 \pm 0.02 \text{ for } -\log c_{H^+} = 1.20 \text{ and}$$
$$-\log k = 3.24 \pm 0.02 \text{ for } -\log c_{H^+} = 4.54.$$

From previous experiments performed at 30° C [6] we find $-\log k = 0.39$ and $-\log k = 3.21$, respectively, in satisfactory agreement herewith.

Finally, table 3 contains the results of the last series of experiments at hydrogen ion concentrations of the same order of magnitude as K_0.

The values of $-\log K_0$ are calculated for each temperature by means of the equations (13), (14), and (15). The mean uncertainty of these figures can be estimated to be about 0.2, and if this is taken into consideration, we find that no noticeable variation of K_0 with temperature can be observed. This means that we can put $Q_0 = 0$.

The Committee on Medical Research and the Medical Research Council [5] give $-\log K_0 = 2.8$. The values found in the present experiments are in good agreement with this statement; it should, however, be kept in mind that the present experiments were performed in 0.5 *M* NaCl, while the Anglo-American values must be supposed to be valid for an ionic strength zero.

Obviously, the procedure outlined above is not suited for a determination of Q_0. The significance of the above account lies, thus, only in the fact that for the practical calculation of the rate of inactivation of penicillin by means of equation (7) we can put $Q_0 = 0$ within the range of temperature investigated here, *i. e.* 10°—50° C.

$$E_A = -R \frac{d\ln k}{d\frac{1}{T}} = E_{A+} + (E_{A\pm} - E_{A+}) \frac{\frac{k_\pm}{K_\pm} K_0}{\frac{k_\pm}{K_\pm} K_0 + \frac{k_+}{K_+} c_{H^+}} \qquad (20)$$

Since $k_\pm/K_\pm$ and k_+/K_+ are dependent on the temperature, also E_A will be dependent on the temperature if c_{H^+} is of the same order of magnitude as K_0. E_A will, however, always lie between E_{A+} and $E_{A\pm}$, because the numerical value of the fraction will always be between 0 and 1. At a given hydrogen ion concentration we find from equation (20) and equations (14) and (15) that E_A approaches $E_{A\pm}$ at very high temperatures and, inversely, that E_A approaches E_{A+} at very low temperatures, since $E_{A\pm} > E_{A+}$.

In order to investigate the magnitude of the numerical variation of E_A within a narrower temperature range, we put $c_{H^+} = K_0$. From equation (20) we obtain, applying the values for the constants given above,

at 10° C $E_A = 19\ 900$ cal
» 50° C $E_A = 20\ 390$ »

The difference between these two values is of the same order of magnitude as the uncertainty with which E_A may be determined experimentally, and hence it will be permissible within this temperature range to reckon with a constant value of E_A.

At 30° C we obtain, by inserting the constants into (20)

$$E_A = -R \frac{d\ln k}{d\frac{1}{T}} = 17\ 570 + \frac{3410}{1 + 200\, c_{H^+}} \qquad (21)$$

The two first series of experiments are performed at hydrogen ion concentrations which are supposed to be so high and so low, respectively, relative to K_0 that E_A approximately will assume its lowest and its highest value. Equation (21) offers a possibility of examining whether these suppositions are fulfilled with sufficient accuracy. By inserting we obtain

$E_A = 17\ 820$ cal for $-\log c_{H^+} = 1.20$
$E_A = 20\ 960$ cal for $-\log c_{H^+} = 4.54$

The difference between these values and those found experimentally is considerably smaller than the mean uncertainties and, correspondingly, the above mentioned suppositions can be assumed to be fulfilled with sufficient approximation.

In order to estimate the applicability of equation (21) to the calculation of the velocity constants at different temperatures, $-\log k$ was calculated for the experimental conditions prevailing during the experiments of table 3. As a basis of the calculations were used the velocity constants at 30° C read from the curve given previously [6]. The results are shown in the last column of the table, from which it appears that the calculated values are in good agreement with those found experimentally, if it is taken into account that the mean uncertainty of the experimental determination of $\log k$ is ca. 0.02—0.04.

SUMMARY

On the basis of previous investigations into the velocity of inactivation of penicillin by acids as a function of the hydrogen ion concentration and, moreover, of a theory for the reaction mechanism, an equation for the inactivation

velocity at different temperatures is deduced (7). For high and for low hydrogen ion concentrations, this equation is reduced to expressions conformable to Arrhenius' equation.

By measurements of the velocity of the process at different temperatures, Arrhenius' critical increments are determined for high and for low hydrogen ion concentrations. These terms are shown to have somewhat different numerical values and to be independent of the temperature within the range investigated.

On the basis of velocity determinations at intermediate hydrogen ion concentrations, it is shown that, within the accuracy necessary here, the heat of dissociation for the acid dissociation of the penicillin molecule can be assumed to be zero. From this, an equation (21) is deduced which can be applied to the calculation of the rate of inactivation at different hydrogen ion concentrations and at temperatures between 10° and 50° C. The velocity constants determined by means of this equation are in agreement with those found experimentally.

The expenses for the performance of the present experiments were defrayed by *Medicinalfabrikantforeningen*. The penicillin employed was kindly put at the author's disposal by Professor K. A. Jensen. Cand.act. Helge Brodersen has performed the statistical calculations.

I am greatly indepted to Professor J. N. Brönsted Ph. D. for valuable advice and criticism.

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Nuclear Oxidation of Furfural

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It has been demonstrated that 1,4-addition products come into existence when furan is oxidized under proper conditions with the following reagents:

a. Bromocyanogen [1].
b. Atmospheric oxygen [2].
c. Nitric acid in acetic acid anhydride [3] (Marquis' reagent [4]).
d. Osmium tetroxide and hydrogen peroxide in alcohol [5] (Milas' procedure [6]).
e. Bromine in alcohol [7] (Meinel's procedure [8]).
f. Lead tetraacetate [7].
g. Bromine in acetic acid in the presence of potassium acetate [9].
h. Peracids.

It was concluded that, in general, reactions between furans and oxidizing reagents as a rule are initiated by a 1,4-addition of the reagent to the double bonds of the furan nucleus, the only alternative being a so-called direct β-substitution [7].

In this communication the oxidation of furfural is discussed with special regard to the structure of addition products which have earlier been obtained from furfural by oxidation. The preparation of the stable 2,5-dimethoxy-2,5-dihydrofurfural diacetate is described and the structure proved by oxidative degradation.

Furfural has frequently been oxidized to maleic acid or to compounds, which are formed by further action of the reagent on maleic acid. In some cases maleic acid aldehyde has been isolated together with formic acid [10, 11] or carbon dioxide [12]. An explanation of the formation of these compounds based upon the conception of 1,4-addition has earlier been given (Milas [12], Dunlop, Stout and Swadesh [11] and Clauson-Kaas [7]). The well known oxidations of furfural to mucobromic and mucochloric acid with warm aqueous solutions of the halogens belong to the same type of reactions.

Cross, Bevan and Heiberg [13] have oxidized furfural in water with Fenton's reagent at about 40°. The authors suggest that 3-hydroxyfurfural and 3-hydroxyfuroic acid are formed by the reaction. We believe that their 3-hydroxyfurfural, which was isolated as a phenylhydrazone in a 93 % yield, calculated on furfural, actually is maleic acid aldehyde. The proposed hydroxyfuroic acid, which was isolated as a barium salt, was obtained in a 5 % yield. It is highly improbable, that the acid is 3-hydroxyfuroic acid and we suggest that it has the formula I and is a product of hydrolysis of an intermidiate 2,5-hydroxy-2,5-dihydrofuroic acid II. In this connection it should be mentioned that Milas [12] by the oxidation of furfural with vanadium pentoxide and sodium chlorate isolated the barium salt of a dicarboxylic acid with the formula $C_5H_4O_5$ which might have been formed by oxidation of I and consequently be a ketoglutaconic acid (III).

By the oxidation of furfural with Fenton's reagent, formic acid and acetic acid as well as compounds, which could be oxidized to iodoform, were also shown to be present in the reaction mixture. The identification of formic acid is in agreement with our interpretation of the reaction mechanism, but we are not able to explain how the other products may arise from the oxidation of furfural. Probably their formation is due to a content of 5-methylfurfural in the furfural employed.

Cross, Bevan and Briggs [14] oxidized furfural with Caro's acid. Formic acid and succinic acid were obtained together with the barium salt of an acid with the formula $C_5H_6O_5$ and what was claimed to be 5-hydroxyfurfural, precipitated as phenylhydrazone and methylphenylhydrazone. We believe that the aldehyde isolated from this oxidation also is maleic acid aldehyde, while the acid probably is *a*-ketoglutaric acid. The formation of this acid as well as of succinic acid has previously been formulated as the result of a 1,4-elemination of water from the intermediate 2,5-dihydroxy-2,5-dihydrofurans [7].

Hughes and Acree [15] oxidized furfural with bromine in water at low temperature. Addition of phenylhydrazine to the reaction mixture yielded a red bis-phenylhydrazone of what was proposed to be a ketodihydrofurfural. We believe that furfural by this reaction is oxidized to 2-pentene-1,5-dial-4-one (IV) which in some way or other has condensed with two moles of phenylhydrazine.

The formation of all above mentioned oxidation products of furfural may be expressed by the following sequence of reactions (see pag. 417; *cf.* Milas [12], Dunlop, Stout and Swadesh and Clauson-Kaas [7]).

Scheibler, Jeschke and Beiser [16] have obtained an epoxide of furfural diacetate in an analytically pure state by the action of perbenzoic acid (yield 8 %). This compound was indifferent to bromination and catalytic hydrogenation

Oxidation of furfural.

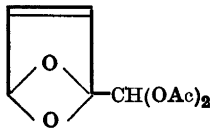
and was therefore assumed to possess a formula containing a diepoxycyclobutane ring and no double bonds. However, in view of what is known about the inertness of the 3,4-double bonds of certain 2,5-dihydrofurans [7], the oxide may as well be a 1,4-addition compound with the formula V which would be consistent with the findings of Böeseken *et. al.*[10] that furfural is oxidized with peracetic acid to maleic acid aldehyde (yield 40 %), maleic acid (7 %) and formic acid (100 %).

We have not repeated the experiments of Scheibler, Jeschke and Beiser but investigated the action of peracids on furan. It was found that when equimolar amounts of furan and peracid interact, the bis-phenylhydrazone of malealdehyde may be precipitated in a pure state by addition of phenylhydrazine to the reaction mixture. The highest yield obtained was 23 % of the theoretical amount. In spite of numerous preparations under various conditions it was not possible to improve this figure, but in judging the yield the lability and reactivity of the substances involved in the reaction should be considered.

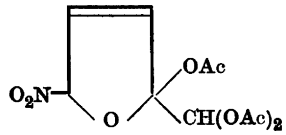
The isolation of malealdehyde-bis-phenylhydrazone proves, that the peracids have been added to furan to yield 2,5-epoxy-2,5-dihydrofuran or its equivalent whereafter the addition product, either spontaneously or by the action of phenylhydrazine rearranges to malealdehyde.

The isolation of several oxidation products formed by the interaction of peracids and furans has been reported by Böeseken *et al.* and by Milas and McAlevy [17], but the experimental evidence from these investigations is not sufficient to permit any deductions concerning the mechanism of this type of reaction. It therefore seems, that peracids behave like other oxidizing agents towards furans. It is of special interest in this connection to mention that this is also the case with atmospheric oxygen according to the experiments of Ciamician and Silber [18], Schenk [2] and Dunlop, Stout and Swadesh.

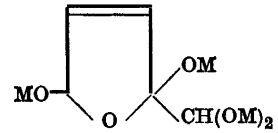
So far two stable addition products of furfural have been isolated in good yields and analyzed. Gilman and Wright [19] prepared the nitroacetate of furfural diacetate by nitration of this compound according to the directions of Marquis, and Meinel [18] obtained dimethoxy-dihydrofurfural dimethylacetal by methoxylation of furfural with methanolic bromine. We have earlier proposed formulas VI and VII for these compounds although other formulas were suggested by the above authors [3, 7].



V



VI

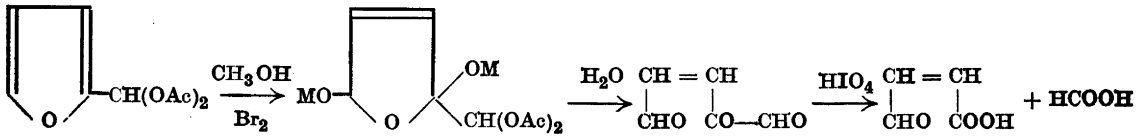


VII

Meinel reported that furfural diacetate can not be methoxylated, but we encountered no difficulties in preparing a dimethoxydihydrofurfural diacetate

by the action of bromine in methanol on furfural diacetate. The addition compound was a colourless, extremely viscuous oil which could be purified by distillation in vacuum. The yield of the pure product was as high as 81 %. The addition of two methoxy groups was confirmed by analysis, methoxy, acetoxy and molecular weight determination.

In order to elucidate the structure, the dimethoxydihydrofurfural diacetate was hydrolyzed and oxidized with one mole of periodic acid. Maleic acid aldehyde was hereby isolated as phenylhydrazone (yield 70 %) and methylphenylhydrazone (yield 71 %). The identification of maleic acid aldehyde proves that the addition compound is 2,5-dimethoxy-2,5-dihydrofurfural diacetate (VIII). The periodic acid oxidation at the same time supports the theory, that the oxidation of furfural to maleic acid aldehyde proceeds through a 1,4-addition product.



VIII

The methylphenylhydrazone of maleic acid aldehyde melted at about the same temperature as the methylphenylhydrazone prepared by Cross, Bevan and Briggs. This substantiates the opinion expressed above that the 5-hydroxyfurfural of these authors is in fact maleic acid aldehyde.

EXPERIMENTAL

Oxidation of furan with peracids

The action of peracetic acid and perbenzoic acid on furan was investigated under various conditions. Moist and dry chloroform, ether, acetic acid anhydride and water were tested as solvents and the temperature was varied from zero to 40°. When the peracid had disappeared, phenylhydrazine was added and the malealdehyde bis-phenylhydrazone filtered off and weighed. Several preparations yielded about 20 % of the theoretical amount of the hydrazone, but this figure was never surpassed substantially. No difference in the action of the two peracids was observed. Details of a typical experiment are given below:

280 mg of freshly distilled furan were added to 9.8 ml of 0.42 *M* perbenzoic acid in moist chloroform and the mixture left standing over night at zero. The next day 95 % of the peracid had disappeared. 2.00 ml of the reaction mixture were added to a solution of 0.2 ml of phenylhydrazine in 0.3 ml of alcohol. The hydrazone precipitated immediat-

ely and was filtered off, washed with benzene and dried in vacuum; yield 44 mg = 20 %. Yellow crystals, m. p. 172° (Kofler stage, corr.); previously [3] found 171°.

$C_{16}H_{16}N_4$ (264) Calc. N 21.21 Found N 21.23

2,5-Dimethoxy-2,5-dihydrofurfural diacetate

Preparation. 19.8 g (0.1 mole) of furfural diacetate were methoxylated and the reaction product isolated in the usual manner [7]. The methanolic solution of bromine was added during 45 minutes and the temperature kept at about 15°. The etheral layer was shaken with 50 ml of a saturated solution of acid potassium carbonate instead of the normal salt employed for the isolation of dimethoxydihydrofuran. The final product was distilled in vacuum at 1 mm and obtained as a practically colourless, very viscuous oil; b. p. 122—26°; yield 21 g = 81 %. The freezing point depression of a benzene solution was measured.

$C_{11}H_{16}O_7$ (260)	Calc.	C	50.74	H	6.21	CH_3COO	45.3	CH_3O	23.8	Mol.wt. 260
	Found	»	50.44	»	6.21	»	45.8	»	23.5	» 252

Hydrolysis and oxidation with periodic acid; identification of maleic acid aldehyde. 260 mg of 2,5-dimethoxy-2,5-dihydrofurfural diacetate and 240 mg of potassium metaperiodate were weighed into a 50 ml volumetric flask. 10 ml of 0.1 *N* sulfuric acid were added and the flask filled to the mark with water and shaken for one hour at room temperature. At this time the mixture was homogenous. The solution was left standing for 40 hours at room temperature and was then heated for 5—6 hours at 75° on the water bath. After cooling 25 ml of the mixture were added to a solution of 0.30 ml of phenylhydrazine and 0.30 ml of glacial acetic acid in 10 ml of water. The hydrazone, which precipitated almost immediately, was filtered off after shaking for two minutes, washed with water and dried in vacuum over phosphorous pentoxide at 80°; yield 66 mg = 70 %. The crude product was recrystallized from acetone-benzene; yield 45 mg = 47 %; m. p. 149° (tube, corr.). When recrystallized once more from acetone-ligroin the melting point was raised to 150°. (Fecht [20] 158—59°; Ciamician a. Silber [18] 157°; Milas [12] 157.5—58°; Böeseken *et. al.* [10] 155°). The phenylhydrazone prepared by Cross, Bevan a. Briggs melted at 155°.

The fraction melting at 149° was analyzed:

$C_{10}H_{10}O_2N_2$ (190)	Calc.	C	63.16	H	5.32	N 14.74
	Found	»	62.52	»	5.28	» 14.45

The methylphenylhydrazone was prepared in the same manner by adding 25 ml of the reaction mixture to a solution of 0.30 ml of methylphenylhydrazine and 1 ml of glacial acetic acid in 10 ml of water; yield 72 mg = 71 %. The crude product was washed with ligroin and recrystallized from methanol-water and then twice from benzene-ligroin; faintly yellow crystals; yield 28 mg = 28 %; m. p. 145° (tube, corr.). The methylphenylhydrazone prepared by Cross, Bevan a. Briggs melted at 143°.

$C_{11}H_{12}O_2N_2$ (204)	Calc.	C	64.67	H	5.93	N 13.72
	Found	»	65.30	»	6.02	» 13.28

We are indebted to Mr. O. Rosenlund Hansen and Miss Muriel Schmidt for the microanalyses.

During the investigation we have received financial aid from *Tuborg Fondet* (N. Clauson-Kaas) and *Det teknisk-videnskabelige Forskningsraad* (J. Fakstorp).

We wish to thank the Director of the Chemical Laboratory of the University of Copenhagen, Professor Dr. A. Langseth, for his kind interest in our work.

SUMMARY

The oxidation of furfural is discussed. 2,5-Dimethoxy-2,5-dihydrofurfural diacetate is prepared by methoxylation of furfural diacetate and the structure is proved by oxydative degradation.

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Enzymatic Breakdown of Polymetaphosphate

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In the course of studies on phosphatases Kitasato found in 1928 that »taka-phosphatase» from *Aspergillus oryzae* was also capable of liberating orthophosphate from metaphosphate [1, 2]. This observation was subsequently confirmed by other workers, and it was also shown that yeast extract and some organ extracts also possess the power of liberating orthophosphate from metaphosphate and »triphosphate» [3, 4, 5].

While investigating phosphorus metabolism in moulds, Mann [6] observed powerful metaphosphatase activity in extracts from *Aspergillus niger*. Mann made at the same time the interesting observation that extracts from *A. niger* contained not only pyrophosphate but also metaphosphate.

Experiments have thus been described in the literature which demonstrate the existence of metaphosphatase. The activity has been demonstrated by determinations of the free orthophosphate liberated from the metaphosphate. There seem, however, to be no published experiments giving any indication as to how this enzymatic breakdown proceeds. We have now carried out some experiments, one of the objects of which was to ascertain that metaphosphatase breaks down polymetaphosphate of very high molecular weight. Another object of our experiments was to ascertain if the metaphosphatase breaks down high molecular polymetaphosphate by a random hydrolysis of -P-O-P- bonds, or whether the breakdown proceeds in such a way that one orthophosphoric acid residue at a time (or a few such residues. *e. g.* as pyrophosphoric acid) is split off from the ends of the metaphosphate chains. The postulated processes have an analogy in the breakdown of starch by the different amylases. We have also made preliminary experiments with the idea of finding out if any of the metaphosphate found by Mann in *A. niger* has such a high molecular weight as to be capable of determination in the ultracentrifuge, for comparison in this way with the polymetaphosphates of synthetic origin.

We have also attempted to demonstrate metaphosphatase activity in extracts of some micro-organisms other than *A. niger* and *A. oryzae.*

The substrate for our experiments has been the well-defined polymetaphosphate of very high molecular weight that has been described by Malmgren [7] and Malmgren and Lamm [8, 9]. These polymetaphosphates are built up from long chains which may be formulated as follows:

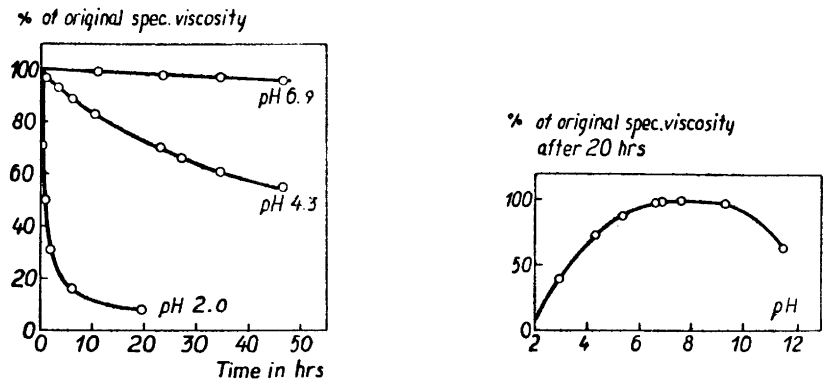
- - -O-PO(ONa)-O-PO(ONa)-O-PO(ONa)-O-PO(ONa)- - -

The molecular weight of these substances can be considerable, extending up to several millions. Two different preparations [7, 8, 9], designated as K11 and K14, have been employed. The molecular weight of K11 is about 2,000,000 and of K14 about 1,500,000 (as measured in buffer solutions of ionic strength 0.4). n in the formula $(NaPO_3)_n$ thus has magnitudes of the order 15,000—20,000. These substances were obtained by heating KH_2PO_4. The resulting potassium polymetaphosphate was dissolved, for the experiments, in buffer solutions with a large excess of sodium ions.

At low pH values the spontaneous breakdown velocity of the polymeta phosphate is quite considerable. We carried out a series of preliminary experiments to measure the velocity of breakdown of the polymetaphosphate in some buffers at different pH's. In this way it was hoped to ascertain in which pH region one should work to ensure that the spontaneous breakdown velocity of the polymetaphosphate is insignificant. Of course it might also be of interest to do experiments with metaphosphatase at pH values where the spontaneous breakdown of polymetaphosphate is considerable, but under these circumstances it would be necessary to make allowance for it. For the present purpose the viscosity at 25.0° C was determined after various intervals for three different solutions having the following composition (ionic strength of NaCl + the buffer substances = 0.3):

I. 0.5 % polyphosphate (K14) + 1.17 % NaCl in citrate buffer — pH 2.0.
II. 0.5 % polyphosphate (K14) + 1.17 % NaCl in acetate buffer — pH 4.3.
III. 0.5% polyphosphate (K14)+1.17% NaCl in phosphate buffer — pH 6.9.

In fig. 1 the y axis shows the percentage of the original specific viscosity $(\eta/\eta_0\text{-}1)$ and the x axis the time in hours. In fig. 2 the percentage of the original specific viscosity reached after 20 hours is plotted against the pH of the solutions. (The curve has been supplemented with 5 extra points obtained in rther experiments.) It is evident from the curves that one should avoid caroying out enzyme experiments at pH values that are too low; under these



Figs. 1 and 2. Breakdown of polymetaphosphate at different pH values.

conditions the polymetaphosphate undergoes spontaneous decomposition quite rapidly.

All the enzyme experiments were done at 25.0° C (except those with *P. expansum*). The enzymatic breakdown of the polymetaphosphate was followed by means of viscosity measurements, ultracentrifuge studies, dialysis experiments and orthophosphate determinations.

The viscosity measurements were made by determining the running time in ordinary Ostwald viscosimeters. As these polymetaphosphate solutions have a comparatively high viscosity, it is easy to follow the first phase of the breakdown by viscosity measurements. The viscosity of the polymetaphosphate solutions has been measured relative to that of the buffer solutions, and from the relative viscosity (η/η_0)the specific viscosity (η/η_0-1) has been calculated. In the graphs the % of the original specific viscosity has been plotted against the time elapsing after the enzyme was added to the polymetaphosphate solution. For present purposes it can be assumed that the specific viscosity provides a qualitative measure of the degree of polymerisation of the polymetaphosphate.

The centrifugations were done in the ultracentrifuge of Svedberg. In these experiments both sedimentation constants and the quantities of sedimenting polymetaphosphate were determined (the latter from the area under the »peak» in the sedimentation diagram). For details of the experimental procedure in the ultracentrifugations, reference is made to [10].

For determining the liberated orthophosphate we have used a method of determination recently published by Lowry & Lopez [11]. The reaction is based on the reduction of phosphomolybdate by ascorbic acid at pH 4. This

method has the advantage that the determination of orthophosphate can be done in such conditions that easily hydrolysable phosphorus compounds (*e. g.* labile esters of phosphoric acid) do not split off orthophosphate, that would interfere with the analytical results. To give a suitable pH to the solutions for analysis, they were diluted with an acetate buffer of ionic strength 0.2 and pH 4.0. The readings were taken at a wave-length of 720 mμ. To convince ourselves that pyrophosphate and metaphosphate do not, in this method, interfere with the determination of orthophosphate, we analysed for orthophosphate known mixtures of orthophosphate, pyrophosphate and polymetaphosphate. These experiments showed that we could determine orthophosphate with satisfactory accuracy in the presence of 100 times as much pyrophosphate or polymetaphosphate.

A number of experiments have been done with extracts from *Aspergillus niger* and with »clarase» (enzyme preparation from *A. oryzae*). As they all gave results pointing towards the same conclusion, we will confine ourselves to describing a few of these experiments.

Experiment with »clarase»

For this experiment a »clarase» preparation was used (»taka-diastase» from *A. oryzae*, commercial preparation), which we purified by dialysis against distilled water and then dried *in vacuo* from the frozen state. In the experimental series (no. I) a solution was used containing 0.44 % polymetaphosphate (K14) and 0.12 % »clarase» in a maleic acid sodium maleate buffer to which had been added 1.17 % NaCl (ionic strength of NaCl + buffer substance = 0.3, pH 6.6). In the blank series (no. II) a solution of the same composition was used, except that here the enzyme solution had been kept for 30 min at 100° C before it was added to the polymetaphosphate solution.

The breakdown of the polymetaphosphate in the two solutions was followed by viscosity measurements, orthophosphate determinations and dialysis experiments. The viscosity fell markedly faster in the solution containing the active enzyme. After 48h the specific viscosity of solution no. I had sunk to 47 % of its original value: for solution no. II the corresponding value was 92 %. During the same period only traces of free orthophosphate-P had been formed, corresponding to less than 0.5 % of the total P present in the solution. Thus at the beginning only insignificant amounts of orthophosphate are split off from the ends of the polymetaphosphate chains.

To show that it is not either the case that the first phase of the breakdown proceeds in such a way that two (pyrophosphate) or a few phosphoric acid residues at a time are split off from the ends of the polymetaphosphate chains,

a dialysis experiment was done (also at 25.0° C) in parallel with the above and employing the same solutions. 10 ml of each of the polymetaphosphate solutions (the one containing unheated, the other heated enzyme) were transferred to cellophane sacs; the two sacs were then placed each in a separate cylinder containing 25 ml of the buffer solution. At suitable intervals phosphate determinations were carried out on the liquid surrounding the dialysis sacs. In these the phosphate was determined both directly and after boiling with dilute sulfuric acid, under such conditions as to break down any pyrophosphate or polymetaphosphate of low molecular weight that had dialysed out. This experiment showed that after 48 h only quite insignificant amounts of pyrophosphate or low-molecular, dialysable polymetaphosphate had been formed (less than 1 % of the total P present).

It is thus apparent that the first phase of breakdown due to the action of this enzyme does not involve the splitting off of one, two, or a few phosphoric acid residues from the ends of the polymetaphosphate chains. (If this were the case, the marked fall in viscosity should be accompanied by the liberation of considerable quantities of orthophosphate). It seems that the splitting occurs here and there, probably at random along the chains, leading chiefly at first to the formation of comparatively large fragments.

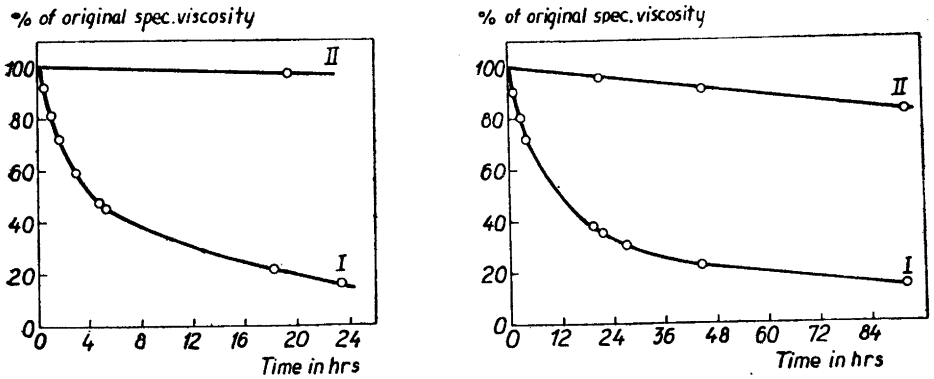
The experiment also shows that the »clarase» has a fair activity even when dialysed against distilled water. It thus appears that the enzyme does not readily loose any co-enzyme that may be necessary, or else, that it requires only very small quantities of such a co-enzyme.

Experiment with *Aspergillus niger* enzyme I

Aspergillus niger (from the Institute of Plant Physiology, Uppsala) was cultivated at 33° C on a medium of the following composition: 100 g sucrose, 4 g KH_2PO_4, 4 g K_2HPO_4, 0.7 g $MgSO_4 \cdot 7H_2O$, 2 g $(NH_4)_2SO_4$, 0.2 g NaCl, 0.2 g $CaCl_2$, 0.2 g enzymically degraded casein, a trace of a vitamin preparation (containing *inter alia* vitamin B_1), a trace of $FeSO_4$, 1 l tap water.

After 4 days growth the mould was ground up in the culture medium. The mixture was then filtered. The filtrate was neutralized with NaOH, and then dialysed against ice-water in a cellophane sac. After dialysing for 24 h some of the solution in the sac was taken out and used as enzyme solution for the experiment. Some more of the solution was kept at 100° C for 30 min for use as a blank.

A polymetaphosphate solution was prepared containing 0.5 % of K11 and 1.17 % NaCl (0.2 *M*) in a maleic acid-sodium maleate buffer (combined ionic strength of NaCl and buffer substances = 0.3, pH = 6.6). For the



Figs. 3 and 4. Breakdown of polymetaphosphate by A. niger enzym.

experimental series (no. I) 7 ml of this solution of K11 was mixed with 1 ml of the enzyme solution. The blank series (no. II) was made up in the same way, except that in this case the heat-inactivated enzyme solution was used. The alteration of viscosity with time for the two solutions is shown in fig. 3. When, after 23 h, the specific viscosity had fallen to 16 % of its original value, only traces of free orthophosphate-P had been formed (less than 0.5 % of the total quantity of P present).

Experiment with *Aspergillus niger* enzyme II

For this experiment the same enzyme solution as in the previous experiment was used, except that the dialysis had been continued for a further 48 h. A polymetaphosphate solution with the same composition as in the previous experiment was prepared. In series (no. I) 20 ml of this polymetaphosphate solution was taken and mixed with 2 ml of enzyme solution (pH 6.6). The solution for the blank series (no. II) was prepared in the same way, except that for this series an enzyme solution was used that had been inactivated by heating. In this case the breakdown was followed by viscosity determinations and phosphate determinations, and also by dialysis experiments and ultracentrifugations. The fall of the specific viscosity with time is shown in fig. 4. When the specific viscosity had sunk to 14 % of the original value, here again only traces of free orthophosphate-P had been formed (less than 0.5 % of the total P present).

In parallel with this experiment a dialysis experiment was done at the same temperature (25° C) and employing the same solutions. 12 ml of each of

the polymetaphosphate solutions were transferred each to a separate cellophane sac, and the two sacs were then placed in separate cylinders, each containing 20 ml of the buffer solution. At suitable intervals phosphate was determined in the liquid outside the cellophane sacs, both directly and after hydrolysis with acid such as to break down to orthophosphate any pyrophosphate or polymetaphosphate of low molecular weight that had dialysed out. When, after 44 h, the specific viscosity of the solution had sunk to 23 % of its original value, only insignificant amounts of dialysable polymetaphosphate-P had dialysed out of the sac (less than 1 % of the total P present). After 92 h when the specific viscosity had sunk to 14 % of its original value, some few percent of the polymetaphosphate had been reduced to such small molecules as to have dialysed out. (Nevertheless, there were still only traces of orthophosphate.)

In parallel with these experiments 3 ultracentrifugation experiments were also carried out on solution no. I at different times, as well as an ultracentrifugation of the blank solution. The results are given in table 1. (The sedimentation constant, *s*, is given in Svedberg units.)

Table 1. Breakdown of polymetaphosphate by A. niger enzym.

Series no.	Time (h) after beginning of experiment	% of original specific viscosity	Sedimentation constant (*s*)
I	5	70	10.6
I	20	37	10.2
I	95	14	8.7
II	22	95	10.6

The sedimentation constant for solution no. I falls somewhat with time. As expected, however, it does not fall as markedly as the specific viscosity. (For thread-like molecules the sedimentation constant does not vary so sharply with the chain-length as does the specific viscosity.) Calculations were made, from the area under the »peak» of the centrifugation diagram, of the quantity of high-molecular polymetaphosphate recovered as sedimenting material. The same amount of material was observed in this way in both the first two centrifugations of solution I, and on centrifuging the solution II from the blank experiment. In the third centrifugation of solution I (after 95 h) there was also found practically the same amount of sedimenting substance. (The value found was a few percent lower, which would be in agreement with the dialysis experiment. However, the accuracy of quantity deter-

minations from centrifugation diagrams is not sufficient to permit of drawing quantitative conclusions from an alteration of only a few percent.) The centrifugation diagrams for solution I also showed that the substance becomes more and more polydisperse as the enzyme action proceeds. Since, therefore, practically all the polymetaphosphate seems to remain in a comparatively high-molecular form, it is seen that these centrifugation experiments support the conclusions as to the nature of the breakdown which we obtain from the viscosity and phosphate determinations. (Centrifugation experiments have also been done in connection with some of our other experiments; results of a similar character were obtained.)

Experiments with enzyme from *Penicillium expansum*

Several experiments have also been done with extracts from *P. expansum*, which showed that this micro-organism also produces a metaphosphatase with properties similar to those of the metaphosphatase from *A. niger*. We will confine ourselves to describing a few of the experiments with an extract from this organism.

P. expansum (no. 593 from the National Collection of Type Cultures maintained in Britain by the Medical Research Council) was cultivated at 25° C on a medium of the following composition: 40 g glucose, 0.8 g $(NH_4)_2SO_4$, 0.8 g KH_2PO_4, 0.2 g $MgSO_4 \cdot 7H_2O$, 0.3 g enzymically degraded casein, traces of a vitamin preparation containing, *inter alia*, vitamin B_1, and 360 ml tap-water.

After 5 days growth the mould was ground up in the culture-medium with sand. The mixture was filtered, and the filtrate was neutralized with NaOH and dialysed against ice-water. A sample was removed after 4 h and was used as enzyme solution in some experiments, designed to ascertain in what pH region the optimum lay for this metaphosphatase.

Four different buffer solutions were prepared, containing 0.5 % polymetaphosphate (K11) and 1.17 % NaCl (0.2M). (Ionic strength of NaCl + buffer substances = 0.3). The pH of the various solutions was as follows:

A. Acetate buffer	pH 4.3
B. Acetate buffer	pH 5.4
C. Phosphate buffer	pH 6.9
D. Borax-soda buffer	pH 8.9

To 10 ml of each of these solutions 2 ml of enzyme solution was added, and the breakdown of the polymetaphosphate was followed viscosimetrically at

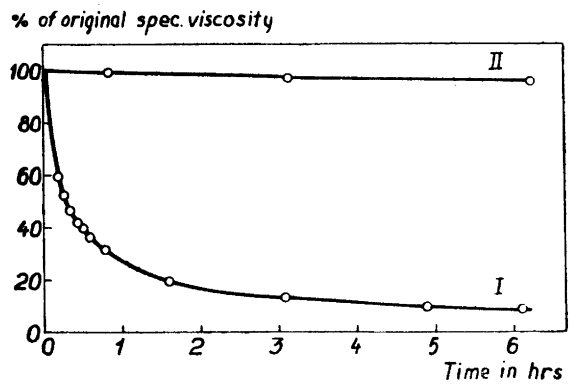


Fig. 5. Breakdown of polymetaphosphate by P. expansum enzym.

20.3° C. This showed that the breakdown occurred rapidly at low pH, but only slowly at pH 6.9 and over. Table 2 illustrates the result:

Table 2. Enzymatic breakdown at different pH values.

pH	Time from beginning of experiment	% of original specific viscosity
4.3	45 min	20
5.4	45 min	32
6.9	18 h	85
8.9	18 h	95

Like the metaphosphatase found by Mann in *A. niger* this metaphosphatase also has its optimum at an acid pH.

In the course of breakdown of polymetaphosphate by this enzyme it is likewise the case that at first no measureable quantities of orthophosphate are liberated. This is demonstrated by the following experiment: The same enzyme solution was used as in the preceeding experiment, except that it had been dialysed for a further 48 h. Some of the enzyme solution was inactivated by heating for use as a blank. A solution was made up as in the previous experiment with 0.5 % KI1 and 1.17 % NaCl in acetate buffer (pH 5.4). In the experimental series (I) 9 ml of this solution was treated with 2 ml enzyme solution. For the blank series (II) heat-inactivated enzyme solution was used instead. The fall of specific viscosity with time is shown by fig. 5 (Temperature 20.3° C).

When, after 24 h, the specific viscosity of mixture I had fallen to 4 % of its original value, only traces of free orthophosphate-P had yet been formed (less than 0.5 % of total P present).

Experiment with enzyme from *Schizosaccharomyces pombe*

A single experiment with an extract from *Schizosaccharomyces pombe* showed a weak effect at pH 5.6.

Attempts to isolate metaphosphate from *A. niger*

Preliminary experiments having the object of isolating the metaphosphate found by Mann in *A. niger* have shown that it is perhaps not impossible that a fraction of the naturally occurring metaphosphate may possess a relatively high molecular weight. We intend to publish these experiments at a later date.

DISCUSSION

These experiments confirm the observations of Kitasato and Mann that metaphosphatase occurs in *A. oryzae* and *A. niger*. It has also been possible to demonstrate activity in extracts from *P. expansum*. This phosphatase has its optimum at a rather low pH. All the metaphosphatases studied have possessed the power of breaking down polymetaphosphate of very high molecular weight. It is not always evident what was the molecular weight of the metaphosphate used in the experiments with metaphosphatase in the previous literature but it seems that low molecular substances have been used. (Kitasato says that he used hexa-metaphosphate $(NaPO_3)_6$ and Mann says that he used metaphosphate and hexa-metaphosphate. Neuberg and Fischer used triphosphate $Na_5P_3O_{10}$.) In our experiments we have used preparations of polymetaphosphate having molecular weights of more than a million. Thus in these experiments a colloid of purely inorganic character has been degraded by enzymatic means. The polymetaphosphate has been broken down by metaphosphatase through hydrolysis of -P-O-P- links at scattered points along the chains, so that comparatively large fragments are at first formed; the hydrolysis does not take the form of a splitting off of one or a few phosphoric acid residues at a time from the ends of the chains.

The experiments that have been done show that the well-defined polymetaphosphates of extremely high molecular weight, that have previously been studied in this laboratory, are well suited for use as substrates even in

the study of metaphosphatase preparations of low activity, since the breakdown is easily followed by viscosimetric means.

It has not yet proved possible to answer the question whether some micro-organisms may perhaps be capable of synthesizing and storing a polymetaphosphate of comparatively high molecular weight. Experiments have been initiated with the object of finding out whether or not this is so.

SUMMARY

The enzymatic breakdown of polymetaphosphate of very high molecular weight with enzymes from micro-organisms has been studied by viscosity measurements, ultracentrifugations, dialysis experiments and orthophosphate determinations. It has been demonstrated that the breakdown proceeds through the scission of scattered -P-O-P- links in the polymetaphosphate chains, so that comparatively large fragments are at first formed; it is not the case that the hydrolysis occurs through the removal of one (or a few) orthophosphoric acid residues at a time from the ends of the polymetaphosphate chains.

The authors wish to thank Prof. T. Svedberg and Prof. A. Tiselius for the privilege of carrying out this work in their laboratories.

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On the Use of a Weight Burette for Measuring Out Bromine Water

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In experiments on the rate of bromination, different amounts of bromine water of known concentration and fixed temperature (18° C) were to be added to solutions in a reaction vessel. Pipettes for bromine water have been devised by Ramberg [1] and by Biilmann [2]. The author of this paper used an ordinary weight burette with a stopcock at the bottom. The burette was closed at the top by a ground-on glass bell having a bore which, when the bell was rotated to a certain position, communicated with a corresponding bore in the top part of the burette. The tip below the stopcock was closed by a ground-on glass cup. The burette was divided in ml, from 0 to 100, the total volume being 130—140 ml. Before use the burette was charged with bromine water to above the zero mark and placed in the water thermostat. When temperature equilibrium was established, the burette was taken from the thermostat, and bromine water was tapped out until the surface was on a level with the zero mark. The burette was closed and shaken vigorously for about 15 seconds. It was again left in the thermostat for some time after which it was opened for a moment at the top bore, closed again and, once more, shaken vigorously. It was weighed, and was now ready for use. After each delivery of bromine water it was shaken, weighed and placed in the thermostat. The first and the last two or three samples taken from the burette were analyzed. They were added to potassium iodide and titrated with thiosulphate. In the following, we shall deduce an interpolation formula which may be used for computing the concentrations of the intermediate portions of bromine water.

Let the total volume of the burette be v ml, the volume above the zero mark a ml. From the burette are taken altogether n portions of bromine water weighing x_1, x_2. . . x_n g and containing, respectively, c_1, c_2. . . c_n gramme-equi-

valents of bromine per kg. s is the density of the bromine water, α the Bunsen absorption coefficient of bromine in water. We express that the total amount of bromine in the burette, in the vapour phase and in the liquid phase, when p—1 portions have been taken out, is equal to the total amount in the two phases when p portions have been removed, plus the bromine in portion p. We obtain

$$\left(a+\frac{x_1+\ldots+x_{p-1}}{s}\right)\frac{c_p\,s}{\alpha}+\left(v-a-\frac{x_1+\ldots+x_{p-1}}{s}\right)c_p\,s$$

$$=\left(a+\frac{x_1+\ldots+x_p}{s}\right)\frac{c_{p+1}\,s}{\alpha}+\left(v-a-\frac{x_1+\ldots+x_p}{s}\right)c_{p+1}\,s+x_p\,c_p$$

Rearranging the terms we find

$$c_{p+1}=c_p\left(1-\frac{1}{\alpha-1}\frac{x_p}{u-(x_1+\ldots+x_p)}\right) \tag{1}$$

where

$$u\equiv\left(\frac{\alpha}{\alpha-1}v-a\right)s \tag{2}$$

The total decrease of the concentration of bromine from the first to the last portion is only a few per cent. Equation 1 may therefore with sufficient accuracy be written as follows

$$c_{p+1}=c_p-\gamma\frac{x_p}{u-(x_1+\ldots+x_p)} \tag{3}$$

where

$$\gamma\equiv\frac{c_1+c_n}{2\,(\alpha-1)} \tag{4}$$

Using equation 3 we find

$$c_p=c_1-\gamma\,F_{p-1} \tag{5}$$

where

$$F_{p-1}\equiv\frac{x_1}{u-x_1}+\frac{x_2}{u-(x_1+x_2)}+\ldots+\frac{x_{p-1}}{u-(x_1+\ldots+x_{p-1})} \tag{6}$$

By means of formula 5 we may calculate c_p when v, a, s, and α are known. According to Winkler[3], α is at 18° C 23.4. We prefer, however, to use this

value of α only for the calculation of u by means of formula 2, while γ is determined for each run directly from the analyses of the first and last samples taken. The following example may serve as a test of formula 5 and an illustration of its application.

The total volume of the burette was $v = 136.9$, the volume above the zero mark $a = 19.0$. The bromine water was about 0.08 normal, its density [4] at 18° $s = 1.004$. Hence, $u = 124.5$. 14 samples of bromine water were taken from the burette. They were all titrated with 0.04 normal sodium thiosulphate. The results are given in the table. F_{p-1} was computed from the weighings by means of formula 6. With the aid of a calculating machine, this took only a few minutes. The first three and the last two titrations were used for determining the constants c_1 and γ of equation 5. The best agreement is obtained when the values $c_1 = 0.07736$ and $\gamma = 0.00324$ are chosen. We may now calculate c_p from formula 5. The results are given in the next to the last column of the table. The last column shows Δ, the difference between the amount of bromine found by analysis and that calculated from formula 5, expressed in ml 0.04 N thiosulphate. The agreement is satisfactory, not only for the samples used for fixing c_1 and γ, but also for the other portions (nos. 4—12). When the value of γ found here is used, we calculate from equation 4 the Bunsen absorption coefficient $\alpha = 24.2$ in good agreement with $\alpha = 23.4$ found by Winkler [3]. The average of α from 118 experiments, where the bromine water was used for kinetic measurements, was 22.9. The probable error of the single determination was $\pm$ 0.9.

Table 1.

p	x_p	F_{p-1}	c_p found	c_p form. 5	Δ
1	8.24	0	0.07726	0.07736	— 0.02
2	8.06	0.0709	0.07726	0.07713	+ 0.03
3	7.88	0.1454	0.07687	0.07689	0.00
4	9.71	0.2239	0.07673	0.07663	+ 0.02
5	8.20	0.3311	0.07627	0.07629	0.00
6	6.72	0.4306	0.07588	0.07596	— 0.01
7	4.07	0.5194	0.07586	0.07568	+ 0.02
8	2.38	0.5762	0.07516	0.07549	— 0.02
9	5.38	0.6106	0.07521	0.07538	— 0.02
10	5.17	0.6948	0.07499	0.07511	— 0.02
11	8.02	0.7829	0.07470	0.07482	— 0.02
12	8.26	0.9412	0.07415	0.07431	— 0.03
13	8.74	1.1359	0.07372	0.07368	+ 0.01
14	9.60	1.3955	0.07278	0.07284	— 0.01

Three sources of error in the method may be mentioned. (1) Insufficient saturation of the vapour phase. (2) When the burette is shaken after the removal of bromine water, the pressure in the burette increases slightly owing to evaporation of bromine. When the burette is opened at the top before the next portion is taken out, a small amount of bromine excapes. (3) If the temperature of the air that enters the burette while bromine water is running out is different from that of the thermostat, a change of pressure in the burette will take place when it is left in the thermostat, and this will either diminish or augment the loss of bromine when the next sample is taken. It holds for all three kinds of error that they will affect rather the values found for γ and a than those for c_p when the computation is carried out as described above.

When the loss of bromine owing to error (2) is taken into account, it may be shown that formulae 2, 4, and 5 remain the same, while a correction must be added to the right side of formula 6. This correction is given by the expression

$$\frac{6\,(c_1 + c_n)}{a + 6(c_1 + c_n)} \left(\frac{x_1^2}{u-(x_1 + x_2)} + \ldots + \frac{x_{p-2}}{u-(x_1 + \ldots + x_{p-1})} \right)$$

A recalculation of the example in the table using this correction gave the same values of c_p; only changes of one in the 5th decimal were found. a, however, increased from 24.2 to 24.9.

SUMMARY

An ordinary weight burette may be used for measuring out bromine water of fixed temperature (near room temperature). A formula is given which makes it possible to calculate the concentration of the bromine water delivered by the burette when the concentrations of the first and last portions taken from the burette are determined by titration.

I wish to express my thanks to the head of the laboratory, Professor Niels Bjerrum, for his kind interest in my work.

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The Decomposition of Nitroacetic Acid in Concentrated Aqueous Solutions of Some Non-Electrolytes

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Nitroacetic acid decomposes in aqueous solution into nitromethane and carbon dioxide. The first kinetical investigations of this reaction were carried out simultaneously by Heuberger [1, 2] and by the author of this paper [3]. The reaction is of the first order when the hydrogen ion concentration is kept constant. Nitroacetic acid is a dibasic acid. Its first dissociation constant is 0.0210 at 18° C and zero ionic strength (this paper and [4]). The second dissociation constant is, according to Heuberger [2], about 10^{-9}. Both the undissociated acid and the divalent ion, $^{-}OON{:}CH \cdot COO^{-}$, are stable, while the univalent ion decomposes spontaneously. The maximum velocity constant is therefore found in solutions where practically all the nitroacetic acid is transformed into the univalent ion, *e. g.* in acetate buffers. In more acid solutions, say in hydrochloric acid, the velocity constant is smaller and proportional to the degree of dissociation of the nitroacetic acid.

There are three possible forms of the univalent nitroacetate ion, namely

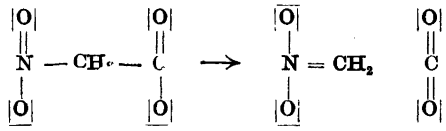
$$O_2N \cdot CH_2 \cdot COO^{-},\ HOON : CH \cdot COO^{-},\ \text{and}\ {}^{-}OON : CH \cdot COOH$$

The author of this paper has shown [4] that the first, and probably only the first, of these splits off carbon dioxide directly, and that the primary reaction product is the aci-form of nitromethane. The rate determining step is therefore

$$O_2N \cdot CH_2 \cdot COO^{-} \longrightarrow {}^{-}OON : CH_2 + CO_2$$

The ion formed takes up a proton, and, finally, the aci-nitromethane rearranges into normal nitromethane. It was suggested as a hypothesis that the decomposition takes place when a valency electron in an activated nitroacetate ion

is displaced from the oxygen atom of the carboxylate group through the molecule to the oxygen atom of the nitro group as illustrated in the following scheme where a line denotes a pair of electrons



In this paper, the decomposition is examined in aqueous solutions of dioxane, urea, glucose, and sucrose. In the first part, the velocity constant for the decomposition of the nitroacetate ion is determined. From a study of the influence of a gradual exchange of water molecules with those of the non-electrolyte, we may hope to find out whether water takes a direct part in the reaction as suggested by Heuberger [2], and whether the rate depends upon the dielectric constant of the medium. The urea solutions have a higher, the other solutions studied a lower dielectric constant than pure water. In the second part of the paper, the dissociation constant of nitroacetic acid is determined from the velocity constant in solutions containing hydrochloric acid in addition to the non-electrolyte.

The measurements were carried out at the temperature 18.00° C. The initial concentration of nitroacetic acid was always about 0.02 molar. The reaction was followed by observing the pressure above the solution during the reaction. The experimental procedure was the same as in earlier papers [4, 5]. The nitroacetic acid was prepared in the same way as earlier [3]. It was recrystallized twice from chloroform. Two preparations (melting points 92 and 93° C) were used. They gave concordant results. Dioxane which had been purified two years earlier [6] was again refluxed with sodium and distilled in an all-glass apparatus fitted with a column containing glass beads. The freezing point of the fraction used for the measurements was 11.65° C, while pure dioxane freezes [7] at 11.80°. The urea was partly a Kahlbaum preparation »for scientific purposes», partly the same recrystallized twice from alcohol. The two preparations gave concordant results. The sugars were Merck's purest anhydrous glucose and a high-grade, large-crystalline cane sugar.

THE VELOCITY CONSTANT FOR THE DECOMPOSITION OF THE NITROACETATE ION

The solutions examined in this part of the paper contain, in addition to the non-electrolyte, 0.100 molar acetic acid and 0.100 molar sodium acetate. The ratio between the dissociation constants of nitroacetic acid and acetic acid is,

in dilute aqueous solution, more than 10^3. Owing to the similarity in structure of the two acids, this ratio will probably not change much when going from pure water to the solutions investigated. That this holds for the dioxane solutions, is seen from table 4. We may therefore conclude that the nitroacetic acid, in all the solutions containing the acetate buffer, has been practically completely transformed into the univalent ion.

The results of the measurements are given in table 1. k^* is the velocity constant per minute based on decadic logarithms.

Table 1. The decomposition of nitroacetic acid in aqueous solutions of non-electrolytes at 18.0° C. The solutions contain in addition 0.100 molar acetic acid and 0.100 molar sodium acetate.

Dioxane Molarity	Molality	Mole fraction	k^*
0.000	0.000	0.00000	0.02482
0.495	0.522	0.00931	0.02661
0.999	1.099	0.01942	0.02864
1.998	2.411	0.04162	0.03450
3.006	4.028	0.06766	0.04327
4.003	6.014	0.09776	0.05578

Urea Molarity	Molality	Mole fraction	k^*
0.998	1.057	0.0187	0.02672
2.005	2.228	0.0386	0.02890
3.004	3.516	0.0596	0.03145
3.998	4.936	0.0817	0.03392

Glucose Molarity	k^*
0.500	0.02434
1.000	0.02380
1.500	0.02342
2.000	0.02317

Sucrose Molarity	Per cent	k^*
0.250	8.27	0.02473
0.500	16.02	0.02453
0.750	23.34	0.02453
1.000	30.21	0.02453
1.250	36.75	0.0244
1.500	42.90	0.0244
2.000	54.35	0.0243

When the sucrose concentration was more than 1 molar, the equilibrium between the carbon dioxide of the solution and the gas phase was not established sufficiently quickly to prevent a supersaturation effect. When log P for these experiments was plotted against the time t (P being the difference between the final pressure reading and the reading at the time t), the points did not, as usually, all fall close to a straight line, but formed a curve which rapidly approached a straight line with increasing t. This effect has been discussed earlier [8]. The velocity constant k^* is here the numerical value of the slope of the straight line approached. Owing to this complication, the velocity constants for solutions containing more than 1 molar sucrose are not quite as accurate as those for the other solutions.

In the experiments on solutions of urea, the pressure did not keep completely constant when all the nitroacetic acid was decomposed, but increased slowly, possibly owing to a slow hydrolysis of urea. This increase was, however, too slow to have any importance for the accuracy of the measurements.

It is seen from table 1 that the velocity constant for the decomposition of the nitroacetate ion increases with increasing concentrations of dioxane and urea, while it decreases a little when glucose is added. Sucrose has almost no effect. Even when more than half of the water has been replaced by sucrose, the decrease of k^* is only 2 per cent. These results show that the decomposition does not necessarily proceed *via* a chemical reaction with a water molecule.

The measurements show no dependence between the velocity of decomposition and the dielectric constant (ε) of the medium. In the dioxane solutions examined, ε decreases [9] from 81 to 50, in the urea solutions it increases [10] to about 88, while in both cases k^* increases. In the sucrose solutions, k^* is nearly constant, while ε decreases [11] from 81 to about 45. In the glucose solutions, k^* decreases slightly, while ε decreases [11] from 81 to about 40.

In fig. 1, the open circles show k^* for urea solutions plotted against the mole fraction (N_U) of urea. The points fall close to a straight line of which the equation is

$$k^* = 0.02482 + 0.110\ N_U$$

The solid circles represent k^* for dioxane solutions plotted against the mole fraction of dioxane. Here a curved line is obtained. If we, however, plot against the mole fraction N_D' of a hypothetical tetrahydrate of dioxane (crossed circles), the relation is again linear. It is represented by the equation

$$k^* = 0.02482 + 0.1940\ N_D'$$

It is uncertain whether this result has any real significance. In favour of the assumption that a hydrate of dioxane is formed speak the following facts. A

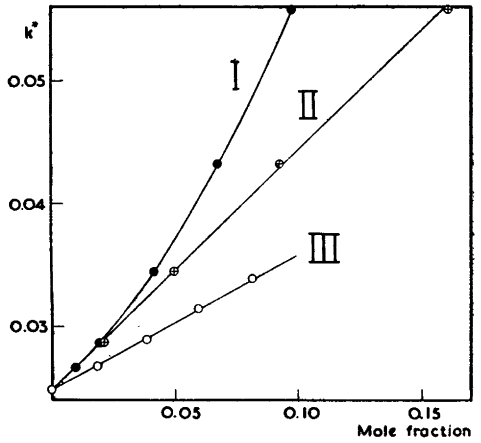


Fig. 1. The velocity constant k^* for the decomposition of the nitroacetate ion at 18° C. (1) in solutions of dioxane, plotted against the mole fraction of dioxane (I)*, and against the mole fraction of dioxane tetrahydrate* (II)*; (2) in solutions of urea, plotted against the mole fraction of urea* (III).

considerable contraction is found when dioxane and water are mixed (Hovorka, Schaefer, and Dreisbach [12]), and the viscosity-concentration curve for mixture, of dioxane and water shows a maximum (Geddes [13], Mariani [14]). While Geddes explains the maximum by the assumption of a pentahydrate of dioxanes Mariani concludes that a tetrahydrate is formed.

THE DISSOCIATION CONSTANT OF NITROACETIC ACID

The decomposition was studied in solutions of hydrochloric acid to which had been added either dioxane or glucose. The velocity constant is proportional to the degree of dissociation α of the nitroacetic acid. During the reaction, α increases a little and approaches a constant value α_∞ corresponding to an infinitely small concentration of nitroacetic acid. It has been shown earlier [4] how the velocity constant $\alpha_\infty k^*$ may be computed from the measurements.

The results are given in table 2. α_∞ has been computed by dividing $\alpha_\infty k^*$ with k^* found for an acetate buffer containing the non-electrolyte in the same concentration (table 1). The dissociation constant of nitroacetic acid is $K = a\alpha_\infty/(1-\alpha_\infty)$, where a is the concentration of hydrochloric acid. $-\log K$ found in this way is given in the next to the last column of table 2. $-\log K$ varies with the concentrations of both the non-electrolyte and the hydrochloric acid. In order to find $-\log K_a$, where K_a is the dissociation constant corresponding to pure aqueous solution of the non-electrolyte, extrapolations to zero ionic strength are carried out by means of formulae of the following form

$$-\log K = -\log K_a - A\mu^{\frac{1}{2}} + B\mu - C\mu^{\frac{3}{2}}, \qquad (1)$$

Table 2. The dissociation constant K of nitroacetic acid in aqueous solutions of dioxane and glucose at 18.0° C computed from the velocity constant $a_\infty k^*$ for the decomposition in solutions containing a molar hydrochloric acid.

Moles/l	a	$a_\infty k^*$	a_∞	μ	—log K found	—log K formula 1
Dioxane						
0.000	0.2003	0.003898	0.1571	0.2022	1.428	1.428
	0.09923	0.006428	0.2590	0.1024	1.460	1.460
	0.04049	0.01074	0.4327	0.0458	1.510	1.510
	0.04047	0.01074	0.4327	0.0458	1.510	1.510
	0.02009	0.01461	0.5886	0.0268	1.542	1.540
	0.02009	0.01465	0.5902	0.0270	1.539	1.540
0.999	0.2003	0.003370	0.1177	0.2017	1.573	1.573
	0.09923	0.005637	0.1968	0.1018	1.614	1.615
	0.09923	0.005646	0.1971	0.1017	1.613	1.615
	0.04047	0.00995	0.3474	0.0448	1.667	1.665
	0.02009	0.01432	0.5000	0.0259	1.697	1.698
1.998	0.2003	0.002866	0.08307	0.2014	1.741	1.741
	0.09923	0.004845	0.1404	0.1011	1.790	1.790
	0.04043	0.008867	0.2570	0.0436	1.854	1.853
	0.02009	0.01344	0.3896	0.0246	1.892	1.893
3.006	0.2003	0.002342	0.05413	0.2009	1.941	1.941
	0.09923	0.003954	0.09138	0.1004	2.001	2.002
	0.04051	0.007445	0.1721	0.0426	2.075	2.073
	0.02009	0.01185	0.2734	0.0226	2.122	2.123
4.003	0.2003	0.001837	0.03293	0.2007	2.166	2.167
	0.2003	0.001833	0.03286	0.2007	2.167	2.167
	0.09923	0.003112	0.05579	0.1000	2.232	2.234
	0.04049	0.005817	0.1043	0.0417	2.326	2.322
	0.02009	0.00953	0.1708	0.0218	2.383	2.385
Glucose						
1.000	0.2003	0.003028	0.1272	0.2018	1.535	1.535
	0.09931	0.005046	0.2120	0.1018	1.573	1.573
	0.04107	0.008780	0.3689	0.0453	1.620	1.620
	0.02009	0.01247	0.5239	0.0257	1.655	1.655
2.000	0.2003	0.002282	0.0985	0.2015	1.660	1.659
	0.09921	0.003875	0.1672	0.1012	1.701	1.704
	0.04107	0.006982	0.3013	0.0444	1.752	1.745
	0.02009	0.01046	0.4514	0.0249	1.782	1.786

where μ is the ionic strength. We assume that the Debye-Hückel limiting law holds for the solutions when μ is sufficiently small. It follows from this law that A may be calculated from the dielectric constant of the medium by means of the formula

$$A = 2 \times 1.824 \times 10^6 (\varepsilon T)^{-\frac{3}{2}},$$

where T is the absolute temperature. The dielectric constants for the dioxane solutions are found by interpolation from the data of Åkerlöf and Short [9], those for the glucose solutions from the determinations of Fürth as given by the International Critical Tables [11]. The other three constants of equation 1, namely B, C, and $-\log K_a$, are chosen so as to obtain the best agreement with $-\log K$ found from the measurements. The ionic strengths used for the computation are to be found in the fifth column of table 2. They are average values corresponding to solutions where one third of the nitroacetic acid originally added (about 0.02 molar) has been decomposed. ε and the constants of equation 1 are given in table 3. In the last column of table 2, the values of $-\log K$ calculated from equation 1 are presented.

Table 3. The dissociation constant K of nitroacetic acid in aqueous solutions of dioxan and glucose at 18.0° C.

$$-\log K = -\log K_a - A\mu^{\frac{1}{2}} + B\mu - C\mu^{\frac{3}{2}}.$$

Moles/l	Per cent	ε	A	B	C	$-\log K_a$
Dioxane						
0.000	0.00	81.13	1.006	1.103	0.218	1.677
0.999	8.73	73.28	1.170	1.767	1.143	1.845
1.998	17.34	65.45	1.388	2.031	1.223	2.065
3.006	25.91	57.62	1.680	2.772	2.035	2.320
4.003	34.29	49.93	2.082	3.396	2.307	2.625
Glucose						
1.000	16.90	59.6	1.596	3.218	2.61	1.839
2.000	31.82	40.5	2.850	7.806	7.81	2.072

$-\log K_a$ for nitroacetic acid in solutions of dioxane is given in the third column of table 4. A linear relation

$$-\log K_a = 1.677 + 0.1607\ m, \qquad (2)$$

where m is the molality of dioxane, is found to hold. $-\log K_a$ calculated from formula 2 is given in the fourth column of table 4.

Table 4. The dissociation constants of nitroacetic acid and acetic acid in aqueous solutions of dioxane at 18.0° C and zero ionic strength.

Molarity	Molality	—log K_a found	—log K_a form. 2	—log K_a form. 5	—log K_a acetic acid	Diff.
0.000	0.000	1.677	1.677	1.677	4.757	3.08
0.999	1.086	1.845	1.852	1.848	4.970	3.13
1.998	2.381	2.065	2.060	2.056	5.212	3.15
3.006	3.969	2.320	2.315	2.314	5.484	3.16
4.003	5.923	2.625	2.629	2.637	5.800	3.18

If the change in K_a for an uncharged acid when passing from one solvent to another were determined only by the change in electrostatic energy of the ions, and if the ions were unsolvated, rigid spheres (radii r_c and r_a) in a continuous medium, the following formula would hold (*cf.* Bjerrum and Larsson [15], Wynne-Jones [16], Brønsted [17])

$$-\log K_a = -\log K_{ao} + \frac{0.4343\ e^2}{kTr_m}(\varepsilon^{-1} - \varepsilon_o^{-1})$$

where e is the charge of the electron, k the Boltzmann's constant, r_m the average radius of the ions defined by

$$\frac{2}{r_m} = \frac{1}{r_c} + \frac{1}{r_a}$$

while K_{ao} is the dissociation constant and ε_o the dielectric constant in a standard solvent, say water. If the values of e, k, and T are introduced we obtain

$$-\log K_a = -\log K_{ao} + \frac{249}{r_m}(\varepsilon^{-1} - \varepsilon_o^{-1}) \qquad (3)$$

at 18° C., where r_m is expressed in Ångström's units.

If we take into account that the protons combine with water molecules forming hydroxonium ions, but assume that dioxane does not react with protons, we must replace K_a in formula 3 by $K_a \frac{a_{wo}}{a_w}$ or by $K_a \frac{p_o}{p}$, where a_w is the activity and p the partial vapour pressure of the water in the solution, a_{wo} the activity and p_o the vapour pressure of pure water. Adequate data for calculating this expression for the solutions examined here are, however, not available.

Instead of the activity we, therefore, use the molar concentration of water. We replace K_a in formula 3 by $K_a \frac{c_{wo}}{c_w}$. Since

$$\frac{c_{wo}}{c_w} = \frac{m}{c}$$

where m is the molality and c the molarity of the non-electrolyte added, we obtain

$$-\log\left(K_a \frac{m}{c}\right) = -\log K_{ao} + \frac{249}{r_m}(\varepsilon^{-1} - \varepsilon_o^{-1}) \qquad (4)$$

If this formula is valid, there is a linear dependence between $-\log\left(K_a \frac{m}{c}\right)$ and ε^{-1}. In order to test this on the data for nitroacetic acid in dioxane-water mixtures, $-\log\left(K_a \frac{m}{c}\right)$ has been plotted against ε^{-1}. The points fall close to a straight line of which the equation is

$$-\log\left(K_a \frac{m}{c}\right) = 1.677 + 102.6\ (\varepsilon^{-1} - \varepsilon_o^{-1}) \qquad (5)$$

$-\log K_a$ calculated from formula 5 is presented in the fifth column of table 4. From the equations 4 and 5 we calculate $r_m = 249/102.6 = 2.4$ Å.

The dissociation constant of acetic acid in mixtures of dioxane and water has been determined electrometrically at different temperatures by Harned and Fallon [18]. The values given in the next to the last column of table 4 have been found by interpolation from their data. It is seen that the difference between $-\log K_a$ for acetic and nitroacetic acid, presented in the last column of table 4, is nearly constant and always greater than 3.

Table 5. The ratio f between the dissociation constant of nitroacetic acid in solutions of glucose calculated from formula 5 and that found from the measurements.

Molarity	Molality	$-\log K_a$ form. 5	$-\log K_a$ found	f
1.000	1.129	2.187	1.839	0.449
2.000	2.591	3.058	2.072	0.103

It is seen from table 5 that the dissociation constant of nitroacetic acid measured in solutions of glucose is much greater than that calculated from the

dielectric constant and the water concentration by means of the formula 5, holding for dioxane solutions. This difference is by no means surprising and may have different reasons. If, for instance, the protons combine not only with the water but also with the glucose molecules, an increase of K_a for glucose solutions will result. If this were the only reason for the difference, and if we further assume that no other cations than hydroxonium ions are formed to any appreciable extent in the dioxane solutions, the ratio f (given in the last column) between K_a calculated from formula 5 and K_a measured would be equal to the fraction of the protons which have formed hydroxonium ions ($f = c_{H_3O^+}/a$).

SUMMARY

The kinetics of the decomposition of nitroacetic acid into nitromethane and carbon dioxide were studied (1) in acetate buffers containing dioxane, urea, glucose, or sucrose; and (2) in solutions of hydrochloric acid containing dioxane or glucose.

From the experiments in acetate buffers, the velocity constant k^* for the decomposition of the nitroacetate ion was computed. k^* increases when dioxane is added (124 per cent in 4 molar dioxane). Urea causes a smaller increase (37 per cent in 4 molar urea). When glucose is added, a moderate decrease of k^* is observed (7 per cent in 2 molar glucose), while sucrose has very little influence (2 per cent decrease in 2 molar sucrose). There is evidently no relation between k^* and the water concentration or the dielectric constant of the solution.

From the experiments on solutions of hydrochloric acid, the dissociation constant K_a for nitroacetic acid at zero ionic strength was computed. For the dioxane solutions, the formula $-\log K_a = 1.677 + 0.1607\ m$, where m is the molality, is found to hold, and there is a linear relation between $-\log \left(K_a \frac{c_{wo}}{c_w} \right)$ and the reciprocal of the dielectric constant. Here $\frac{c_{wo}}{c_w}$ is the ratio between the molar concentrations of water in pure water and in the solution. When solutions of the same dielectric constant are compared, $\left(K_a \frac{c_{wo}}{c_w} \right)$ is much greater in glucose than in dioxane solution. This may partly be due to the formation of glucosonium ions.

I wish to express my thanks to the head of the laboratory, Professor Niels Bjerrum, for his kind interest in my work.

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Preliminary communication

On the Nitration of Retene

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In spite of several attempts to prepare a well defined nitro compound of retene (1-methyl-7-isopropyl-phenanthrene) by direct nitration, these have not been followed by success [1, 2].

However, we have succeeded in preparing a nitroretene in pure state by nitration of retene under mild conditions followed by chromatographic separation and molecular distillation of the reaction product. The nitro compound obtained has been identified as the 9-nitro compound.

Experimental. 75 ml of concentrated nitric acid (about 65 %) was slowly added to a suspension of 20 g of retene in 200 ml *n*-butyric acid. The temperature was not allowed to rise above —6° C.

The clear orange coloured reaction mixture was poured into 1.5 l of water and the precipitated oil was dissolved in 600 ml of petroleum ether (b. p. 60—80° C). After the solution had been dried with sodium sulphate it was chromatographed on 150 g of aluminium oxide (activated by treatment with sodium hydroxide). Several zones were observed including a purple and a yellow one.

By elution of the yellow zone with benzene and dilution of the eluate with petroleum ether we obtained, on chilling the solution in an ice box, 1.2 g of a yellow crystalline compound (m. p. 115—120° C, corr.).

The compound was then submitted to a molecular distillation at 5×10^{-4} mmHg. The distance between the heated and the cooled surface was 1 cm. At 62—67° C a yellow compound, which crystallized slowly on standing, was removed by distilling and at 72—77° C a yellow crystalline compound was collected. This compound was recrystallized from petroleum ether and melted at 123.5—125° C (corr.).

$C_{18}H_{17}O_2N$ (279.3)
Calc. N 5.02 Found N (Dumas) 5.08

A mixture of our compound and 9-nitro retene (m. p. 123—125°), prepared by deaminating 9-nitro-3-aminoretene[2] melted at 123—125° C (corr.).

A detailed description will be published later.

We are indepted to Docent, Dr. K. J. Karrman, University of Lund, who has drawn our attention to the problem and who has suppiled us with pure retene and a sample of 9-nitroretene.

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Retene Investigations

X. Oxidation of Retene with Hydrogen Peroxide

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Oxidation of retene to well defined products offers serious difficulties. Potassium permanganate has no effect either in acid or alkaline solution [1]. With diluted nitric acid a mixture of different nitro-compounds is obtained and possibly carboxylic acids as well [2]. With potassium ferricyanide the side chains are oxidized to carboxyl groups [3], but this treatment is not practical and the yield is small. The best known and most widely used oxidizing agent is chromic acid in glacial acetic acid which converts retene to retene quinone with a yield of 35—40 % [4]. Bichromates, on the other hand, give a very low yield of quinone.

From a technical point of view the use of chromic acid and glacial acetic acid proves too expensive especially when the relatively poor yield of quinone is taken into consideration. Retene quinone in itself is not a product of technical use but only as an intermediate useful for the manufacture of dyes, dicarboxylic acids, etc. We, therefore, considered it suitable to make a more systematic investigation of the possibilities of oxidizing retene to carboxylic acids in a more satisfactory manner.

Hydrogen peroxide was chosen as oxidizing agent on the grounds that its technical significance is constantly increasing. It is now produced industrially at as high a concentration as 90 %. Hydrogen peroxide is seldomy used for oxidation of hydrocarbons but in the literature, however, we have found a few examples of such oxidations. Thus, there is a statement to the effect that phenanthrene can be oxidized with hydrogen peroxide to diphenic acid [5]. Cyclo-hexane is oxidized by hydrogen peroxide to cyclo-hexandiol in the presence of selenium dioxide [6].

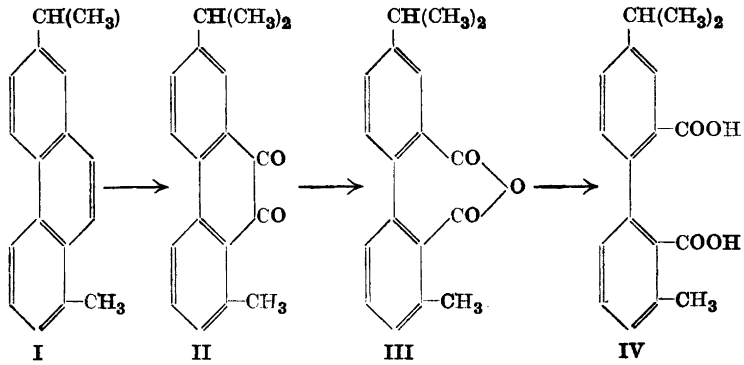
It is generally known that ortho-quinones may be oxidized with hydrogen peroxide with the formation of dicarboxylic acids [7]. In this manner, also

retene quinone has been oxidized. This oxidation was first carried out by Bamberger and Hooker [8], who, however, failed to obtain any crystalline acid, and later by Fogelberg [9], who succeeded in obtaining retenediphenic acid in crystalline form. He obtained after the oxidation a sticky mass which was converted to the anhydride. After hydrolysis of the anhydride the crystalline acid was obtained. Finally Adelson, Hasselstrom and Bogert [10] have worked out a method which gives retenediphenic acid in a yield of 65 % from retene quinone. But since retene quinone is only obtained in a yield of approximately 40 % from retene, the yield of retenediphenic acid from retene is only 20—25 %. The object of this investigation is the production of retenediphenic acid in a better yield from retene by oxidation with hydrogen peroxide.

OXIDATION OF RETENE IN GLACIAL ACETIC ACID SOLUTION

Hydrogen peroxide reacts very strongly with retene dissolved in glacial acetic acid. The reaction mixture first turns dark red, but after a few hours it takes on a lighter colour. According to Charrier and Moggi [5], who investigated the oxidation of phenanthrene, this phenomenon may be explained by supposing that quinone-like products are initially formed and that these are then oxidized to carboxylic acids. As a matter of fact we have isolated carboxylic acids from the crude reaction product at approximately 90 % yield. The acid fraction purified by conversion to the anhydride turned out, for the most part, to be retene-diphenic acid (IV).

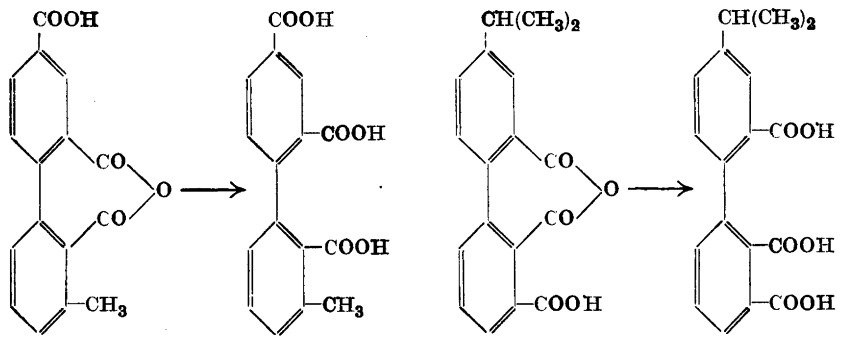
The reaction may be supposed to take place in the following manner. In the first stage of oxidation retene quinone as indicated by the red colour is presumably formed. In the reaction product we have, however, also noted retenediphenic acid anhydride and it is possible that the anhydride is not only a by-product but also a genuine intermediary product of the oxidation of retene to retenediphenic acid. In this respect it is interesting to note that by the oxidation of camphor quinone with hydrogen peroxide, camphoric acid anhydride is the principal product. It seems, therefore, likely that the reaction takes place mainly in the following manner:



I II III IV

If the oxidation product is extracted with a bicarbonate solution and the diphenic acid is then precipitated with sulphuric acid, a smaller quantity of diphenic acid is obtained than when the reaction product is boiled first with sodium hydroxide, and then the acid precipitated. Retenediphenic acid anhydride, being relatively stable, reacts very slowly with bicarbonate solution.

The crude reaction product is more or less dark coloured and of resinous appearance and cannot be brought to crystallization. It may also be assumed that the side chains are partially oxidized to carboxyl groups, since the analysis of the crude acid reveals a higher content of oxygen than that calculated for retenediphenic acid. The fact that the equivalent weight is higher than calculated may be explained by assumpting that a part of the reaction product consists of anhydride acids. The presence of the following anhydride acids (V and VII) is very likely.



V VI VII VIII

If the crude oxidation product is boiled with alkali, lower values for the equivalent weight are found. This can only be explained by assuming that the above-named anhydride acids are saponified to tribasic acids (VI and VIII). When all these things are taken into consideration the quantity of anhydride acids is relatively small compared to the quantity of retenediphenic acid. We have not succeeded in isolating the anhydride acids in a pure state.

OXIDATION IN ETHANOL AND DIOXANE SOLUTION IN THE PRESENCE OF SELENIUM DIOXIDE

If one attempts to oxidize retene with hydrogen peroxide in ethanol solution no oxidation takes place and one obtains for the most part unchanged retene. Almost the same result is obtained in dioxane solution, the yield of acids being extremely poor. If, on the other hand, retene is oxidized with hydrogen peroxide in the two solvents mentioned above and in the presence

of small quantities of selenium dioxide, retenediphenic acid is obtained in quite a good yield. Selenium dioxide, therefore, has a very obvious effect on the oxidation of retene. As oxidation in glacial acetic acid (acid medium) runs smoothly even in the absence of selenium dioxide, it might be surmised that the effect of selenium dioxide depends solely on the fact that it acidifies the reaction mixture when ethanol and dioxane are used as solvents. That this is not the case was clearly demonstrated by experiments with sulphuric acid instead of selenium dioxide. No oxidation was observed (table 1).

Table 1. Oxidation of retene with hydrogen peroxide in different solvents.

Solvents	Added substance (in small quantities)	Reaction products in %		
		Unchanged retene	Neutr. oxidation products	Acid. oxidation products
Ethanol	—	95	—	2
»	SeO_2	—	35	90
»	H_2SO_4	95	—	2
Dioxane	—	90	—	10
»	SeO_2	—	10	85
Acetic acid	—	—	10	90
» »	SeO_2	—	20	85

In acetic acid solution selenium dioxide has, as mentioned above, no noticeable influence on the oxidation. The neutral oxidation products contain approx. 13 % oxygen, the acids approx. 24 %.

OXIDATION OF PHENANTHRENE

In the literature we have only found one reference which deals with the oxidation of phenanthrene with hydrogen peroxide [5]. For the sake of comparison, therefore, we considered it to be of interest to carry out a few oxidation experiments with phenanthrene. The conditions in this case are much simpler than with retene, since the formation of anhydride acids (V and VII) is of course excluded. We immediately obtained diphenic acid in crystalline form. From the fraction of the oxidation products which is insoluble in bicarbonate we have isolated diphenic acid anhydride. This supports the above-mentioned conclusion concerning the course of the oxidation. If the oxidations of phenanthrene and retene are compared it is found that retene gives a higher yield of acids than phenanthrene. This is shown in table 2.

Table 2. Oxidation of retene and phenanthrene with hydrogen peroxide in glacial acetic acid.

Substance	Oxidation product in percentage of the theoretical yield	
	Neutral	$NaHCO_3$-soluble
Phenanthrene	25	50
Retene	10	90

SOME DERIVATIVES OF RETENEDIPHENIC ACID

Seeing that retenediphenic acid has now become a readily available substance, we considered it appropriate to prepare from it a few simple derivatives which have not been prepared previously.

Neutral methyl esters. Neutral esters of retenediphenic acid and simple aliphatic alcohols have not previously been prepared. On the other hand, Adelson and others [10] prepared glyptal-like esters from retenediphenic acid and glycerol or borneol, obtaining light brown, resin-like masses.

Retenediphenic acid reacts easily with methanol with the formation of the acid ester. To prepare the neutral ester a solution of retenediphenic acid in methanol was saturated with hydrochloric acid and then boiled for several hours. But in spite of the fact that the neutral esters may be easely prepared in this manner from both phthalic acid [11] and diphenic acid [12], we obtained from retenediphenic acid the neutral ester in a yield of only approximately 25 %. Therefore the chloride of retenediphenic acid was first prepared with phosphorous pentachloride and then the chloride was reacted with methanol. In this manner the neutral ester was obtained in a yield of approx. 70 %.

It is likely that the presence of a methyl group in ortho-position to one of the carboxyl group has a hindering effect on its esterification.

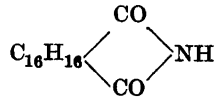
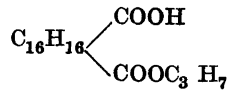
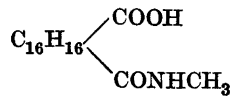
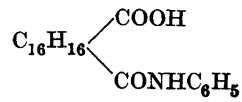
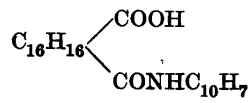
The neutral methyl ester of retenediphenic acid has the consitency of a thick oil which does not appear to crystallize.

The *imide* was obtained by heating the monoamide, the latter being formed by the reaction of ammonia and retenediphenic acid anhydride.

Different amines react easily with retenediphenic anhydride to form the N substituted monoamides. In this manner we have prepared methyl, phenyl and naphthyl derivatives.

Bromination of retenediphenic acid anhydride gave a monobromo derivative. Although the analysis agrees with the calculated values it is presumably a mixture of isomers because the melting-point is far from sharp. On saponification more alkali is consumed than theoretically calculated and a quantity bromine is split off.

Summary of newly prepared substances.

Name	Formula	Property
Retenediphenic acid dimethyl ester	$C_{16}H_{16}(COOCH_3)_2$	light yellow oil
» » imide		—
» » monopropyl ester		sticky mass
» » methylamide		m. p. 165—166° C
» » anilide		m. p. 251—252° C
» » α-naphtyl amide		m. p. 125—127° C

EXPERIMENTAL

Oxidation of retene with hydrogen peroxide in glacial acetic acid

25 g retene were dissolved in 100 ml warm glacial acetic acid and to the boiling solution were added 70 ml 30 % hydrogen peroxide in small quantities. The mixture was boiled for 12 hours. The solution was first very dark red, but grew considerably lighter toward the conclusion of boiling. The solvent was first evaporated under ordinary pressure and then *in vacuo*. The residue, 30.8 g, was divided into two equal parts (15.4 g each).

I. The one half was boiled with sodium hydroxide.

1. Neutral oxidation product 1.2 g (not investigated).
2. Acid oxidation product 14.1 g (= 90 %, assuming that the whole is retenediphenic acid, $C_{18}H_{18}O_4$).

$C_{18}H_{18}O_4$	Calc. C	72.5	H	6.08	O	21.5 (diff.)	Equiv.wt.	149
	Found »	70.0	»	5.91	»	24.1 »	» »	144

Both the equivalent weight determined by titration and the elementary analysis (oxygen excess) show that a quantity of tri- or quadribasic acids is present (oxidation of side chains).

II. The second half was boiled with a bicarbonate solution.

1. Neutral oxidation product 4.4 g (not investigated).
2. Acid oxidation product 10.9 g (= 70 %, assuming that the whole is retenediphenic acid).

The greater quantity of the neutral part in this case shows that anhydride is present in the reaction product.

The acid fraction was analysed:

$C_{18}H_{18}O_4$	Calc.	C 72.5	H 6.08	O 21.5	(diff.)
	Found	» 71.5	» 5.83	» 22.7	»

Titration (direct) gave an equic. weight of 178, calc. 149 (for retenediphenic acid). After boiling with sodium hydroxide an equivalent weight of 145 was found. This indicates as mentioned above the presence of anhydride or possibly a lactone group.

Preparation of the anhydride

40 g of the oxidation product soluble in alkali was boiled with 80 ml acetic acid anhydride for 2 hours under reflux. After the solvent had been removed, 36 g of reaction product were obtained. The product was crystallized from ether. (Yield 22 g.) After recrystallization from acetic acid a pure product was obtained with m. p. 111—112° C. Retenediphenic acid anhydride melts according to Fogelberg at 111—112° C.

$C_{18}H_{16}O_3$	Calc.	C 77.1	H 5.76
	Found	» 77.0	» 5.77

The retenediphenic acid anhydride was boiled with sodium hydroxide, after which the equiv. weight agreed with the calculated value. The melting-point for the retenediphenic acid thus obtained (187—189° C) agrees with the values in the literature.

Oxidation in ethanol and dioxane solutions

5 g of retene dissolved in 80 ml of ethanol or dioxane were boiled with 20 ml 30 % hydrogen peroxide for 20 hours with or without addition of small quantities of selenium dioxide or sulphuric acid. Retene, when precipitated, was filtered off, the solvent evaporated, and the residue treated first with bicarbonate solution and then with a solution of sodium hydroxide. The solutions were then acidified with sulphuric acid and the precipitated acids dissolved in ether. In this manner the quantities of neutral oxidation products and of oxidation products soluble in bicarbonate were determined. (See table 1.)

Dimethyl ester of retenediphenic acid

1. *Boiling with methanol containing hydrochloric acid.* 10 g of retenediphenic acid were dissolved in 50 ml of methanol and the solution was saturated with gaseous hydrochloric acid at 0° C. The reaction mixture was allowed to stand over night,after which it was boiled for 3 hours under reflux. The remaining methanol was distilled off and theresidue dissolved in ether. The ethereal solution was first washed with water and later with a bicarbonate solution. This was finally acidified and the precipitated oil collected in ether. In this manner the neutral and the acid esterification products were separated from one

another; (yield 2.3 g and 7.2 g respectively). The neutral part containing dimethyl ester was distilled at 1 mm Hg 230—250° C. A light-brown viscous oil was obtained.

$C_{20}H_{22}O_4$	Calc. C 73.6	H 6.80
	Found » 74.2	» 6.94

2. *Esterification with acid chloride.* 5.0 g of retenediphenic acid (crude product) and 7.0 g of phosphorous pentachloride were heated in a waterbath and boiled until the gas evolution had ceased and a clear solution was formed. The reaction mixture was allowed to react with methanol for 3 hours. After the remaining methanol had been distilled off, the residue was dissolved in ether and the ether solution washed with water and a solution of bicarbonate. After the ether had been distilled off, the residue was distilled at 1 mm Hg (230—250° C). Yield 3.4 g of a light brown oil (68 % of the theoretical yield).

$C_{20}H_{22}O_4$	Calc. C 73.6	H 6.80
	Found » 73.0	» 6.65

n-*Propyl ester*, $C_{16}H_{16}\begin{cases}COOC_3H_7\\COOH\end{cases}$ To 1 g of retenediphenic acid anhydride was added an excess of n-propyl alcohol and the mixture was boiled for a short time. After the solvent had been evaporated, a tough, almost colourless mass was obtained, which could not be crystallized.

Calc. C 74.0	H 7.11
Found » 73.3	» 7.03

N-Phenyl retenediphenicamide, $C_{16}H_{16}\begin{cases}CONHC_6H_5\\COOH\end{cases}$ Equal parts of retenediphenic acid anhydride and aniline were heated together, after which the excess of aniline was removed with diluted hydrochloric acid. The crude product was recrystallized from ethanol. M. p. 251—252° C.

Calc. C 77.2	H 6.21
Found » 77.2	» 6.14

N-α-Naphthyl retenediphenicamide, $C_{16}H_{16}\begin{cases}CONHC_{10}H_7\\COOH\end{cases}$ was obtained in a similar manner from retenediphenic acid anhydride and α-naphtyl amine. M. p. 125—127° C.

Calc. C 79.4	H 5.96
Found » 79.2	» 5.82

N-Methyl retenediphenicamide, $C_{16}H_{16}\begin{cases}CONHCH_3\\COOH\end{cases}$ was obtained from methylamine hydrochloride and retenediphenic acid anhydride in the presence of an excess of alkali. The crude product was recrystallized from ethanol. M. p. 165—166° C.

Calc.	C 73.3	H 6.80
Found	» 72.9	» 6.64

Oxidation of phenanthrene. 5 g of phenanthrene, 50 ml of glacial acetic acid and 25 ml 30 % hydrogen peroxide were boiled for three hours. The neutral and acid fractions of the reaction products were isolated in the previously described manner. Yield: neutral fraction 1.4 g, acid fraction 3.7 g. The acid reaction product was analysed.

Diphenic acid, $C_{14}H_{10}O_4$	Calc.	C 69.4	H 4.17
	Found	» 69.4	» 4.17

On titration 88.6 mg of substance consumed 7.25 ml of 0.1007 *N* sodium hydroxide, theoretical value for $C_{14}H_{10}O_4$, 7.20 ml.
Both from the analysis and the titration it is clear that a very pure diphenic acid is immediately obtained by the oxidation of phenanthrene. The melting point of diphenic acid was 227—228° C as compared to 228—229° C as reported in the literature [9].

SUMMARY

Retene, dissolved in acetic acid, was oxidized by hydrogen peroxide to a dibasic acid, retenediphenic acid, in a yield of approx. 70 %. In ethanol or dioxane solution retene was oxidized only in the presence of small quantities of selenium dioxide. Selenium dioxide has no effect on the oxidation of retene in acetic acid solution.

Some simple derivatives of retenediphenic acid have been prepared.

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Note on the Synthesis of Di-[ω-phenyl-polyene]-diketones

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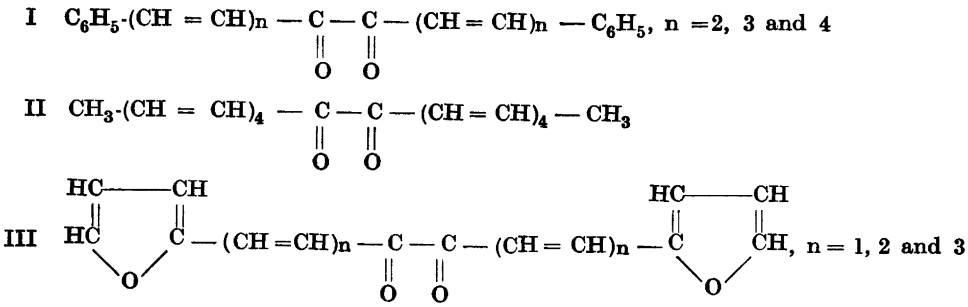
In 3 papers from 1945 and 1946 Karrer and his collaborators[1] have described the preparation and the properties of some polyene-α-diketones *viz.*



In the autumn of 1940 Sörensen [2] put forward the hypothesis that »visual purple» was the endiol-protein-komplex of β-carotene, in which the two central hydrogen atoms were substituted with hydroxyl. The free dihydroxy-β-carotene would rearrange to the ketolform which was postulated to be the »visual yellow». The enolization had to be carried out through a specific protein, stabilizing the endiol through complex salt formation quite in analogy with the reversible passage:

astaxanthin + protein ⇌ ovoverdin

of the lobsteregg chromoproteid, the main difference being the different positions of the ketol-grouping.

In astaxanthin two such ketolgroupings symmetrically frame the polyene system, whilst in »visual yellow» only one occupies the central carbon atoms of the β-carotene-skeleton. This difference is, however, important, as in »visual yellow» the ketol grouping interrupts the conjugation and so reduces

the U. V.-Spectrum to that of vitamin-A-aldehyde whereas the enolization to the endiol-grouping in »visual purple» re-establishes the conjugated carotenoid spectrum.

With the intention of synthesizing model substances for this theory, Samuelsen carried out the synthesis of some of the diketones of Karrers first series (I). Samuelsen gave a lecture about his synthesis of the ω-diphenyl-polyene-diketones with n = 1, 2 and 3 at the meeting of Norsk Kjemisk Selskap, Trondhjems Avdeling on April 29th 1941. Of course, no information about our work could reach professor Karrer.

The synthesis of the simplest of these diketones, 1.6-diphenyl-hexadiene-1,5-dione-3,4 or »cinnamil» which was not prepared by Karrer and Cochand[1] is described in the experimental part. Otherwise we confine ourselves to a tabular comparison of the m. p. stated by Karrer and Samuelsen in 1941. Oxaal in 1946, without knowledge of the Swiss publications, repeated and to some extent improved the procedure of Samuelsen.

n	Yield diketone			M. p.* diketone			M. p. quinoxaline	
	P. K.	E. S.	Fr. O.	P. K.	E. S.	Fr. O.	P. K.	E. S.
1	— %	8.5 %	10.5 %	—	165—166	166	—	194°
2	25 »	45 »	57 »	188.5—189	190—191	191—192	202—203	201°
3	17 »	15 »	—	197	200—201	—	162—163	—
4	5.6 »	—	—	201—202	—	—	—	—

The agreement in m. p. of the diketones and their quinoxalines is quite good. Samuelsen used the piperidonium acetate catalysator of Kuhn, Badstübner and Grundmann[3] which seems to give yields superior to piperidine alone which Karrer has employed. Oxaal has tried to raise the yield of »cinnamil» to that of diphenyl-decatetraene-dione, but could only improve the original yield by a few percent. Probably the reason for these bad yields is some side reaction between »cinnamil» and piperidoniumacetate. All the diphenyl-polyene-diketones give dark reaction products when heated in alcoholic solution with the catalysator; we have, however, got no crystallizing reaction product. Whereas diphenyl-decatetraenedione crystallizes very readily from the reaction medium and so in part escapes further destruction, »cinnamil» only crystallizes on dilution and cooling and therefore is all the time hazardized through side reactions.

* All m.p. are uncorrected.

EXPERIMENT

Cinnamil = 1,6-diphenyl-hexadiene-1,5-dione-3,4

To 0.4 mole benzaldehyde (42.5 g) and 0.1 mole diacetyl (8.6 g) diluted with 40 ml 57 per cent ethanol was given 0.02 mole glacial acetic acid and 0.02 mole piperidine. The mixture was heated to 81—83° C for 2 hours and cooled down through addition of 100 ml of ethanol. Immediately 1.5 g cinnamil separated; concentration of the mother liquor and cooling gave further 1.25 g pure cinnamil. Yield 10.5 per cent calculated on diacetyl. M. p. 166 uncorr, 169 corr.

21.3 mg gave 10,1 mg H_2O, 64.3 mg CO_2.

C_{18} H_{14} O_2 (262.3)	Calc.	C 82.41	H 5.38
	Found	» 82.33	» 5.31

Cinnamil mostly crystallizes in dense needles 3—5 mm long with sawtoothed edge, once however it occured in very fine, flexible, hairy crystals of some cm length. As the morphological difference was very great we were surprised that both preparations proved to be cinnamil: analysis, m. p. and Debye-Scherrer diagrams proved the identity of the two samples. Later on we learned that diphenyl-decatetraene-dione too, which ordinarily crystallizes in six-sided plates through the action of alcali is contaminated with small amounts of some other substance which cause diphenyl-decatetraene-dione to crystallise in long, thin needles. Obviously the crystal habitus of some of the polyenediketones are very sensible to impurities which are formed in alcaline medium.

2,3-Di[β-phenyl-vinyl]-quinoxaline

To 0.52 g cinnamil and 0.22 g *o*-phenylenediamine in 35 ml benzene was added a few drops glacial acetic acid and the mixture heated under reflux for 15 min. 2/3 of the solvent was distilled off and replaced with an equal amount petrol ether. 2.3-di-[β-phenyl-vinyl]-quinoxaline crystallises in slightly yellow needles, m. p. 194° uncorr.

15.9 resp. 28.6 mg gave 49.8; 91.15 mg CO_2 and 7.7; 15.1 mg H_2O.
N(Kjeldahl) 5.6 resp. 6.3 mg used 3.36; 3.76 ml 0.01 *N* H_2SO_4.

$C_{24}H_{18}$ N_2 (334.4)	Calc.	C 86.18	H 5.43	N 8.38
	Found	85.42, 86.92	» 5.42, 5.57	» 8.40, 8.35

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Electrometric Investigation of Equilibria between Mercury and Halogen Ions

III. The „Millimolar" Potentials of Mercury and the Solubility Product of Mercury (I) Chloride

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In Part I[1] of this series the »millimolar potentials» E_{10}, E_{1X}, and E_{20} were defined as the emf of the cells

$$-\text{CE}/\,/Hg_2^{2+}/Hg\,+ \qquad E = E_{10} + 29.58 \log (Hg_2^{2+}) \qquad (1)$$

$$-\text{CE}/\,/X^-,\ Hg_2X_2/Hg\,+ \qquad E = E_{1X} - 59.16 \log (X^-) \qquad (2)$$

$$-\text{CE}/\,/Hg_2^{2+},\ Hg^{2+}/Pt\,+ \qquad E = E_{20} + 59.16 \log (Hg^{2+}) - 29.58 \log (Hg_2^{2+}) \qquad (3)$$

when all concentrations appearing in the expression for E are 1 mC *. These millimolar potentials are defined under the special experimental conditions we have chosen: at a temperature of 25° C with $(H^+) = 10$ mC and $(ClO_4^-) + X_{\text{total}} = 500$ mC in the right electrode vessel, and with the reference electrode

$$\text{CE} = Hg,\ Hg_2Cl_2/4\ \text{C NaCl}/0.5\ \text{C NaClO}_4/ \qquad (4)$$

to the left.

The millimolar emf:s are connected with the equilibrium constant for

$$Hg + Hg^{2+} = Hg_2^{2+} \qquad k_0 = (Hg_2^{2+})\ (Hg^{2+})^{-1}$$

*) C = mole/l; mC = mmole/l.

by the relation

$$E_{20} - E_{10} = 59.16 \log k_0 \qquad (5)$$

The solubility products of the Hg_2X_2 can be calculated from E_{10} and E_{1X} by

$$E_{1X} - E_{10} = 29.58 \log k_s ; k_s = (Hg_2^{2+})\ (X^-)^2 \qquad (6)$$

A knowledge of these quantities was necessary for the following calculations on Hg^{2+}—X^- complexes. Now, the four calomel electrodes used — »A», »F», »G» and »I» — had slightly different potentials, as was found for example by direct comparison, connecting them all to a vessel with 4 C NaCl. Moreover these differences varied slightly with time. Accordingly, the values of E_{10} and E_{20} also varied slightly.

In order to make measurements at different times and with different CE:s comparable, we »standardized» all emf:s relative to E_{10}, which was measured every now and then. Where it was necessary a correction was introduced — generally different for the different CE:s — so as to make E_{10} equal to E_{10}^{st} = 434.5 mV. These corrections never exceeded 2 mV.

TITRATIONS FOR DETERMINING THE REDOX POTENTIAL E_{20}

To determine E_{20}, titrations were made with Pt electrodes, where S (the original solution in the titration vessel) contained Hg_2^{2+} and T (the solution added during the titration) contained Hg^{2+}. Generally T contained Hg_2^{2+} too in the same concentration as in S, which facilitated the calculation. The first point of such a titration, corresponding to the »pure Hg_2^{2+}» solution, is rather well defined (within 1 or 2 mV) as there is always a certain amount of Hg^{2+} left in a solution prepared by shaking to equilibrium with metallic Hg.

E_{20} and the Hg^{2+} correction could be calculated in two ways:

1) An approximate value of E_{20} was obtained from a point with high (Hg^{2+}); with this E_{20}, the (Hg^{2+}) in the »Hg_2^{2+}» solution was calculated. Then a better value of E_{20} was calculated, and so on.

2) They could also be found by direct calculation. We assume that S contains $(b - \frac{\delta}{2})$ mC Hg_2^{2+} and δ mC Hg^{2+}, and that T contains $(b - \frac{\delta}{2})$ mC Hg_2^{2+} and $(a + \delta)$ mC Hg^{2+}, where a and b are known from the analyses of total Hg content. After addition of v ml of T to 100 ml of S

$$(Hg_2^{2+}) = b - \tfrac{\delta}{2},\ (Hg^{2+}) = \delta + av\ (100 + v)^{-1}$$

and according to (3)

$$E(v) = E_{20} + 59.16 \log [\delta + av (100 + v)^{-1}] - 29.58 \log (b - \tfrac{\delta}{2})$$

$$E(0) = E_{20} + 59.16 \log \delta - 29.58 \log (b - \tfrac{\delta}{2})$$

$$\Delta E = E(v) - E(0) = 59.16 \log [1 + av\delta^{-1} (100 + v)^{-1}] \qquad (7)$$

δ is obtained directly from (7)

The ratio $(Hg_2^{2+})/(Hg^{2+})$ was usually about 100—120 in our »Hg_2^{2+}» solutions, which is not far from the equilibrium constant k_0 obtained by us (see (18)). It cannot be expected to agree exactly, as the equilibrium was established under very different conditions.

Table 1 gives a typical titration for E_{20}, showing the validity of (3).

Table 1. Titration for E_{20}. Pt electrode, CE »G».
$S = (4.863 - \frac{\delta}{2})\ Hg_2^{2+}$, δHg^{2+}.
$T = (4.863 - \frac{\delta}{2})\ Hg_2^{2+}$, $(19.47 + \delta)\ Hg^{+2}$.

v ml	E mV	δ mC	(Hg^{2+}) mC	E_{20} mV
0	458.65		0.0437	
5.00	537.8	0.0446	0.971	(558.8_2)
9.97	554.2	0.0439	1.809	559.2_3
15.00	563.45	0.0439	2.583	559.3_3
20.00	569.7	0.0437	3.289	559.3_7
25.00	574.3	0.0437	3.937	559.3_4
30.00	578.0	0.0436	4.537	559.4_0
35.00	580.95	0.0437	5.092	559.3_9
40.03	583.4	0.0437	5.160	559.3_5

When v ml of T had been added the emf was E mV. From $E(0)$ and $E(v)$, δ was calculated by means of formula (7). If an average value $\delta = 0.0437$ mC is accepted, (Hg_2^{2+}) was 4.841 during the entire titration, whereas the calculated values of (Hg^{2+}) are given in the fourth column. Finally E_{20} was calculated by means of (3). The mean value (excluding the value at 5.00 ml where a small error in v causes a large error in E_{20}) was $E_{20} = 559.3_4 \pm 0.1$ mV (not standardized).

THE MERCURY ELECTRODE IN $Hg_2^{2+} - Hg^{2+}$ SOLUTIONS

When a Hg electrode and a solution containing Hg_2^{2+} and Hg^{2+} are in equilibrium in accordance with (4), E can equally well be expressed by (1) or by (3), as in this case the Hg can also be regarded as a redox electrode. One might of course also use the expression for a Hg^{2+}/Hg electrode, which is easily derived from (1) and (3):

$$- \text{CE} \,\|\, \text{Hg}^{2+} \,|\, \text{Hg} + \,; E = \tfrac{1}{2}(E_{10} + E_{20}) + 29.58 \log (\text{Hg}^{2+}) \qquad (8)$$

For the following investigation it was of interest to know how a Hg electrode would behave in solutions where the concentrations of Hg_2^{2+} and Hg^{2+} do not correspond to equilibrium with Hg. One might imagine that the emf would in such cases, depending on the rate of the different relevant reactions, be determined principally by the concentration of Hg_2^{2+} (1), or by that of Hg^{2+} (8), or that Hg would act chiefly as a redox electrode (3). Actually none of these was found to be the case.

To settle the question, a titration was made with both Hg and Pt electrodes, where S contained 4.93 mC Hg_2^{2+} and 0.05 mC Hg^{2+}, and T contained 5.00 mC Hg^{2+}. After equilibrium the concentration of Hg_2^{2+} should therefore be practically unchanged. The results are shown in Table 2. With the first small additions, E_{Hg} scarcely changed at all and even after adding 55 ml, so that $(Hg_2^{2+}) \approx$

Table 2. Mercury electrode in Hg_2^{2+}—Hg^{2+}.
$S = 4.93$ Hg_2^{2+} 0.05 Hg^{2+}, $T = 5.00$ Hg^{2+}.

v ml	t	E_{Hg}	E_{Pt}
0		454.55	(~455)
2	3m	454.5	
	6m	»	
	10m	»	
5	5m	454.45	
	10m	454.5	
	16m	»	
15	10m	454.45	
	15m	454.5	
35	10m	454.3	
55	5m	454.15	556.25
	16m	454.1	555.3
	21m	»	554.95
	25m	»	554.6
	72m	454.4	550.45
	104m	454.35	546.35
	144m	»	543.75
	24h	454.45	508.7
	36h	454.5	500.8

The times t are counted from the last addition of T. For each value of v the last E was always read immediately before the next addition of T. E_{Hg} and E_{Pt} are not standardized.

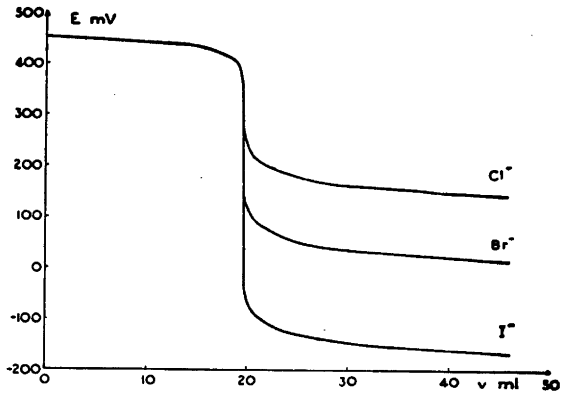

Fig. 1. Titration of 4.9 mC Hg_2^{2+} with 50 mC X^- (=Cl^-, Br^-, and I^-).

3.2 and $(Hg^{2+}) \approx 1.8$, E_{Hg} was only about 0.5 mV lower than the original value, although E_{Pt} had increased by 100 mV. Within an hour, E_{Hg} had moreover returned to a value about 0.1 mV below the original one, and afterwards remained practically constant. During the next few days, the solution was stirred continously, and gradually approached equilibrium as is seen from E_{Pt}, which drifts slowly towards E_{Hg}.

Thus E_{Hg} kept all the time to within a few 0.1 mV of *the value corresponding to the final equilibrium between the solution and Hg metal*, $(Hg_2^{2+}) = 4.94$—4,95 mC. In evaluating the potentials of Hg electrodes, we have, therefore, thought it justified to assume that, so long as there are considerable amounts of Hg^{2+} or Hg_2^{2+} ions present, the layer of solution close to the mercury is in equilibrium with the metal in accordance with (4).

TITRATIONS FOR DETERMINING E_{10} AND E_{1X}

In order to obtain E_{10} and E_{1X} we made titrations with a Hg electrode and with the solutions $S = (b - \frac{\delta}{2})$ mC Hg_2^{2+}, δ mC Hg^{2+} and $T = c$ mC X^-. The general course of the $E(v)$ curves with X = Cl, Br, and I is brought out by Fig. 1. Before the equivalence point the curves are practically identical for the three halogens; the latter part of the curve, where X^- is in excess, is highest for Cl^- and lowest for I^-, corresponding to the low solubility of Hg_2I_2.

In the first rather flat part of the curve, there is an excess of Hg_2^{2+}. In this part, the only complexes of bivalent mercury that need be considered are HgX^+ and HgX_2; the concentrations of HgX_3^- and HgX_4^{2-} can be neglected. It is, moreover, assumed that soluble complexes of mercury (I) such as Hg_2X^+ can be neglected. It can be proved that the concentration of free halogen, X^-, is negligible.

If it is observed that there may be a transition $Hg^{2+} + Hg = Hg_2^{2+}$; the following equations are found to be valid:

$$(Hg_2^{2+}) + (Hg_2X_2)_{solid} + (Hg^{2+}) + (HgX^+) + (HgX_2) = (Hg^I)_{total} + (Hg^{II})_{total} = \\ = 100\ (b + \tfrac{\delta}{2})\ (100 + v)^{-1}$$

$$2(Hg_2X_2)_{solid} + (HgX^+) + 2\ (HgX_2) = (X)_{total} = vc\ (100 + v)^{-1}$$

from which it follows that

$$(Hg_2^{2+}) + (Hg^{2+}) + \tfrac{1}{2}\ (HgX^+) = [100(b + \tfrac{\delta}{2}) - \tfrac{vc}{2}]\ (100 + v)^{-1} = -\tfrac{1}{2}\ X_e \qquad (9)$$

Here X_e is the concentration of halogen in excess over that needed for forming Hg_2X_2 and HgX_2. In the first part of the titration curve, X_e is negative.

The results in Table 2 make it probable that close to the electrode there is equilibrium between bivalent, monovalent and metallic mercury. Under this assumption it is found from (4) and (6) and the definitions of $\varkappa_i$ in Part I that

$$(Hg^{2+}) = k_o^{-1}\ (Hg_2^{2+}); \quad (HgX^+) = \varkappa_1 \sqrt{k_s}\ k_o^{-1}\ \sqrt{(Hg_2^{2+})} \qquad (10)$$

If we insert the values of $\varkappa_1$, k_s and k_o that were found later on during this investigation, we find, expressing all concentrations in mC

$$(Hg^{2+}) = 0.0077\ (Hg_2^{2+});\ \tfrac{1}{2}\ (HgCl^+) = 0.0024\ \sqrt{(Hg_2^{2+})}$$

$$\tfrac{1}{2}\ (HgBr^+) = 0{,}0028\ \sqrt{(Hg_2^{2+})};\ \text{and}\ \tfrac{1}{2}\ (HgI^+) = 0.016\ \sqrt{(Hg_2^{2+})} \qquad (11)$$

(The values for Br and I are preliminary). The millimolar potential E_{10} can be calculated from E by means of (1) which gives

$$E_{10} = E - 29.58 \log (Hg_2^{2+}) = E - 29{,}58 \log (-\tfrac{1}{2}X_e) + \delta E \qquad (12)$$

where according to (9) and (10)

$$\delta E = 29.58\ \log\ [-\tfrac{1}{2}\ X_e\ (Hg_2^{2+})^{-1}] = 29.58\ \log\ [1 + (Hg^{2+})\ (Hg_2^{2+})^{-1} + \\ + \tfrac{1}{2}\ (HgX^+)\ (Hg_2^{2+})^{-1}] \approx 12.85\ [(Hg^{2+})\ (Hg_2^{2+})^{-1} + \tfrac{1}{2}\ (HgX^+)\ (Hg_2^{2+})^{-1}] = \\ = 12.85\ k_o^{-1} + 6.42\ \varkappa_1\ k_o^{-1} \sqrt{k_s}\ (Hg_2^{2+})^{-\frac{1}{2}} \qquad (13)$$

As (Hg_2^{2+}) is only slightly smaller than $(-\tfrac{1}{2}\ X_e)$, the correction δE is rather small. By inserting the numerical values into (12) we find *e. g:*

$(Hg_2^{2+}) = 4$ mC $\quad \delta E = 0.115$ mV for Cl and Br, 0.20 mV for I
$(Hg_2^{2+}) = 1$ mC 0.13 mV for Cl and Br 0.30 mV for I
$(Hg_2^{2+}) = 0.25$ mC 0.16 mV for Cl and Br 0.50 mV for I (14)

At $v = 0$, before any X^- has been added, δE is in all cases 0.10 mV.

Table 3 gives a few typical titrations of Hg_2^{2+} with Cl^-. The constancy of E_{10} shows that our fundamental assumptions are probably true, and especially that complexes Hg_2Cl^+ are not formed in considerable amounts.

Table 3. Titrations for E_{10} and E_{1X}.
$S = 4.832$ mC Hg_2^{2+}, 0.040 mC Hg^{2+}.
$T = 50.00$ mC Cl^-.

v ml	$\frac{1}{2} X_e$ mC	29.58 log (Hg_2^{2+}) mV	E mV					E average	E_{10}
				CE »A»		CE »G»			
0	4.872	20.24	453.85	453.9	453.7	454.8	454.9	454.8_3	434.5_9
2	4.286	18.59		452.2	452.1	453.1	453.1	453.1_3	434.5_4
4	3.723	16.78		450.4	450.4	451.4	451.3	451.3_8	434.6_0
6	3.181	14.75		448.35	448.3	449.3	449.3	449.3_1	434.5_6
8	2.659	12.44		446.0	446.0	446.9	446.9	446.9_5	434.5_1
10	2.156	9.75	443.4	443.3	443.3	444.7	444.2	444.3_8	434.6_3
12	1.671	6.50			440.2	441.1	440.9	441.0_7	434.5_7
15	0.975_7	—0.45	433.3					(434.3)	(434.7_5)
17	0.531_6	—8.26	425.5					(426.5)	$(434\ 7_6)$
19	0.102_5	—29.45	403.9					(404.9)	(434.3_5)
	X_e	59.16 log X_e mV							E_{1X}
20	0.213	—39.70	239.9					(240.9)	(201.2_0)
21	0.625	—12.08	212.85					(213.8_5)	(201.7_7)
30	4.043	35.89	164.7					(165.7)	(201.5_9)
35	5.745	44.92	155.9	155.7				156.8_0	201.7_2
40	7.326	51.16	149.1	149.2	150.0	150.8	150.8	150.5_8	201.7_4
45	8.797	55.87		144.3	144.3	145.3		145.3_0	201.1_7
50	10.171	59.59		140.5	140.9	141.9	141.9	141.8_0	201.3_9

For each value of v (the volume added), X_e and 29.58 log (Hg_2^{2+}) have been calculated with the aid of (9), (12) and (14). The emf:s were measured with two different CE:s, »A» and »G»; Before the average was taken, 1.0 mV was added to the emf:s with »A». Average of the values for 0—12 ml and 30—50 ml:

$$E_{10} = 434.5_7, \quad E_{1X} = 201.5_2$$

The equilibrium conditions for Hg_2X^+ would be

$$Hg_2X_2(s) + Hg_2^{2+} = 2Hg_2X^+ \qquad K = (Hg_2X^+)^2(Hg_2^{2+})^{-1} \tag{15}$$

The Hg_2X^+ present would cause an additional term $\frac{1}{2}(Hg_2X^+)$ to the left in (9) and thus an addition to δE

$$\delta' E = 29.58 \log [1 + \tfrac{1}{2}(Hg_2X^+)\,(Hg_2^{2+})^{-1}] \approx 6.42\ (Hg_2X^+)\ (Hg_2^{2+})^{-1} = \\ = 6.42 \sqrt{K}\,(Hg_2^{2+})^{-\frac{1}{2}} \tag{16}$$

If the amount of Hg_2X^+ were considerable, there would be a decrease in the calculated E_{10} between $v = 0$ (where no X has been added) and, for example, $v = 2$ ml. Actually no such decrease could be proved to exist for Cl, Br or I. In any case it never seemed to amount to as much as 0.2 mV, which according to (16) means that K is less than 5.10^{-6}C. Even if this value were attained, the k_{12} calculated in Part II[2] for Hg^{2+} —Cl^- and Br^- would not be significantly changed. (The value of k_{12} for I^- is in any case rather uncertain). In the »E_{max} titration» to be described in Parts IV and V, no error at all would be caused by disregarding Hg_2X^+.

During our investigations the difference E_{20}—E_{10} was determined on several occasions, using different stock solutions and different CE:s. The results are summarized in Table 4; each pair of values given was measured with the same CE and within a short time interval.

Table 4. Measurements of E_{20} and E_{10}.

Month	CE	E_{20}	E_{10}	E_{20}—E_{10}
7. 1945	»G»	559.7	434.7	125.0
»	»A»	559.6	434.8	124.8
5. 1946	»G»	559.1	434.0	125.1
»	»G»	559.2	434.5	124.7
7. 1946	»A»	559.1	433.6	(125.5)*
»	»G»	559.3	434.6	124.7
8. 1946	»A»	558.3	433.6	124.7
»	»A»	558.6	433.6	125.0
»	»G»	559.5	434.6	124.9
9. 1946	»A»	559.2	433.9	(125.3)*
10. 1946	»A»	558.9	433.9	125.0
				Average 124.9 ± 0.2

We have excluded the measurements of E_{20} made with one particular stock solution of Hg^{2+} which was prepared in November, 1945, and proved later on to have been contaminated.

With two exceptions, marked with an asterisk, the difference $E_{20}-E_{10} = 59.16 \log k_0$ is seen to lie within the limits

$$59.16 \log k_0 = 124.9 \pm 0.2 \text{ mV} \tag{17}$$

With the first less accurate apparatus (see Part I), Inger Brattsten, *legitimerad apotekare*, found $E_{20}-E_{10} = 558.0-432.7 = 125.3$ mV, whereas Mrs Inga Westöö found 558.0 —433.4 = 124.6 mV. These values are seen not to be very far from our final average.

From (17) follows

$$\log k_0 = 2.111_2 \pm 0.003_4 \quad ; \quad k_0 = 129.2 \pm 1.0 \tag{18}$$

This constant has previously been determined analytically by Ogg (1898)[3] who found 100—120 (the temperature was not mentioned) and by Abel (1901)[4] who found 120 at 25° C. Latimer [5] calculated k_0 for infinite dilution from the standard potentials of mercury; he found 81, but as the extrapolation of the redox potential to infinite dilution is uncertain to within about 5 mV, this k_0 is not very accurate. As the activity factors of the two bivalent ions can be expected approximately to cancel out one another, k_0 should not vary very much with the ionic strength. However, the effect of varying acidity may be considerable and this is probably the chief reason for the incertainty of an extrapolation to infinite dilution.

From (17) we find, standardizing E_{10} to 434.5 mV

$$E_{10}^{st} = 434.5 \text{ mV} \qquad E_{20}^{st} = 559.4 \pm 0.2 \text{ mV} \tag{19}$$

From (8) and (19) we find for any Hg electrode

$$E^{st} = 496.95 \ (\pm 0.10) + 29.58 \log (Hg^{2+}) \tag{20}$$

This expression will prove useful in subsequent work.

E_{1X} AND k_s FOR Hg_2Cl_2

After the equivalence point, there is a new flat part of the titration curve $E(v)$ in Fig. 1. From this part of the titration it is possible to calculate E_{1X} and thus k_s for Hg_2X_2 by means of (2) and (6). The excess of halogen

$$X_e = [vc - 200(b + \tfrac{\delta}{2})] \ (100 + v)^{-1} \tag{9a}$$

is positive and one might ask if it is permissible to assume $X_e = X$, (or (X^-), the concentration of free X^- ions). Certainly all previous experimental evidence speaks against the existence of considerable amounts of soluble complexes $Hg_2X_3^-$ and the like at moderate X^- concentrations. However, in equilibrium with Hg metal, solid Hg_2X_2, and X^- there is always a certain amount of HgX_2, HgX_3^-, and HgX_4^{2-}, and it can be shown that

$$X_e = (X^-) + (HgX_3^-) + 2(HgX_4^{2-}) = X\,(1 + \varkappa_3 k_s k_o^{-1} + 2\,\varkappa_4 k_s k_o^{-1} X) \qquad (21)$$

If the numerical values of the equilibrium constants for Cl are inserted, it is found that the two constant factors to the right in (21) are about 10^{-5} and $2 \cdot 10^{-7}$ mC^{-1}, and can thus be neglected. Consequently it seems permissible to use X_e instead of (X^-) in (2).

In the latter part of Table 3 are given particulars of a few titrations, whereas Table 5 gives average values of E_{10} and E_{1X} for a number of titrations. As in

Table 5. Measurements of E_{10} and E_{1X}.

Month	E_{10}	E_{1X}	$E_{10}-E_{1X}$
7. 1945	434.7	201.9	232.8
12. 1945	434.3	201.1	233.2
»	434.2	200.9	233.3
6. 1946	435.5	202.2	233.3
7. 1946	433.6	200.5	233.1
»	434.6	201.5	233.3
		Average	233.1 ± 0.3

each titration the E_{1X} values for different v often differ by 0.2—0.3 mV, whereas the difference in E_{10} or E_{20} in the same titration are usually only 0.1 mV, it seems safe to give here broader limits of error,

$$E_{10} - E_{1X} = -29.58 \log k_s = 233.1 \pm 0.3 \text{ mV} \qquad (22)$$

from which follows

$$\log k_s = 0.120 \pm 0.010 - 8;$$

$$k_s = (1.32 \pm 0.03) \cdot 10^{-8}\ mC^3 = (1.32 \pm 0.03) \cdot 10^{-17}\ C^3 = (Hg_2^{2+})\ (Cl^-)^2 \qquad (23)$$

$$E_{1X}^{st} = 201.4 \pm 0.3 \text{ mV} \qquad (24)$$

According to Brodsky and Scherschewer (1926) the activity product of Hg_2Cl_2 at 25° C is $K_s = 1.15 \cdot 10^{-18}$. If this value is assumed to be correct, the product of the activity factors in our solutions should be about 0.1, which seems reasonable.

Experimental note: After the equivalence point, the emf was at first higher than the final value, which was not attained until after several hours. Our observations could be explained by the assumption that the precipitate is at first very fine-grained and therefore more soluble than are larger grains. Thus when the solution was stirred before equilibrium had been reached, the potential was too high (both small and large grains smashed against the mercury surface). If the stirrer was stopped, the emf sank to a value close to the final equilibrium emf, as the larger grains are the first to sink down to the mercury surface.

In one instance the titration of Hg_2^{2+} was started in the evening. When a certain excess of Cl^- had been added, after about an hour the emf was 181.0 mV with vigorous stirring, 180.8 mV with moderate stirring, and 180.6 mV if the stirring was stopped for a while; these emf:s could be reproduced at will. Next morning, the emf was still 180.6 mV without stirring but increased to 180.7 mV if a vigorous stirring was applied.

SUMMARY

Under the experimental conditions chosen: 25° C, $(H^+) = 10$ mC, $(X^-) + (ClO_4^-) = 500$ mC, the following »millimolar potentials» (see 1—3) were found:

$E_{10}^{st} = 434.5$ (standard value), $E_{20}^{st} = 559.4 \pm 0.2$, $E_{1X}^{st} = 201.4 \pm 0.3$ (for Cl)

The following equilibrium constants were calculated:

$Hg^{2+} + Hg = Hg_2^{2+}$	$k_o = 129.2 \pm 1.0$
$Hg_2Cl_2(s) = Hg_2^{2+} + 2\ Cl^-$	$k_s = (1.32 \pm 0.03).10^{-17}$ C³

It was shown that a mercury electrode in a solution containing Hg^{2+} and Hg_2^{2+} quickly acquired the potential corresponding to the final equilibrium between the solution and Hg metal. During a titration of Hg_2^{2+} with halogen X^-, corrections should be made for the presence of Hg^{2+} and its complexes with X^-. Expressions are given for these corrections. No appreciable amounts of soluble complexes Hg_2X^+ could be detected.

The effect of stirring on the electrode potential of Hg in a newly precipitated suspension of Hg_2X_2 in an X^- solution is discussed.

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Electrometric Investigation of Equilibria between Mercury and Halogen Ions

IV. Redox Titrations of Hg(I,II) Solutions with Halogen Ions

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If a solution S containing Hg^{2+}, Hg_2^{2+}, and an excess of solid Hg_2X_2 is titrated with a solution T of a halogen ion $X^- = Cl^-$ or Br^-, and the redox emf is plotted against the volume of T added, a curve is obtained similar to those in Fig. 1. (For I^-, the curve is complicated by precipitation of HgI_2). E first rises slowly to a maximum and then falls off rather rapidly. The curve is steepest in the neighbourhood of the point where almost all mercury is present either as HgX_2 or as Hg_2X_2(X_e = O, see below), and then gradually flattens out. Incidentally, similar titration curves were obtained by Müller and Aarflot [1], who concluded that it is impossible to determine Hg^{2+} and Hg_2^{2+} separately by titration with halogen.

It will now be demonstrated that such titrations, together with the data collected in Parts I—III [2, 3, 4] of this series, will give sufficient information

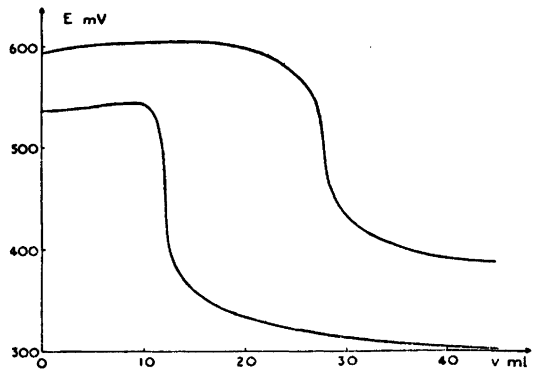

Fig. 1. Redox titrations with excess of Hg_2Cl_2. Upper curve: S = 10 mC Hg^{2+}, 1 mC Hg_2^{2+}, T = 10 mC Hg^{2+}, 100 mC Cl^-. Lower curve: S = 0.93 mC Hg^{2+}, 2.0 mC Hg_2^{2+}, T = 0 93 mC Hg^{2+}, 50 mC Cl^-.

for calculating the complex products $\varkappa_1$, $\varkappa_2$, $\varkappa_3$, and $\varkappa_4$ for Hg^{2+} and the halogen ion in question. To be specific, $\varkappa_1$ and $\varkappa_2$ can be calculated from the maximum value of E, whereas $\varkappa_3$ and $\varkappa_4$ are obtained from the lower part of the curve.

In our experiments, we always tried to use the same total concentration a of Hg^{II} in S and T. For the calculations it is convenient to introduce the quantity X_e, the concentration of halogen in excess over that needed for forming HgX_2 and Hg_2X_2 (cf. III,9):

$$X_e = (X_{total}) - 2(Hg^{II})_{total} - 2(Hg_2^{2+})_{total} \tag{1}$$

In the first part of the curve, X_e is negative. It increases during the titration and passes through zero at the steepest point. In the latter part it is then positive.

If we intended to study the first part of the curve, X_e in S was of course negative. If, on the other hand, only the latter part of the curve was to be studied, it was also possible to begin with a positive X_e in S.

THE MAXIMUM VALUE OF E.

The emf of the cell can be written according to (I, 5) or (III, 3)

$$E = E_{20} + 29.58 \log[(Hg^{2+})^2 (Hg_2^{2+})^{-1}] \tag{2}$$

As solid Hg_2X_2 is present, according to (III,6) and (I,1)

$$(Hg^{2+})^2 (Hg_2^{2+})^{-1} = (Hg^{2+})^2(X^-)^2 k_s^{-1} = (HgX^+)^2 \varkappa_1^{-2} k_s^{-1} \tag{3}$$

By (2), (3), and (III,6)

$$E = E_{20} + E_{10} - E_{1X} + 59.16 \log[(HgX^+) \varkappa_1^{-1}] \tag{4}$$

Thus E is a function of (HgX^+) only, which might have been expected from the fact that the electrode reaction can be written $2\,HgX^+ + 2e^- = Hg_2X_2$ (s)

If the total concentration of Hg^{II} remains constant $= a$, it was shown in (II,6) that the maximum value of $(HgX^+) = \alpha_1 a$ is reached when $(Hg^{2+}) = (HgX_2)$ and

$$(HgX^+)_{max} = a \sqrt{k_{12}} (2 + \sqrt{k_{12}})^{-1} = a \varkappa_1 \varkappa_2^{-\frac{1}{2}} (2 + \sqrt{k_{12}})^{-1} \tag{5}$$

(See II,1.) At this point E will also have a maximum. By inserting (5) into (4) we find

$$E_{max} - 59.16 \log a = E_{20} + E_{10} - E_{1X} - 59.16 \log (2 + \sqrt{k_{12}}) - \\ - 29.58 \log \varkappa_2 \qquad (6)$$

If our assumptions are correct, ($E_{max} - 59.16 \log a$) should thus remain constant when a is varied. As all quantities in the right hand side of (6) except $\varkappa_2$ are known from previous measurements, we can obtain $\varkappa_2$ from E_{max} and a, and thus by means of (II,1) also $\varkappa_1$.

E AS A FUNCTION OF X_e (FOR NEGATIVE X_e)

We shall now derive a relation between the two experimental quantities: E which is measured directly and X_e which can easily be calculated if we know the composition of the solutions S and T and the volume v of T added to 100 ml of S.

In the first part of the titration, where X_e is negative, the only soluble molecules that need be considered are Hg^{2+}, HgX^+, HgX_2 and Hg_2^{2+}:

$$(Hg_2^{2+})_{total} = (Hg_2^{2+}) + (Hg_2X_2)_{solid}$$
$$(Hg^{2+})_{total} = (Hg^{2+}) + (HgX^+) + (HgX_2) = a \qquad (7)$$
$$(X^-)_{total} = 2(Hg_2X_2)_{solid} + (HgX^+) + 2(HgX_2)$$

With (1) we find

$$-X_e = 2(Hg_2^{2+}) + 2(Hg^{2+}) + (HgX^+) \qquad (8)$$

We introduce the parameter

$$z = X\sqrt{\varkappa_2} = (HgX_2)^{\frac{1}{2}}\ (Hg^{2+})^{-\frac{1}{2}} \qquad (9)$$

and remember that $k_{12} = \varkappa_1^2\ \varkappa_2^{-1}$ according to (II,1)

This and the equilibrium conditions gives:

$$(HgX^+) = (Hg^{2+})\ \varkappa_1 X = (Hg^{2+})\ z\sqrt{k_{12}} \qquad (10a)$$
$$(HgX_2) = (Hg^{2+})\ \varkappa_2 X^2 = (Hg^{2+})\ z^2 \qquad (10b)$$
$$(Hg_2^{2+}) = k_s X^{-2} = k_s \varkappa_2 z^{-2} \qquad (11)$$

By (7) and (10) we find

$$(Hg^{2+}) = a(1 + z\sqrt{k_{12}} + z^2)^{-1};\ (HgX^+) = az\sqrt{k_{12}}\ (1 + z\sqrt{k_{12}} + z^2)^{-1}$$
$$(HgX_2) = az^2\ (1 + z\sqrt{k_{12}} + z^2)^{-1} \qquad (12)$$

If (12) is introduced in (4) we find

$$E = E_{20} + E_{10} - E_{1X} + 59.16\ \log\ a + 59.16\ \log\ [z\ (1 + z\sqrt{k_{12}} + z^2)^{-1}]$$
$$-\ 29.58\ \log\ \varkappa_2 \qquad (13)$$

If (13) is combined with (6) we see that $E = E_{max}$ for $z = 1$ and that

$$E_{max} - E = 59.16\ \log[(z^{-1} + \sqrt{k_{12}} + z)\ (2 + \sqrt{k_{12}})^{-1}] \qquad (14)$$

The relation between X_e' and z is found from (11), (12) and (8):

$$-X_e = 2k_s\varkappa_2 z^{-2} + a(2z^{-1} + \sqrt{k_{12}})\ (z^{-1} + \sqrt{k_{12}} + z)^{-1} \qquad (15)$$

Thus if k_{12}, k_s, and $\varkappa_2$ are known, E and X_e can be calculated by means of (14) and (15) for round values of the parameter z, plotted and compared with a plot of the experimental values.

THE CALCULATION OF q_1 AND q_2.

For the discussion of the lower part of the titration curve we shall transform our expression (2) for E. By means of (I,1) we find

$$E = E_{2X} + 59.16\ \log\ (HgX_2) - 59.16\ \log\ X \qquad (16)$$
$$\text{where } E_{2X} = E_{20} + E_{10} - E_{1X} - 59.16\ \log\ \varkappa_2 \qquad (17)$$

Thus E_{2X} can be calculated from quantities determined by experiments already described.

If the composition corresponds to a point in the lower part of the titration curve, only HgX_2, HgX_3^-, HgX_4^{2-}, and X^- seem to be present in appreciable amount in the solution. From (I,2), the definition of q_1 and q_2, we find

$$(HgX_3^-) = q_1X(HgX_2)\ \ ;\ \ (HgX_4^{2-}) = q_2X^2(HgX_2) \qquad (18)$$

Moreover

$$(HgX_2) + (HgX_3^-) + (HgX_4^{2-}) = a = (HgX_2)\ (1 + q_1X + q_2X^2) \qquad (19)$$
$$X_e = (X^-) + (HgX_3^-) + 2(HgX_4^{2-}) = X[1 + (q_1 + 2q_2X)\ (HgX_2)] \qquad (20)$$

From (19) and (20)

$$X_e = X[1 + a(q_1 + 2q_2X)\ (1 + q_1X + q_2X^2)^{-1}] \tag{21}$$

and from (16) and (19)

$$59.16 \log\ (1 + q_1X + q_2X^2) = E_{2X} + 59.16 \log\ a - E - 59.16 \log\ X = \\ = 59.16 \log r \tag{22}$$

These are the equations needed. Every titration gives a number of pairs (E,X_e). First X_e is inserted instead of X in (22), which gives an approximate value r' of $r = 1 + q_1X + q_2X^2$. By plotting $(r' - 1)X_e^{-1} \approx q_1 + q_2X$ against X_e approximate values of q_1 and q_2 are obtained. By means of these and (21), X_e is calculated for round values of X, and a table of the correction $-(X_e - X)$ for different X_e is constructed. By interpolation in this table, better values of X are calculated, and inserted in (22), and the function

$$F_1 = (r - 1)\ X^{-1} = q_1 + q_2X \tag{23}$$

calculated and plotted against X. The values obtained in this graph will generally be accurate enough; otherwise the procedure can be repeated. The latter part of this calculation reminds one of the method introduced by Leden [5].

From the lower part of the titration curve it is thus possible to find values of q_1 and q_2, from which $\varkappa_3$ and $\varkappa_4$ can be calculated by means of (I,2). In principle it is also possible to deduce from these titration curves whether there are considerable amounts of complexes with more than 4 X^- or of complexes with more than one Hg^{2+} under the experimental conditions. By combining the equilibrium constants $\varkappa_1$, $\varkappa_2$, $\varkappa_3$, $\varkappa_4$, and k_s some other equilibria of interest can be calculated, such as for example

$$\begin{array}{l} Hg_2X_2 + 2Hg^{2+} = Hg_2^{2+} + 2HgX^+ \ ; \ (HgX^+)^2\ (Hg_2^{2+})\ (Hg^{2+})^{-2} = k_s\ \varkappa_1^2 \\ Hg_2X_2 = Hg + HgX_2 \ ; \ (HgX_2) = k_s\ \varkappa_2\ k_0^{-1} \ ; \ E_{1X} - E_{2X} = 59.16 \log\ (HgX_2) \\ 2HgX_2 = HgX^+ + HgX_3^- \ ; \ (HgX^+)\ (HgX_3^-)\ (HgX_2)^{-2} = \varkappa_1\ \varkappa_3\ \varkappa_2^{-2} \\ Hg_2X_2 + X^- = Hg + HgX_3^- \ ; \ (HgX_3^-)\ (X^-)^{-1} = k_s\ \varkappa_3\ k_0^{-1} \end{array} \tag{24}$$

SUMMARY.

When a mixture of Hg^{2+} and Hg_2^{2+} is titrated with Cl^- or Br^-, the redox potential describes a curve similar to those in Fig. 1. It is demonstrated how such curves can be used for calculating the complex constants for Hg^{2+}

$-X^-$; the maximum E in the upper part gives the constants $\varkappa_1$ and $\varkappa_2$, and the lower part gives $\varkappa_3$ and $\varkappa_4$.

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Electrometric Investigation of Equilibria between Mercury and Halogen Ions

V. Complexes between Hg^{2+} and Cl^-

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This paper is concerned with the equilibrium constants of the reactions between Hg^{2+} and Cl^-, leading to the formation of $HgCl^+$, $HgCl_2$, $HgCl_3^-$, and $HgCl_4^{2-}$; from these constants a few equilibria with Hg metal and Hg_2Cl_2 have also been calculated.

In part I[1] of this series, the complex products $\varkappa_i$ were defined by (I,1) ($X = (X^-)$, the concentration of free halogen ions):

$$\varkappa_1 = (HgX^+)(Hg^{2+})^{-1}X^{-1}, \qquad \varkappa_2 = (HgX_2)(Hg^{2+})^{-1}X^{-2}$$
$$\varkappa_3 = (HgX_3^-)(Hg^{2+})^{-1}X^{-3}, \qquad \varkappa_4 = (HgX_4^{2-})(Hg^{2+})^{-1}X^{-4} \tag{1}$$

and the equilibrium constants q_1 and q_2 by (I,2)

$$q_1 = (HgX_3^-)(HgX_2)^{-1}X^{-1} = \varkappa_3\,\varkappa_2^{-1}$$
$$q_2 = (HgX_4^{2-})(HgX_2)^{-1}X^{-2} = \varkappa_4\,\varkappa_2^{-1} \tag{2}$$

In part IV[2] of this series was demonstrated how the complex constants $\varkappa_i$ can be calculated from the data recorded in I—III, together with redox titrations with halogen X^- of a mixture of Hg^{2+} and Hg_2^{2+}, in the presence of an excess of solid Hg_2X_2. The course of the E curve is shown in Fig. 1, part IV; from its first part $\varkappa_1$ and $\varkappa_2$ can be calculated, from its latter part q_1 and q_2, and thus $\varkappa_3$ and $\varkappa_4$.

The apparatus used was described in Part I, where the reasons were also given that made us study these equilibria at constant (H^+) and constant ionic strength.

The measurements recorded in part II[3] led to the equilibrium constant:

$$k_{12} = (HgX^+)^2(HgX_2)^{-1}(Hg^{2+})^{-1} = \varkappa_1^2\,\varkappa_2^{-1} = 1.80 \pm 0.10 \text{ for } X = Cl \qquad (3)$$

In part III[4] are given measurements of the »millimolar potentials» E_{10} and E_{20} and E_{1X} defined by (I,3—5) or (III, 1—3). The average values found were (in mV):

$$E_{10} = 434.5,\ E_{20} = 559.4 \pm 0.2,\ E_{1X} = 201.4 \pm 0.3 \text{ (for Cl)}$$
$$-29.58 \log k_s = 233.1 \pm 0.3 \qquad (4)$$

TITRATIONS FOR E_{max}

In order to obtain $\varkappa_1$ and $\varkappa_2$ we made titrations with Pt electrodes, where v ml of T was added to 100 ml of S, and

S = a mC Hg^{2+}, b mC Hg_2^{2+}, excess of solid Hg_2Cl_2
T = a mC Hg^{2+}, c mC Cl^-

When S was mixed, either the amount of Hg_2^{2+} was chosen rather large, and Cl^- was added so that Hg_2Cl_2 precipitated in the original 100 ml of S, or Hg_2Cl_2 was prepared by precipitation in a separate container, washed, moistened with (490 mC $NaClO_4$, 10 mC $HClO_4$) and added to S. The amount of Hg_2Cl_2 was 1—5 g in 100 ml.

It was demonstrated in part IV that all the information needed can be obtained from E_{max}, the maximum value of E. Sometimes a rather quick titration was made at first without waiting for equilibrium after each new addition of T. This was to ascertain the approximate position of the maximum, which could also be calculated as soon as approximate values of the equilibrium constants had been obtained. Then a new titration was made with the same concentrations but with more careful measurements especially in the neighbourhood of E_{max}.

Generally, after every new addition of Cl^-, the emf was at first 1—2 mV below its final value, which was only obtained after ½—2 hours of stirring.

Table 1 gives the values of E_{max} found for different values of a. The column »corr» gives the correction to be added to all emf:s measured with the CE employed in order to make E_{10} and E_{20} equal to their »standardized» values (4). Some of these measurements were made by Inger Brattsten, *leg. apot.*

It is required by (IV,6) that (E_{max} — 59.16 log a) should be a constant, independent of a. As seen from Table 1, there is a certain variation in the values found for this quantity but this is not systematic with a.

Table 1. Titrations for E_{max}.

a	59.16log a	E_{max}	Corr	E^{st}_{max}—59.16log a
10.00	59.2	605.5	+ 1.8	548.1
10.00	59.2	605.6	+ 1.8	548.2
5.170	42.2	590.0	+ 0.3	548.1
5.100	41.9	589.0	+ 0.3	547.4
2.788	26.3	572.6	+ 1.6	547.9
2.788	26.3	572.4	+ 1.4	547.5
2.180	20.0	568.5	+ 0.3	548.8
				Average 548.0 ± 0.5

We thus feel justified in assuming that we have really obtained the equilibrium assumed in IV, and accept the average value:

$$E_{max} - 59.16 \log a = 548.0 \pm 0.5 \text{ mV} \tag{5}$$

If we insert the numerical values into (IV,6) we find

$$548.0 \pm 0.5 = E_{20} + E_{10} - E_{1X} - 59.16 \log (2 + \sqrt{k_{12}}) - 29.58 \log \varkappa_2 = 559.4 \pm 0.2 + 233.1 \pm 0.3 - 31.0 \pm 0.3 - 29.58 \log \varkappa_2$$

which leads to

$$29.58 \log \varkappa_2 = 213.5 \pm 0.7 \tag{6}$$

$$\log \varkappa_2 = 7.21_8 \pm 0.02_4 \text{ (mC scale)}$$

$$\varkappa_2 = (1.65 \pm 0.10) \cdot 10^7 \text{ mC}^{-2} = (1.65 \pm 0.10) \cdot 10^{13} \text{ C}^{-2} \tag{7}$$

Now from (7) and (3) we find

$$2 \log \varkappa_1 = \log \varkappa_2 + \log k_{12} = 7.21_8 \pm 0.02_4 + 0.25_5 \pm 0.02_5 = 7.47_3 \pm 0.03_5$$

$$\log \varkappa_1 = 3.73_6 \pm 0.01_8 \text{ (mC scale)}$$

$$\varkappa_1 = (5.4_5 \pm 0.2_3) \cdot 10^3 \text{ mC}^{-1} = (5.4_5 \pm 0.2_3) \cdot 10^6 \text{ C}^{-1} \tag{8}$$

We have used (IV,14) and (IV,15) to calculate the course of E during two titrations, where

S = 10.0 Hg^{2+}, 1.0 Hg_2^{2+}, excess of Hg_2Cl_2

T = 10.0 Hg^{2+}, 100 Cl^-

Inserting the equilibrium constants obtained, we find from (IV,14) and (IV,15)

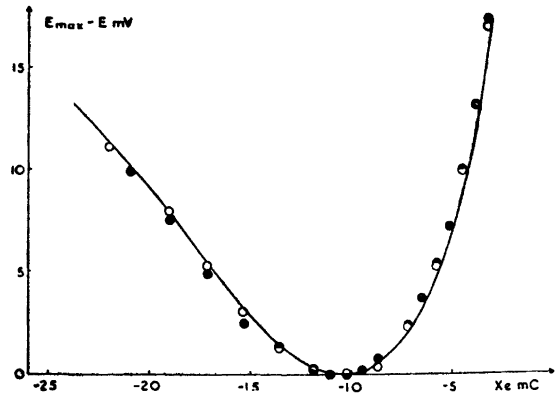

Fig. 1. Titration for E_{max} with S = 10.0 Hg^{2+}, 1.0 Hg_2^{2+}, excess of Hg_2Cl_2, T = 10.0 Hg^{2+}, 100 Cl^-. Filled and open circles: results of two different experiments. Curve = calculated values.

$$E_{max} - E = 59.16 \log[(1.3416 + z + z^{-1}) \cdot 3.3416^{-1}] \quad (9)$$

$$-X_e = 0.436\ z^{-2} + 10(1.3416 + 2z^{-1})(1.3416 + z + z^{-1})^{-1} \quad (10)$$

where z is a parameter which has the value 1 at E_{max}
On the other hand, from the composition of S and T we find that after addition of v ml of T to 100 ml of S, the deficiency in halogen is

$$-X_e = (2200 - 80\ v)(100 + v)^{-1} \quad (11)$$

In Fig. 1, $(E_{max} - E)$ is plotted against $-X_e$. The curve has been drawn through points calculated for round values of z by means of (9) and (10). The circles are the experimental data from the two titrations; X_e has been calculated by means of (11). The experimental points are seen to follow the theoretical curve very well, except that they all seem to be shifted slightly, 0.2—0.3 mC, in the direction of negative X_e. This constant shift seems to indicate that the halogen content was slightly larger than calculated; the most probable explanation seems to be that the Hg_2Cl_2 (which was prepared in these experiments outside the solution) had adsorbed a slight excess of halogen ions.

The good agreement is taken as additional proof that real equilibria have been studied and that the theoretical treatment in Part IV is applicable.

PREVIOUS MEASUREMENTS OF $\varkappa_1$ AND $\varkappa_2$

The most recent measurements of $\varkappa_1$ and $\varkappa_2$ known to us are more than thirty years old and are subject to the objections mentioned in part I in this series.

Luther [5] tried two different ways of calculating $\varkappa_2$. Firstly, from the measurements of other workers on the half-cells $Pt,Hg_2Cl_2(s)/HgCl_2$, Cl^-; $Hg,Hg_2Cl_2(s)/Cl^-$, and Hg/Hg_2^{2+}, and of the equilibrium $Hg^{2+} + Hg = Hg_2^{2+}$ he found $\varkappa_2^{-1} = 1.5 \cdot 10^{-14}$, thus $\varkappa_2 = 6.7 \cdot 10^{13}$ C^{-2} at about 16° C. A second value was calculated from the solubility of AgCl in Hg^{2+} solutions; this calculation was erroneous because Luther did not take into account the formation of $HgCl^+$ ions, the existence of which was proved in the following years by Morse[6]. From the solubility of AgCl in Hg^+ solutions, Morse calculated $\varkappa_1^{-1} = 3.5 \cdot 10^{-8}$, thus $\varkappa_1 = 2.9 \cdot 10^7$ C^{-1} at 25° C. From the distribution of $HgCl_2$ between toluene and aqueous $Hg(NO_3)_2$ solutions he found $k_{12}^{-1} = 0.13$, which with Morse's value of $\varkappa_1$ gives $\varkappa_2 = 10^{14}$ C^{-2} at 25° C. The same value of $\varkappa_2$, 10^{14}, was also found by Sherrill [7] from rather uncertain calculations.

From the solubility of AgCl in solutions containing Hg^{2+} and an excess of Ag^+ or Cl^-, Buttle and Hewitt [8] found $\varkappa_1^{-1} = 5.3 \cdot 10^{-8}$, thus $\varkappa_1 = 1.9 \cdot 10^7$ C^{-1} at 25° C. Drucker (1912) used Luther's first method with more accurate data to recalculate $\varkappa_2^{-1} = 9.4 \cdot 10^{-15}$, thus $\varkappa_2 = 1.06 \cdot 10^{14}$ C^{-2} at 25° C.

TITRATIONS FOR q_1 AND q_2

The titrations for determining q_1 and q_2 were carried out as indicated in part IV, *i. e.* with excess of solid Hg_2Cl_2, excess of Cl^- (positive X_e) and with a constant total concentration a of Hg^{II}.

In Table 2 are given data for two titrations, A1 and A2, with CE »A» and
S = 2.00 mC $HgCl_2$ 6.0 mC Cl^- (20 mC Hg_2Cl_2 (s))
T = 2.00 mC $HgCl_2$ 482 mC Cl^-
Table 3 gives two other titrations, G1 and G2, with CE »G» and
S = 5.00 mC $HgCl_2$ (20 mC Hg_2Cl_2 (s))
T = 5.00 mC $HgCl_2$ 470 mC Cl^-
In all these titrations Hg_2Cl_2 was precipitated in S when it was being mixed.

After each new addition of T, the emf seemed to become constant within 15—30 minutes at lower Cl^- concentrations, within 10 minutes at the higher ones. The emf:s were well defined; different Pt electrodes, however, often showed slightly different emf:s.

In each titration two different platinum electrodes were used. An asterisk shows that the electrode has been »burnt out» in an alcohol flame. After burning, the emf was at first several 10 mV higher than before but rapidly sank and after ½—2 hours attained a value, 1—2 mV higher than that of »old» electrodes. The high value may then be retained for many hours. This is clearly brought out by a comparison of the emf:s of titration A1, where the electrodes were burnt out, with those of A2, where »older» electrodes

Table 2. Titration for q_1 and q_2. $S = 2.00$ mC $HgCl_2$, 60 mC Cl^-; $T = 2.00$ mC $HgCl_2$, 482 mC Cl^-, CE »A».

v ml	X_e mC	X mC	E mV A1		E mV A2		E_{calc} $q_1 = 7, q_2 = 70$
0	6.00	5.91	*335.4	*335.6	331.5	331.5	335.6
2	15.33	15.08	310.2	310.5	306.7	307.1	309.6
4	24.31	23.90	296.5	296.7	293.4	293.7	295.9
6	32.94	32.36	287.3	*	283.9	284.3	286.2
8	41.26	40.53	279.3	(283.7)	276.6	277.0	278.6
10	49.27	48.38	272.7	(275.8)	270.4	270.9	272.2
12	57.00	55.96	266.4	268.8	265.0	265.7	266.8
14	64.46	63.29	261.8	263.6	260.1	260.5	261.8
16	71.66	70.37	*(271.1)	*(268.9)	255.6	256.0	257.4
18	78.61	77.21	254.3	254.1			253.4
20	85.33	83.83	250.1	250.2	248.0	248.2	249.7
25	101.20	99.46	242.1	242.0	240.0	240.4	241.8
30	115.85	113.92	235.3	235.2	233.4	233.7	235.1
35	129.41	127.32	229.6	229.5	227.8	228.0	229.3
40	142.06	139.78	224.7	224.8			224.3
45	153.72	151.39			219.2	219.5	220.0
46	155.97	153.61	219.8	219.8			219.1
50	164.67	162.23	216.7	216.7	215.7	215.8	216.1

Table 3. Titration for q_1 and q_2. $S = 5.00$ mC $HgCl_2$, $T = 5.10$ mC $HgCl_2$, 470 mC Cl^-, CE »G».

v ml	X_e mC	X mC	E mV G1		E mV G2		E_{calc} $q_1 = 8.5$	E_{calc} $q_1 = 7$
8	34.81	33.33	308.5	309.3	307.5	307.3	308.9	309.9
10	42.73	40.88	301.4	301.7	300.7	300.6	301.7	302.9
12	50.36	48.15	*(298.9)	295.2	294.7	294.7	295.7	296.9
14	57.72	55.18	291.3	290.1	290.2	290.4	290.5	291.8
16	64.83	61.98	286.9	285.5	285.5	285.5	285.5	287.2
18	71.69	68.54	282.0	281.0	281.3	281.3	281.6	283.1
20	78.33	74.91	278.2	277.2	277.5	277.5	277.8	279.3
25	94.00	89.99	270.1		269.3	269.3	269.5	271.1
30	108.46	103.95	263.0	262.4	262.1	262.1	262.7	264.2
35	121.85	116.93	257.1	256.5	256.4	256.5	256.7	258.3
40	134.29	129.02	252.2	251.3	251.4	251.4	251.6	253.2
45	145.86	140.29	247.5	247.5	246.9	247.0	247.1	248.6
50	156.67	150.86	243.7	243.7	243.3	243.3	243.2	244.7

were used, and with G1, where one electrode was burnt. During the course of A1, »old» electrodes were sometimes inserted in the solution and then gave potentials close to those of A2. The cause of the difference was thus in the Pt electrodes, not in, for example, errors in the composition of the solution.

The figures in Tables 2 and 3 should give an impression of what sort of accuracy could be attained in our measurements. If the first points are omitted, where the emf:s are sensitive to very small changes in the composition and where moreover the potentials were less steady, then the emf:s are seen to be defined to within one mV in G1 and G2, and to within 2 mV if A1 and A2 are compared. Carter and Robinson [10] seem to have had much worse experience with half-cells $Pt,Hg_2Cl_2/HgCl_2$, Cl^-. Even at high (Cl^-) and after several days their emf:s were unsteady to within several mV. Maybe we owe our more constant results to continual vigorous stirring.
According to (IV,16) (IV,17), (4) and (6):

$$E = E_{2X} + 59.16 \log (HgX_2) - 59.16 \log X \qquad (12)$$

$$E_{2X} = E_{20} + E_{10} - E_{1X} - 59.16 \log \varkappa_2 = 559.4 \pm 0.2 + 233.1 \pm 0.3 - 427.0 \pm 1.4 = 365.5 \pm 1.5 \text{ mV} \qquad (13)$$

The value of E_{2X} can also be found by direct measurements of cells

$$CE//HgCl_2,\ Cl^-/Hg_2Cl_2(s)/Pt$$

at low (Cl^-) where the formation of $HgCl_3^-$ can be neglected or easily corrected for. Such measurements were tried by Inger Brattsten, *leg. apot.*, who found values of E_{2X} (after standardization) ranging from 364.5 to 368 mV, which is in good agreement with (13) in view of the difficulty of the measurements.

The calculations were carried out as indicated in part IV, with the aid of equation (IV,22):

$$59.16 \log r = 59.16 \log (1 + q_1X + q_2X^2) = E_{2x} + 59.16 \log a - E - 59.16 \log X \qquad (14)$$

The individual correction to the emf:s measured with CE »A» was at that time + 1.0 mV, for »G» 0 mV. From the first approximate plotting of $(r'-1)$ X_e^{-1} against X_e, the approximate values $q_1 = 0.007\ mC^{-1} = 7\ C^{-1}$, $q_2 = 0.00007\ mC^{-2} = 70\ C^{-2}$ were chosen. Using these constants better values of $X = (Cl^-)$ were calculated, which are given in Tables 2 and 3. A graph according to (IV,23) was then made of

$$F_1 = (r - 1)X^{-1} = q_1 + q_2X \qquad (15)$$

against X for the different titrations, which is given in Fig. 2.

In Fig. 2, the points for $X<50$ mC can be disregarded, as a small error in E_{2X} or in X with cause a large displacement of these points and moreover less care was for this reason taken in waiting for the equilibrium E.

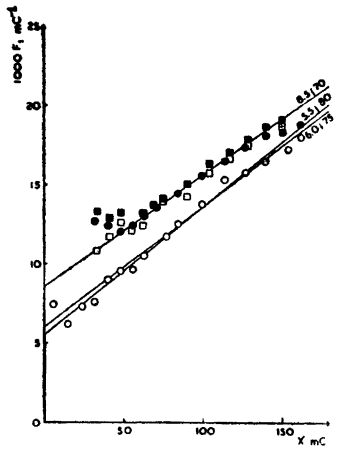

Fig. 2. Function F_1 (X) from different titrations for q_1 and q_2. Squares: titrations with a = 5 mC. Open squares: G1, filled: G2. Circles: titrations with a = 2 mC. Open circles: A1, filled: A2 (see Tables 2 and 3). Upper line: F_1 (X) calculated with q_1 = 8.5 C^{-1}, q_2 = 70 C^{-2}. Lower lines: calculated with q_1 and q_2 = 5.5 and 80, or 6.0 and 75.

It is seen that the points for the titrations A2, G1 and G2 fit rather well to the straight line $F_1 = 8.5 \cdot 10^{-3}X + 70 \cdot 10^{-6}X^2$ corresponding to $q_1 = 8.5 \cdot 10^{-3}\text{mC}^{-1} = 8.5\ \text{C}^{-1}$ and $q_2 = 70 \cdot 10^{-6}\ \text{mC}^{-2} = 70\ \text{C}^{-2}$.

On the other hand, the points of A1 (where the electrodes were burnt) are considerably lower; the two lower lines drawn in Fig. 2 correspond to $q_1 = 5.5\ \text{C}^{-1}$, $q_2 = 80\ \text{C}^{-2}$ and $q_1 = 6.0\ \text{C}^{-1}$, $q_2 = 75\ \text{C}^{-2}$.

An increase or decrease in E_{2X} by 1.5 mV will increase or decrease F_1 by about 1.5 C^{-1} for all except for the lowest X. The inaccuracy in E_{2X} inaccording to (13) means an inaccuracy in q_1 of about 1.5 C^{-1}, whereas q_2 is not appreciably affected.

Of the three lines in Fig. 2 we are personally inclined to prefer the higher one, which was obtained with old electrodes. However, we do not claim the accuracy to be better than

$$q_1 = (7 \pm 3)\text{C}^{-1} = 10^{0.85 \pm 0.15}$$
$$q_2 = (70 \pm 10)\text{C}^{-2} = 10^{1.85 \pm 0.05}$$

Table 2 gives the emf:s calculated for different v assuming the constants be 7 and 70, and Table 3 the emf:s calculated for $q_2 = 70$ and $q_1 = 7$ or 8.5. The agreement is seen to be as good as can be expected.

Inger Brattsten, *leg. apot.*, made similar titrations with the first apparatus (see part I). It was much more difficult to get reproducible results with the earlier type of Pt electrodes than with the present type, where the glass-Pt seal is well above the aqueous solution.

The average of her determinations was $q_1 = 5.5\ \text{C}^{-1}$, $q_2 = 75\ \text{C}^{-2}$ which is well within the limits of accuracy given above.

According to Linhart [11] the equilibrium constant $(Hg_2Cl_4)\ (HgCl_2)^{-2}$ is 0.3 C^{-1} at 25° C. If the order of magnitude is correct, which we do not doubt, the amount of binuclear complexes must be negligible at the low mercury concentrations we have used. Nor is there anything in our results forcing us to assume the formation of binuclear complexes.

PREVIOUS DETERMINATIONS OF q_1 AND q_2

As far as we know, the equilibria of $HgCl_2$ with $HgCl_3^-$ and $HgCl_4^{2-}$ have hitherto been studied only by partition or solubility equilibria and by optical methods. Sherrill [7] measured the partition of $HgCl_2$ between benzene and aqueous KCl solutions at 25° C. He assumed that only $HgCl_4^{2-}$ is formed, but found values of q_2 ranging from 6000 to 82. Out of this range he chose the value 90 which seems to be of the right order of magnitude. Sand and Breest [10] pointed out that Sherrill's measurements could be explained by the formation of $HgCl_3^-$ with $q_1 = 11\ 5\ C^{-1}$. Drucker [9] recalculated q_1 from Sherrill's results, with a correction for the »degree of dissociation» of KCl; he found $q_1 = 13.5\ C^{-1}$. Linhart [13] measured the partition of $HgCl_2$ between benzene and NaCl solution and found $q_1 = 9.8\ C^{-1}$, $q_2/q_1 = 4.9\ C^{-1}$, i e $q_2 = 48\ C^{-2}$ at 25° C. Job [14] from spectrographic measurements concluded $q_1 = 5\ C^{-1}$ (16° C).

Garrett [15] has concluded $q_1 \approx 20$ at 25° C from measurements of the solubility of $HgCl_2$ in chloride solutions. Garrett assumed that the concentration of $HgCl_4^{2-}$ is negligible, which does not agree with our results.

Table 4. Summary of equilibrium constants.

Reaction	Equilibrium constant	log of equil. const. (C scale)
$Hg^{2+} + Cl^- = HgCl^+$	$\varkappa_1 = \sqrt{k_{12}\varkappa_2} = (5.4_5 \pm 0.2_3)10^6 C^{-1}$	$6.73_6 \pm 0.018$
$Hg^{2+} + 2Cl^- = HgCl_2$	$\varkappa_2 = (1.65 \pm 0.10) \cdot 10^{13} C^{-2}$	$13.21_8 \pm 0.02_4$
$Hg^{2+} + HgCl_2 = 2HgCl^+$	$k_{12} = 1.80 \pm 0.10$	0.255 ± 0.025
$HgCl_2 + Cl^- = HgCl_3^-$	$q_1 = (7 \pm 3) C^{-1}$	0.85 ± 0.15
$HgCl_2 + 2Cl^- = HgCl_4^{2-}$	$q_2 = (70 \pm 10) C^{-2}$	1.85 ± 0.05
$Hg^{2+} + 3Cl^- = HgCl_3^-$	$\varkappa_3 = q_1\varkappa_2 = (1.2 \pm 0.5) \cdot 10^{14} C^{-3}$	14.07 ± 0.15
$Hg^{2+} + 4Cl^- = HgCl_4^{2-}$	$\varkappa_4 = q_2\varkappa_2 = (1.2 \pm 0.2) \cdot 10^{15} C^{-4}$	15.07 ± 0.06
$2HgCl_2 = HgCl^+ + HgCl_3^-$	$q_1 k_{12}^{\frac{1}{2}} \varkappa_2^{-\frac{1}{2}} = (2.3 \pm 1.0).10^{-6}$	$(0.37 \pm 0.15) - 6$
$Hg_2Cl_2(s) = Hg_2^{2+} + 2Cl^-$	$k_s = (1.32 \pm 0.03)10^{-17} C^3$	$(0.120 \pm 0.010) - 17$
$Hg_2Cl_2 + 2Hg^{2+} = Hg_2^{2+} + 2HgCl^+$	$k_s k_{12}\varkappa_2 = (3.9 \pm 0.4)10^{-4} C$	$(0.593 \pm 0.036) - 4$
$Hg + Hg^{2+} = Hg_2^{2+}$	$k_0 = 129.2 \pm 1.0$	$2.111_2 \pm 0.003_4$
$Hg_2Cl_2 = Hg + HgCl_2$	$k_s\varkappa_2 k_0^{-1} = (1.7 \pm 0.1)10^{-6} C$	$(0.227 \pm 0.26) - 6$
$Hg_2Cl_2 + Cl^- = Hg + HgCl_3^-$	$q_1 k_s\varkappa_2 k_0^{-1} = (1.2 \pm 0.5)10^{-5}$	$(0.08 \pm 0.15) - 5$
$Hg_2Cl_2 + 2Cl^- = Hg + HgCl_4^{2-}$	$q_2 k_s\varkappa_2 k_0^{-1} = (1.2 \pm 0.2)10^{-4} C^{-1}$	$(0.08 \pm 0.06) - 4$

In Table 4 is given a survey of the equilibrium constants that have been obtained directly by our measurements or that can be calculated from them by

means of, for example, the formulae in Part IV. In the second column is indicated how the other constants are calculated from the primary values: k_{12}, $\varkappa_2$, q_1,q_2, k_s, and k_0. Previous measurements by other workers have been quoted inthe text.

SUMMARY

By electrometric measurements, a number of equilibrium constants, involving Hg^{2+}, $HgCl^+$, $HgCl_2$, $HgCl_3^-$, $HgCl_4^{2-}$, Hg_2^{2+}, Hg_2Cl_2(s), and Hg(l) have been determined. The constants are listed in Table 4. They are valid for the special experimental conditions: 25° C, $(H^+) = 10$ mC, ionic strength 500 mC.

At the beginning of this investigation Inger Brattsten, *leg. apot.*, carried out a large amount of pioneer work, which has proved of great value in this investigation and others to follow. We also wish to thank Mr. Erik Ekedahl, Mr. Gunnar Infeldt and Miss Ingegerd Qvarfort for their valuable aid.

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Deviation from Coplanarity of the Atoms in Structures of Halogen Substituted Benzenes

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The discovery of the quantum mechanical phenomenon of resonance has greatly contributed to the progress in the theory of the chemical bond. The theory has been able to explain well established experimental facts in cases where the older theories failed. On the other hand applications to effects, the existence of which may still be questioned, may bring discredit to the theory. It seems therefore most important to increase our experimental knowledge as far as possible before minor details in molecular structures are discussed using the resonance theory.

In aromatic compounds the resonance phenomena are very strikingly demonstrated. In benzene, for instance, the properties of the equivalent C-C-bonds is explained by assuming that they have 50 % single bond and 50 % double bond character. The C-C-distance in benzene is about 1.40 Å, *i. e.* it has a value between that usually observed for single bonds (1.54 Å) and that observed for double bonds (about 1.34 Å). According to a well-known theory resonance may also exist between double and single bonds in cases where the proportion of single and double bond character differs from that of benzene. If the percentage of double bond character exceeds 50 % the C-C-distance is smaller than 1.40 Å, if the percentage is less than 50 % the distance is greater.

Brockway and Palmer [1] have investigated the structures of some chlorine derivatives of benzene. The ortho Cl-Cl-distance was found to be greater than expected for an undistorted model. The effect was explained partly by assuming a small amount of double bond character of the C-Cl-bonds, resulting in a reduction of the double bond character of the bond between the two adjacent carbon atoms, partly by the assumption that the angle between the C-Cl-bonds is greater than 60°, both bonds remaining in the plane of the carbon ring.

In the hope of being able to contribute to the elucidation of this very interesting problem, we have applied the rotating sector method to it and obtained results which differ somewhat from those obtained by Brockway and Palmer. We think it worth while to give our results in some detail, not only because of the discrepancy just hinted at, but also because the examples treated seem well fitted for a comparison between the visual method and the sector method of electron diffraction.

A survey of the sector method in use for some years in this laboratory has recently been given in this journal [2]. Each of the $\frac{\sigma(r)}{r}$-curves reproduced in present publication was obtained by Fourier synthesis of a mean molecular scattering curve which was the average of a series of scattering curves, each of which was computed from a separate diagram.

The following compounds were studied:
o-*dichlorobenzene*, o-*dibromobenzene*, *hexachlorobenzene*, *hexabromobenzene*, *1,2,3,5-tetrabromobenzene and* p-*dibromobenzene*.

The C-C-bond distance gives rise to pronounced maxima in the $\frac{\sigma(r)}{r}$-curves (at $r = 1.40$—1.405 Å) in all cases except in C_6Cl_6 and p-$C_6H_4Br_2$, where it is disturbed by the influence of higher neighbouring maxima. The C-Br-bond distance is found to be 1.88 Å:

1.89(o-$C_6H_4Br_2$), 1.87 (C_6Br_6), 1.88 (1,2,3,5-$C_6H_2Br_4$ and p-$C_6H_4Br_2$).

The corresponding C—Cl-distance is found equal to 1.72 Å (C_6Cl_6) and 1.735 ($C_6H_4Cl_2$). In each case the C-C and C-halogen bond distance found in the actual molecule will be used as the basis for calculating the dimensions of the models which we are going to discuss. In the two cases where the C-C bond distance was not directly observed, the value 1.40 Å is used. With the exception of p-$C_6H_4Br_2$, all the models representing plane molecules give sets of interatomic distances which are in poor agreement with the values obtained from the $\frac{\sigma(r)}{r}$-curves. The agreement in the case of the plane p-$C_6H_4Br_2$ molecule, however, is very satisfactory. We have calculated the interatomic distances in the other compounds, considering models in which C-halogen bonds are bent out of the plane of the C_6-hexagon, and under these circumstances a much better correspondence between calculated and experimental distances is reached.

In the Figs. 1—6 the experimental $\frac{\sigma(r)}{r}$-curves of the six compounds with

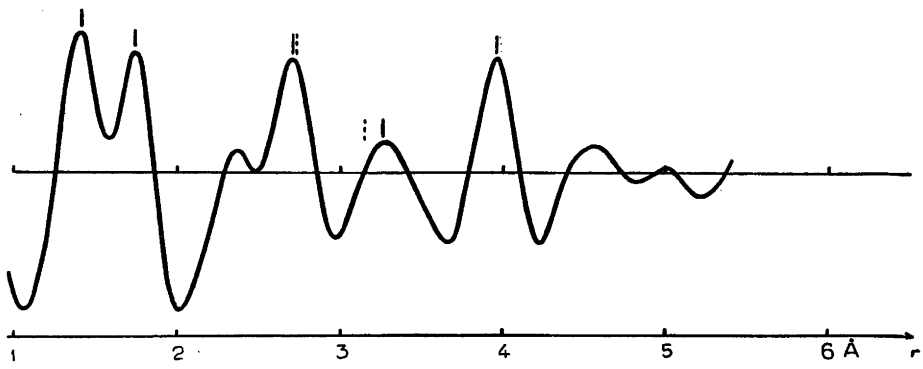

Fig. 1. $\frac{(\sigma r)}{r}$*-curve for o-dichlorobenzene.*

which we are dealing are reproduced. In all these figures the r-values of distances occurring in the models under discussion are indicated by vertical lines, the broken lines corresponding to plane models, the fully drawn lines to models where halogen atoms are outside the plane of the carbon atoms. The Tables 1—6 contain values of the main distances, both experimental and theoretical, of the different molecules. Only such distances as may be determined with some accuracy from the $\frac{\sigma(r)}{r}$- curves, have been tabulated. In the following discussion each compound will be treated separately.

o-*Dichlorobenzene.* (Fig. 1 and Table 1.) The experimental distances tabulated in the second column of the table are obtained directly from the $\frac{\sigma(r)}{r}$-curve. The C_1—C_2 and the C_1—Cl_1-distances are found to be 1.405 Å and 1.735 Å respectively. In the third column of the table theoretical values of the distances occurring in the plane model are tabulated. The fourth column contains theoretical values for a non-planar model in which the two C—Cl-bonds are bent 18° out of the C_6-plane, perpendicular to this plane and in opposite directions. Both the table and figure show that the non-planar model gives a better agreement between experiment and calculation than the plane one.

Table 1. o-$C_6H_4Cl_2$. C_1—C_2 = *1.405 Å.* C_1—Cl_1 = *1.735 Å.*

Distance	Experimental	Theoretical, plane	Theoretical, 18°
C_1—Cl_2	2.70 Å	2.72 Å	2.70 Å
C_1—Cl_3	3.97 »	4.03 »	3.98 »
Cl_1—Cl_2	3.28 »	3.14 »	3.26 »

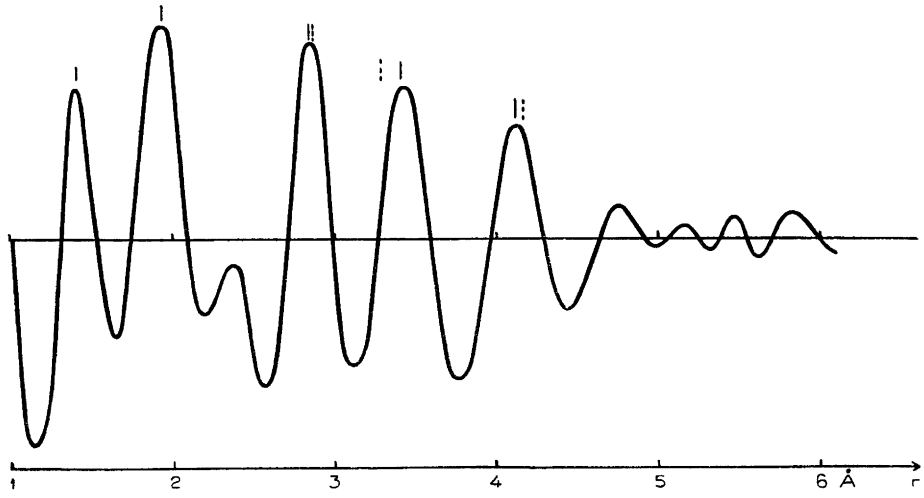

Fig. 2. $\frac{\sigma(r)}{r}$*-curve for o-dibromobenzene.*

o-*Dibromobenzene.* (Fig. 2, Table 2.) The observed C_1—C_2-distance is 1.405 Å, the C_1—Br_1-distance 1.89 Å. From the figure and the table the same conclusion may be drawn as in the case of the corresponding chlorine compound. A bending of the C-halogen bonds like that described for the chlorine compound, and of the same magnitude, leads to the best agreement between calculation and experiment.

Table 2. o-$C_6H_4Br_2$. C_1—C_2 = *1.405 Å.* C_1—Br_1 = *1.89 Å.*

Distance	Experimental	Theoretical, plane	Theoretical, 18°
C_1—Br_2	2.85 Å	2.86 Å	2.84 Å
C_1—Br_3	4.13 »	4.18 »	4.13 »
Br_1—Br_2	3.43 »	3.30 »	3.42 »

Hexachlorobenzene (Fig. 3, Table 3). The carbon-carbon bond distance is observed in the $\frac{\sigma(r)}{r}$-curve, but the presence of the C—Cl-bond distance makes the condition for an accurate determination unfavourable. We have therefore used the value 1.40 Å found in other benzene derivatives as a basis for the calculation of the dimensions of the models. The value 1.72 Å was observed for the C—Cl-bond distance. A bending of the C—Cl-bonds out of the plane of the carbon hexagon is observed in this case as well, but the angle seems to

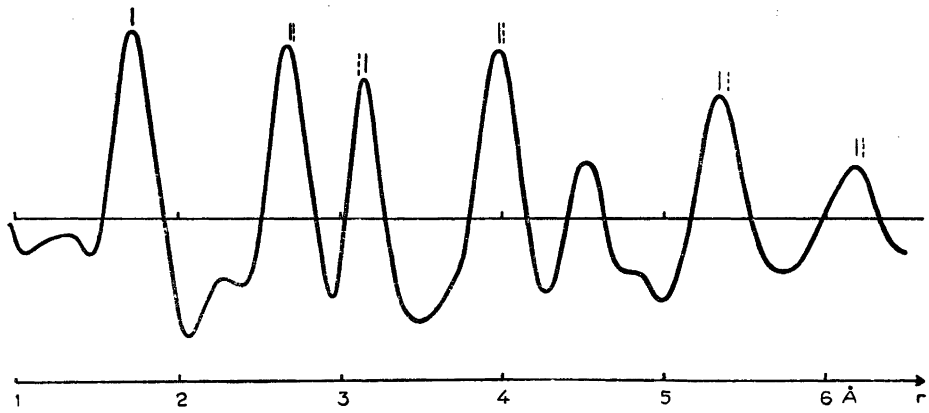

Fig. 3. $\frac{\sigma(r)}{r}$*-curve for hexachlorobenzene.*

be somewhat smaller (about 12° instead of 18°) than in the two compounds mentioned above. Here, also, the figure and the table may best demonstrate the effect.

Table 3. C_6Cl_6. $C_1—C_2 = 1.40$ *Å.* $C_1—Cl_1 = 1.72$ *Å.*

Distance	Experimental	Theoretical, plane	Theoretical, 12°
$C_1—Cl_2$	2.68 Å	2.71 Å	2.70 Å
$C_1—Cl_3$	3.99 »	4.01 »	3.99 »
$C_1—Cl_4$	4.51 »	4.52 »	4.50 »
$Cl_1—Cl_2$	3.15 »	3.12 »	3.16 »
$Cl_1—Cl_3$	5.35 »	5.40 »	5.34 »
$Cl_1—Cl_4$	6.19 »	6.24 »	6.20 »

Hexabromobenzene (Fig. 4, Table 4). The carbon-carbon bond distance is found equal to 1.40 Å, the C—Br-bond distance to 1.87 Å. Here, also, the observed distances are best explained by assuming a bending of the C—Br-bonds of about 12° out of the plane of the benzene ring.

Table 4. C_6Br_6. $C_1—C_2 = 1.40$ *Å.* $C_1—Br_1 = 1.87$ *Å.*

Distance	Experimental	Theoretical, plane	Theoretical, 12°
$C_1—Br_2$	2.81 Å	2.84 Å	2.83 Å
$C_1—Br_3$	4.12 »	4.15 »	4.13 »
$C_1—Br_4$	4.64 »	4.67 »	4.64 »
$Br_1—Br_2$	3.30 »	3.27 »	3.33 »
$Br_1—Br_3$	5.62 »	5.66 »	5.61 »
$Br_1—Br_4$	6.50 »	6.54 »	6.50 »

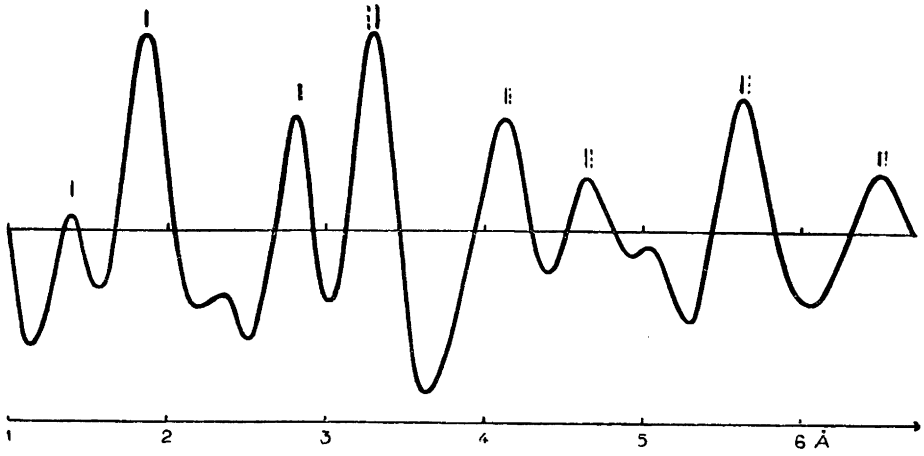

Fig. 4. $\frac{\sigma(r)}{r}$*-curve for hexabromobenzene.*

1,2,3,5-*Tetrabromobenzene* (Fig. 5, Table 5). The C—C and C—Br-bond distances obtained are 1.40 Å and 1.88 Å respectively. There seems to be no reason for the assumption that the C_5—Br_5-bond is bent out of the ring plane. The assumption that the other C—Br-bonds are bent about 15° out of this

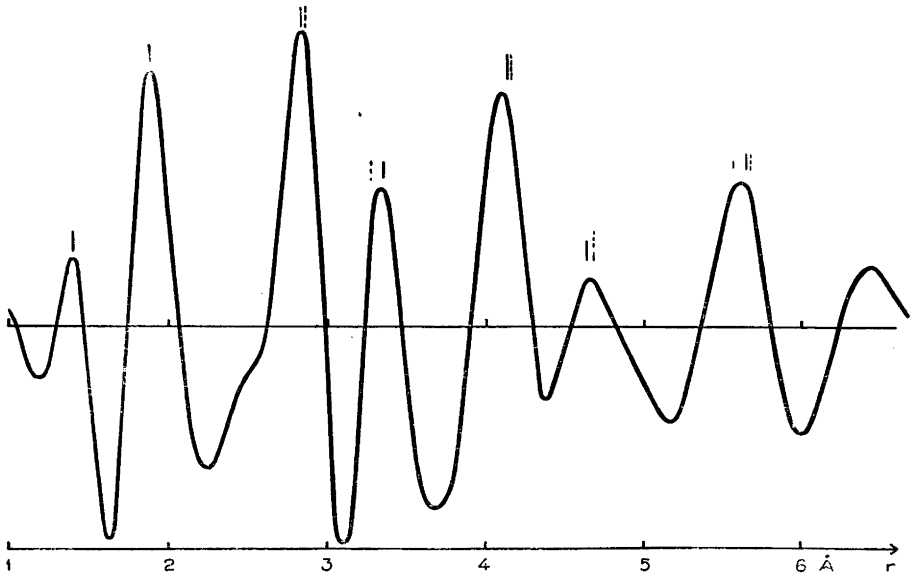

Fig. 5. $\frac{\sigma(r)}{r}$*-curve for 1,2,3,5-tetrabromobenzene.*

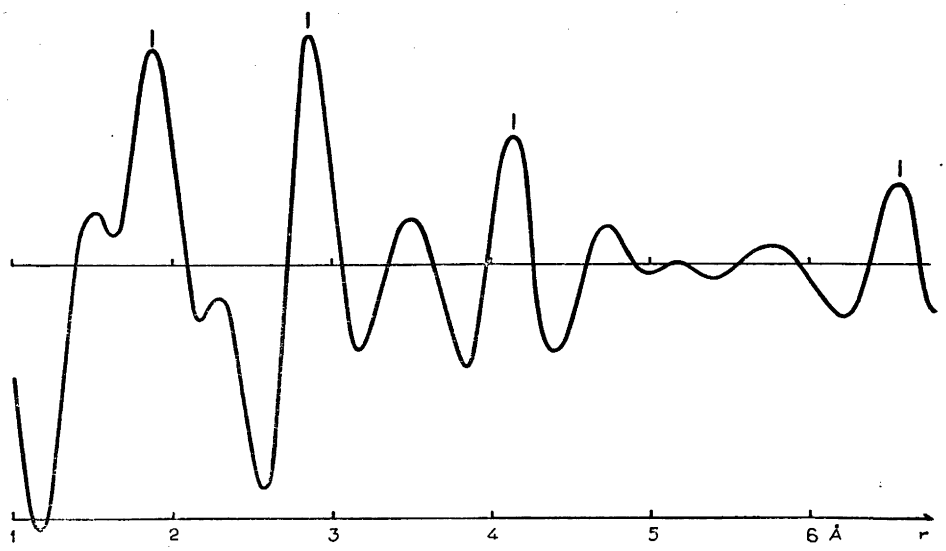

Fig. 6. $\frac{\sigma(r)}{r}$*-curve for p-dibromobenzene.*

plane gives the best agreement between theory and experiment. It follows from this assumption (see Table 5 and Fig. 5) that the C_1—Br_3, C_1—Br_4 and Br_1—Br_3-distances are each split up into two components, lying some hundreths of an Ångström apart.

Table 5. 1,2,3,5-$C_6H_2Br_4$. C_1—C_2 = 1.40 Å. C_1Br_1 = 1.88 Å.

Distance	Experimental	Theoretical, plane	Theoretical, 15°
C_1—Br_2	2.83 Å	2.85 Å	2.83 Å
C_1—Br_3	4.11 »	4.16 »	4.13 Å; 4.16 Å (rel. weights 3 : 1)
C_1—Br_4	4.66 »	4.68 »	4.64 »; 4.68 » (rel. weights 3 : 1)
Br_1—Br_2	3.34 »	3.28 »	3.36 Å
Br_1—Br_3	5.62 »	5.68 »	5.57 »; 5.65 » (rel. weights 1 : 2)

p-*Dibromobenzene* (Fig. 6, Table 6). This compound was investigated in order to check our assumption that the repulsion between *o*-substituted halogen atoms is responsible for the bending of the C-halogen bonds. From table and figure it is obvious that the distances observed are those to be expected for a plane model and that no disturbing effects can be observed.

Table 6. p-$C_6H_4Br_2$. C_1—C_2 = 1.40 Å. C_1—Br_1 = 1.88 Å.

Distance	Experimental	Theoretical, plane
C_1—Br_2	2.85 Å	2.85 Å
C_1—Br_3	4.15 »	4.16 »
Br_1—Br_4	6.56 »	6.56 »

DISCUSSION OF THE RESULTS

From the results of our experiments we have not been able to confirm the presence of systematic variation of the C—C-bond length. Discussing the accuracy of our measurements it should be born in mind that the determination of relative values of distances within the same molecule are more reliable than absolute values of such distances. This follows from the fact that our devices for precision measurements of the voltage (owing to post-war conditions) are not quite satisfactory. The greater differences in C—C and C-halogen distances do not, however, exceed 0.005 Å (C—C), 0.015 Å (C—Cl) and 0.02 Å (C—Br).

The reason for a deviation of the angle between two neighbouring C-halogen bonds from the value of 60° is no doubt chiefly a result of repulsive forces between halogen atoms. The distances to be expected in absence of such deviation are 3.13 Å (Cl—Cl) and 3.28 Å (Br—Br); both values which must be assumed to be smaller than the double van der Waals' radii, which should be at least 3.3 Å and 3.5 Å respectively.

It is striking that the deviation seems to be greater in the case of 1,2-halogen derivatives than in the hexa-compounds. It should be remembered, however, that the entire discussion was based upon the assumption that the bending of the C-halogen bonds takes place in planes normal to the benzene ring. Actually, this must be true in the hexa-derivatives, but in less symmetric molecules, like the *o*-dihalogenbenzenes, the bending will of course have two components, one perpendicular to the benzene ring and one in the plane of the ring. The latter component will influence the distance of 1.2-halogen atoms much more strongly than other distances. The result is that it becomes rather more difficult to determine the magnitude of this »planar effect» than that of the other. In the case of 1,2,3,5-tetrabromobenzene the bending of the C_2—Br_2-bond must be orthogonal to the benzene ring, the C_5—Br_5-bond remaining in the ring plane. The distortion process just mentioned is able to explain the fact that the bending angle, if the bending is assumed to take place perpendicularly to the benzene ring only, is found to be smaller in the C_6X_6 molecules than in the others. It seems very likely that the perpendicular component of bending is in reality very much the same in all cases, and that an additional »planar effect» occurs in less symmetrical molecules.

It is clear that the *o*-dihalogenbenzenes, if not plane, may exist in two optically active forms, but the conversion from the one form to the other must be expected to take place rather frequently so that a separation of the two antipodes will be impossible. It seems very unlikely, however, that this may be treated as an oscillation and that the intermediate forms may contribute

noticeably to the scattering of electrons. Similar considerations should be expected to be applicable to the conversion of the symmetrical molecules where mirror-image forms are indentical.

The so-called »ortho-effect» of dipole moments can of course at least partly be explained by the increase in angle between the ortho C—X-bonds. Thus the determined dipole moment of 1,2,4-trichlorobenzene is 1.25 D, whereas the moment of the undisturbed molecule should be equal to that of the mono-derivative (1.56 D), and the calculated value, if the C_1—Cl_1 and C_2—Cl_2-bonds are bent 18° perpendiculary to the benzene ring, would be 1.45 D. If a bending *in* the plane is also accounted for, the calculated value will be still less than 1.45. In the case of 1,2,3,5-chlorobenzene and the corresponding bromo-compound, the low value of 0.7 D would also be better accounted for if a bending in the benzene ring takes place besides the perpendicular distortion. The fact that induction phenomena will always tend to diminish the dipole moments makes conclusions of this kind rather questionable, however.

The results obtained by us differ markedly from those obtained by Brockway and Palmer who used the visual method. A comparison between the potentialities of the two methods seems therefore justified. We consider it most convenient to choose a special example, and in Fig. 7 we reproduce some theoretical intensity curves (A—F), taken from Brockway and Palmer's paper (Fig. 4, p. 2185), corresponding to a set of slightly different models of the o-dichlorobenzene molecule. The vertical lines give the position of the maxima and minima visually observed from the diffraction diagrams. According to the authors mentioned it is possible to decide which of these alternative models gives the best approximation to the true molecule simply by comparing the position of the maxima and minima with the experimental positions indicated by the vertical lines.

Below the curves A—F we have given an experimental molecular scattering curve using the same s-scale. The general agreement between our experimental curve and the calculated curves is striking. In fact, the positions of the maxima and minima of the theoretical curves are generally in better accordance with those of our experimental curve than with the positions of the maxima and minima visually observed. In spite of this, and of the fact that the whole intensity curve is known and based on photometrically measured intensities, it occurs to us that it would be unjustified, for example, to state that the curve D is better than E.

The question now arises, why the Fourier-transformed curve, $\frac{\sigma(r)}{r}$, may be better suited than the molecular scattering curve for the purpose of picking out the best molecular model. The $\frac{\sigma(r)}{r}$-curve of course expresses the same ex-

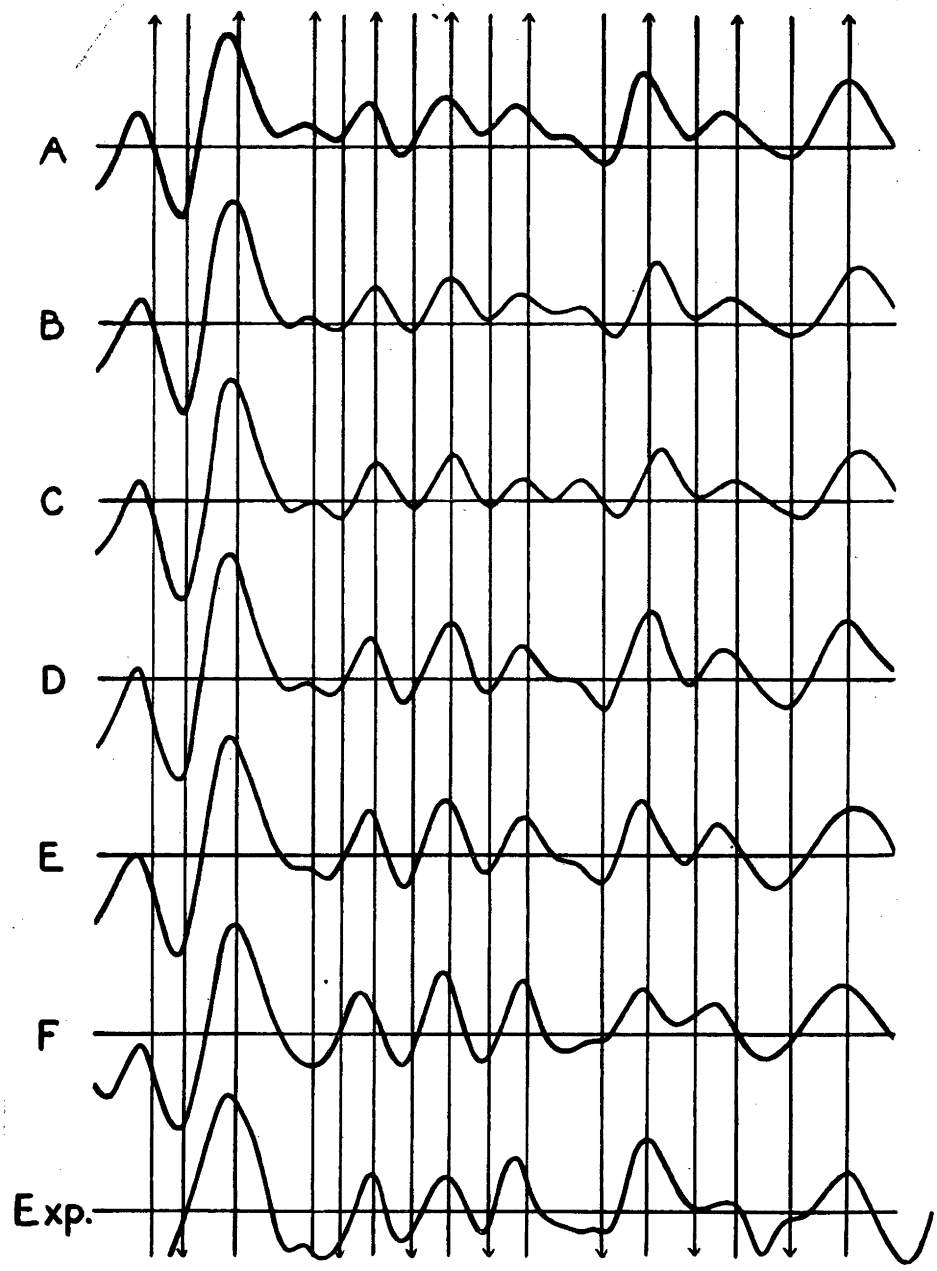

Fig. 7. A . . . F *theoretical intensity curves for* o-*dichlorobenzne according to Brockway and Palmer.* Exp. *experimental curve obtained by the sector method.*

perimental facts as the molecular scattering curve. Nevertheless, the $\frac{\sigma(r)}{r}$ curve has the advantage of concentrating the effect of a certain internuclear distance within a rather short range, usually giving rise to pronounced maxima, whereas the corresponding contribution of the intensity curve is sinusoidal and thus distributed over the whole curve. On the other hand a certain error in the experimental intensity curve will usually be distributed over the entire $\frac{\sigma(r)}{r}$ curve and therefore not lead to serious consequences. Should the error happen to be sinusoidal, however, and range over a greater part of the intensity curve, it will have a noticeable effect on the curve. But an error of this type is in general very improbable.

SUMMARY

The following compounds have been investigated using the electron diffraction method with rotating sector: *o*-$C_6H_4Cl_2$, *o*-$C_6H_4Br_2$, C_6Cl_6, C_6Br_6, 1,2,3,5-$C_6H_2Br_4$ and *p*-$C_6H_4Br_2$. The results are compared with those obtained by Brockway and Palmer [1].

The effects reported by these authors could not be confirmed. Our observations are in favour of non-planar models for these molecules, the single exception being the *p*-dibromobenzene. The forces acting between *o*-halogen atoms tend in general to bring the halogen atoms out of the plane of the benzene ring. Finally a comparison between the visual method and the rotating sector method has been carried out.

We wish to thank cand. real. T. Jacobsen and cand. real. S. Furberg for having carried out part of the preparative work and computation relating to the two dibromobenzenes and hexachlorobenzene.

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ACTA CHEMICA SCANDINAVICA 1 (1947) 500—502

A Polarographic Study on the Catalytic Effect of the Catalase, Peroxidase and Cytochrome C Ferments on the Cathodic Reduction of Hydrogen Peroxide

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The oxygen dissolved in an aqueous solution to a concentration of about 2.5×10^{-4} *M* can be reduced to water at the dropping mercury cathode [1]. The reduction takes place in two stages. The oxygen is first reduced to hydrogen peroxide, which, in turn, is afterwards reduced to water in accordance with the following scheme [2]:

$$O_2 + 2e + 2H^+ \rightarrow H_2O_2$$
$$H_2O_2 + 2e + 2H^+ \rightarrow 2H_2O$$

This process can be followed in detail in the currentvoltage curve obtained in polarographic analysis (Fig. 1).

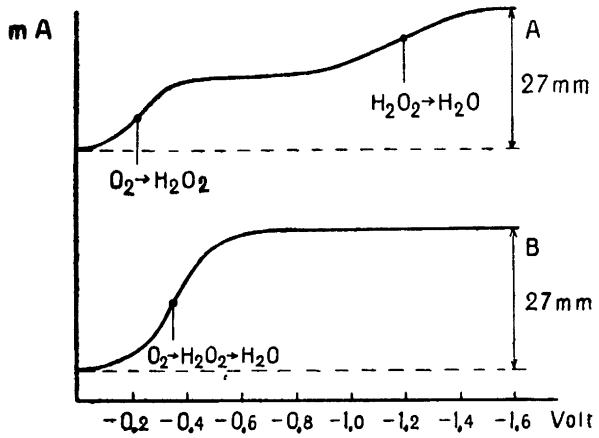

Fig. 1. Polarogram A: phosphate buffer solution pH 7.38. Polarogram B: phosphate buffer solution pH 7.38, containing 500 γ catalase/ml.

If the aqueous solution is mixed with increasing amounts of a hemin proteid, the cathodal reduction of the hydrogen peroxide will take place at a more positive potential. If the hemin proteid percentage is sufficiently increased, the two reduction stages will coincide in the polarogram, as shown by Fig. 1. This activation of the hydrogen peroxide molecule in the presence of hemoglobin and hematin has been studied by Brdicka and Tropp [3]. They found that these substances had the stated effect, which was not the case with porphyrin. The cobalt, nickel and manganese salts of hematoporphyrin were also tested [4]. These salts, however, had not the same catalytic effect as the ferric salt hematin. Brdicka and Tropp try to explain the catalytic effect by the powerful paramagnetic action which the iron in porphyrin position exerts on the likewise paramagnetic hydrogen peroxide. In a comparison between equimolar solutions of hemoglobin and hematin, they found that the hemoglobin had four times as great catalytic effect as the hemin — a difference which they supposed might be due to traces of catalase contained in the hemoglobin solution.

In order to investigate this matter, the present writer made a polarographic analysis of the conditions in the cathodic reduction of hydrogen peroxide in the presence of small amounts of pure catalase, peroxidase and cytochrome c, respectively.

OWN INVESTIGATIONS

The analyses were made with the aid of a polarograph of Leybold's make (no. 1353).

As an indifferent electrolytic conductor I used throughout a $M/15$ phosphate buffer solution pH 7.38. The pure horseradish peroxidase [5] had the activity PZ = 1160, and the catalase preparation, produced from horse liver [6], had the activity Kat. f. = 50000. The investigation was carried out, broadly speaking, in the same way as that of Brdicka and Tropp [3]. That is to say, a series of polarographic analyses were made on solutions with successive falling content of the respective hemin proteid until the concentration was reached where no catalytic affect was any longer obtained. The lowest concentration of the respective hemin proteid at which a catalytic effect could still be observed is shown in Table 1.

DISCUSSION

A comparison between the results obtained by Brdicka and Tropp and those found in the present investigation indicates that the content of hemin proteid in the solutions was of the same order of magnitude, when their catal-

Table 1. The lowest concentration of hemin at which catalytic effect could still be observed.

	Mol. wt.	Number of hemin molecules per hemin proteid molecule	Lowest conc. for catalytic effect		
			γ/ml	mole/ml x 10^{12}	Hemin molarity (mole/litre) x 10^{7}
Hemoglobin	67,000	4	2 *	29.8	1.19
Hematin	592	1	0.08 *	135	1.35
Cytochrome c	13,000	1	4	307	3.07
Peroxidase	44,100	1	10	226	2.26
Catalase	225,000	3	10	44.4	1.33

* According to Brdicka and Tropp.

ytic effect on the reduction of the hydrogen peroxide at the dropping mercury cathode set in. When the hemin content in the solutions was computed it was found that, if account was taken of experimental errors, it was equimolar. Evidently, we are not concerned here either with a specific catalase or specific peroxidase effect, but rather with a catalytic effect which may be entirely ascribed to the hemin component which, probably, has been split off from the protein at the mercury surface.

SUMMARY

The catalytic effect of hematin as well as of the hemin proteids hemoglobin, peroxidase, catalase, and cytochrome c on the cathodic reduction of hydrogen peroxide is directly proportional to the content of hemin in the solution. No potentising of this effect of specific nature in presence of these ferments, as compared whith hemoglobin, could be shown.

This investigation has been facilitated by a grant from *Stiftelsen Thérèse och Johan Anderssons minne*. To Professor H. Theorell and Assistent Professor K. Agner, who have placed pure peroxidase, catalase, and cytochrome c, respectively, at my disposal, I desire to convey my renewed thanks for their great complaisance.

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ACTA CHEMICA SCANDINAVICA 1 (1947) 503—506

Determination of Manganese in Natural Waters

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The heretofore used methods for quantitative determination of manganese in natural waters, especially sea water, are rather slow and complicated. Therefore Prof. Kurt Buch requested the author to investigate these methods, and thus a simple analytical procedure on the basis of two works, the one by Thompson and Wilson [1] and the other by Tschuiko [2] has been worked out.

In natural waters manganese occurs with the valencies 2, 3 and 4. Septevalent manganese is always reduced to a lower valency. The compounds of manganese principally form oxides, insoluble in water, wherefore they must be precipitated together with a collector. The divalent manganese salts are soluble in water but are converted into an insoluble form when the pH-value of the water is raised to about 12. Manganese hydroxide $Mn(OH)_2$ is formed and this is then converted, under the influence of the oxygen dissolved in the water, into insoluble $MnO(OH)_2$. At this pH-value Tschuiko uses magnesium sulfate as coagulating agent. As far as sea water is concerned the question of coagulating agent is simple, for sea salts contain so much magnesium (Mg:Cl = 0.067) that even such a very low salinity as 2.46 ‰ gives the necessary number of Mg-ions. Thus, the pH of the water need only be raised to 12 to bring about coagulation and to effect complete precipitation of $Mn(OH)_2$.

The manganese removed from the initial volume of water concentrates in the hydroxide precipitate which, after decantation of the unnecessary supernatant liquid is dissolved in sulfuric acid and then oxidated to permanganate. Tschuiko uses the reaction of Marshall [3] with alkali persulfates as oxidant in the presence of silver ions, while Thompson and Wilson use alkali periodates. In accordance with the latter alkali periodates have also been used in this research for they give a much clearer and steadier colour, than that obtained with persulfates.

The silver nitrate which has a catalytic effect in Marshall's reaction is not without importance when oxidating with alkali periodates. In the presence of silver ions the oxidation to permanganate proceeds faster than without them and even small amounts of periodates can be used. A great excess of silver nitrate, 10 ml of a 0.2 *N* solution, does not disturb the reaction, but the case is quite reverse when alkali persulfate is used as oxidant, which fact is of importance in the elimination of halide ions present, and to which reference will be made below.

A condition for oxidation to the permanganate stage is that two constituents of sea water, the halide ions and the organic matter, must be eliminated, but fortunately only the former are always present and can be removed in two ways:

1. By precipitating as silver haloides.

2. By evaporating and igniting the hydroxide precipitate dissolved in sulfuric acid.

This last mentioned method is used only when organic matter is present in great amounts and is evinced by the yellow colour of the sulfuric acid wherein the hydroxide precipitate is dissolved.

In order to check the utility of this method varying quantities of manganese, 0.001—0.01 mg were added to one liter of distilled water, which was subsequently made alkalic and then coagulated with magnesium sulfate. The precipitate was dissolved in sulfuric acid after which oxidation to permanganate took place. By comparing the results with a series of colour standards in 50 ml Nessler tubes it was found that exactly the same quantities of manganese were recovered as had been added. The method was further tested by using in stead of distilled water, sea water, freed of its eventual manganese content by coagulating with alkali and removing the precipitate as mentioned above. In this way it is possible to detect variations of 0.001 mg of manganese in a liter of sea water.

PREPARATION OF REAGENTS

(a) *Sodium paraperiodate* is prepared by the method of Wells [4].

(b) *Sulfuric acid.* — Test the acid for manganese in the way suggested by Thompson and Wilson. Dilute one part of the concentrated acid with two parts of distilled water and add sodium paraperiodate so that 10 ml contains 150 mg.

(c) *Potassium hydroxide.* — Guaranteed reagent from E. Merck shows free from manganese. Use a 0.2 *N* solution.

(d) *Silver nitrate.* — Use a 2 *N* and a 0.2 *N* solution. To test the silver nitrate for manganese add to 10 ml of a 2 *N* solution a solution of 1.1 g sodium chloride, which has been recrystallized from water. Filter the silver chloride off and determine the manganese in the filtrate in the usual way.

(e) *Magnesium sulfate.* — »Kahlbaum z. Analyse» shows free from manganese. Dissolve 22.5 g $MgSO_4 \cdot 7H_2O$ in 250 ml distilled water. 10 ml contains 90 mg Mg.

(f) *Standard permanganate solution.* — Dissolve 57.539 mg of recrystallized potassium permanganate in water add 1 ml of 2 *N* sulfuric acid and reduce by the addition of 0.15 g sodium bisulfite. Boil the solution until free of sulfur dioxide, cool and dilute to exactly 200 ml. Dilute 1 ml of this solution containing 0.1 mg of manganese to 60 ml and heat to boiling together with 10 ml sulfuric acid reagent and 2 ml of 0.2 *N* silver nitrate solution. Add 50 mg sodium paraperiodate and place in a water-bath for half an hour, cool, and dilute to exactly 100 ml. This standard solution which should be kept from contact with direct sunlight contains 0.001 mg of manganese per ml.

A liter sample of sea water to be analyzed is poured into a 1 000 ml graduated glass. Add 50 ml of 0.2 *N* potassium hydroxid solution while mixing well and let the now formed precipitate settle for at least 12 hours. Remove the compleatly clear supernatant liquid with a siphon and dissolve the precipitate in 1.5 ml of concentrated sulfuric acid. The continuation of the analysis depends on whether organic matter is present or not.

1. If the solution is colourless precipitate the halide ions with 2 *N* silver nitrate solution. According to this procedure a too great excess of the latter must be avoided. Filter the silver halide through a porcelain filter, Jena 3G4. Heat the filtrate to boiling with 10 ml of sulfuric acid reagent, add 50 mg of sodium paraperiodate, place in a water-bath for half an hour and cool. The total volume should now be 50 ml at the most and is transferred to a 50 ml Nessler tube and compared with a series of colour standards.

2. If organic matter is present, transfer the hydroxide precipitate dissolved in sulfuric acid into a platinum dish and heat the contents carefully until the excess sulfuric acid is expelled and the salts are fused. Raise the temperature and heat the fused salts for a few minutes over a Bunsen burner. Cool the mass, dissolve it in 10 ml of sulfuric acid reagent, heat to boiling, cool again and filter through a porcelain filter, Jena 3G4. Dilute the filtrate to about 75 ml add 2 ml of 0.2 *N* silver nitrate solution and treat further as described under 1.

Samples of surface sea water collected from various parts of the Gulf of Finland, the Gulf of Bothnia and the North Baltic were analyzed for their total manganese content and the results are shown in Table 1.

Table 1. Amount of manganese present in surface sea water from the Gulf of Finland and Bothnia and the North Baltic.

Date	Location	Salinity	mg Mn/l
18.11.46	Rysskär, Helsinki	6.38	0.003
18.11.46	59°06′N, 22°53′O	6.83	0.0015
27.10.46	59° N, 20°30′O	6.71	0.002
27.10.46	58° N, 18°40′O	7.02	0.0015
27.10.46	57° N, 17°30′O	7.16	0.002
28.10.46	56° N, 16°10′O	7.27	0.002
28.10.46	Snyggehuk, Denmark	7.54	0.0015
1.10.46	Helsinki harbour	5.68	0.016
27.12.46	63° 8′N, 21° 7′O	5.59	0.01
8. 1.47	60°16′22″N,21°55′40″O	6.40	0.0075
27. 1.47	Utö	6.98	0.004

The above described method gives the total manganese content in water, but according to investigations carried out by Thompson and Wilson as well as Tschuiko a part of the manganese, especially at the seasides, exists in a suspended, insoluble form. According to Tschuiko the coagulation method offers a chance to determine the last mentioned state, that is if aluminium sulfate is used as coagulating agent in stead of manganese sulfate and the pH is about 8 the divalent manganese does not precipitate from the solution as hydroxide, for in accordance with different investigators the solubility product of manganese hydroxide is $1.3\text{-}4 \cdot 10^{-14}$ which for pH 8 gives a solubility of 7-20 mg Mn per liter, a number considerably exceeding that existent in sea water. The manganese yield obtained in spite of this only proves that a part of the same exists in a suspended or colloidal state.

SUMMARY

A method for the quantitative determination of manganese present in natural waters on the basis of two works, the one by Thompson and Wilson and the other by Tschuiko has been worked out. The manganese is isolate from the water by precipitation with potassium hydroxide. There will generally be sufficient oxygen present in the water to oxidize the precipitate to hydrous manganese dioxide which is highly insoluble. Magnesium hydroxide is used as collector. In the suggested way it is possible to detect variations of 0.001 mg of manganese in a liter of water. The halides and organic matter present in the water must be removed and care must be used to remove all suspended material. It has been found that the silver ions has a catalytic effect when oxidating of manganese to permanganate with alkali periodates.

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On Acid-Base Equilibria in Non-Aqueous Solutions

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The substances, which are included in the proton theory of acids and bases react with each other according to the well known general scheme:

$$\text{Acid} \rightleftharpoons \text{Base} + H^+$$

If they are arranged in a hydrogen bond series, starting with the one that has the greatest tendecy to give up protons, the first member of the series will act as an acid towards all the others, while the last member will act exclusively as a base. All the other members of the series possess an amphoteric character, acting as bases towards the preceding members of the series and as acids towards succeeding members. Thus water acts as a base towards all those substances that give the water lattice as a whole an excess of protons, *viz.*, the classical acids, and as an acid towards those that give the water lattice as a whole a dificit of protons, *viz.*, the classical bases. This solvent effect must be general.

If we therefore choose a substance like acetic acid as a solvent for potential acids or bases, we must have the equilibria:

$$CH_3COOH + \text{Base} \rightleftharpoons CH_3COO^- + \text{Base } H^+$$

and

$$HX + CH_3COOH \rightleftharpoons X^- + CH_3COOHH^+$$

Corresponding to the general Brönsted-Lowry scheme:

$$\text{Acid}_1 + \text{Base}_2 \rightleftharpoons \text{Base}_1 + \text{Acid}_2$$

* Present address.

That acetic acid can act as a base appears from the electrometric titrations of Hall and co-workers [1, 2, 3]. With acetic acid as solvent they obtained neutralization curves of bases, which are unable to form salts in aqueous solutions.

According to the theory, however, neutralization curves ought not to be limited to interaction between recognized bases and acids alone; but the »base» may be a substance that is usually termed an acid. The acidic character of the solvent must in that case lie between that of the »base» and the titrating acid.

In the present investigation dichloro acetic acid was chosen as solvent, as the equilibrium

$$2CHCl_2COOH \rightleftharpoons CHCl_2COO^- + CHCl_2COOH_2^+$$

was assumed.

As will be seen from the figure, a substance like phenol gives no typical neutralization curve in acetic acid. In dichloro acetic acid, however, an unmistakable neutralization curve is obtained.

In acetic acid the following cells were measured:

Pt	$C_6Cl_4O_2$ (sat.) $C_6C_4(OH)_2$(sat.) 0.2 *M* $C_6H_5NH_2$ in CH_3COOH LiCl	CH_3COOH LiCl	$C_6Cl_4O_2$(sat.)$_{22}$ $C_6Cl_4(OH)_2$(sat.) CH_3COOH LiCl	Pt
and				
Pt	$C_6Cl_4O_2$(sat.) $C_6Cl_4(OH)_3$(sat.) 0.2 *M* $C_6H_5NH_2$ in CH_3COOH LiCl	CH_3CCOH LiCl	$C_6Cl_4O_2$(sat.) $C_6Cl_4(OH)_2$(sat.) 0.2 *M* C_6H_5OH in CH_3COOH LiCl	Pt

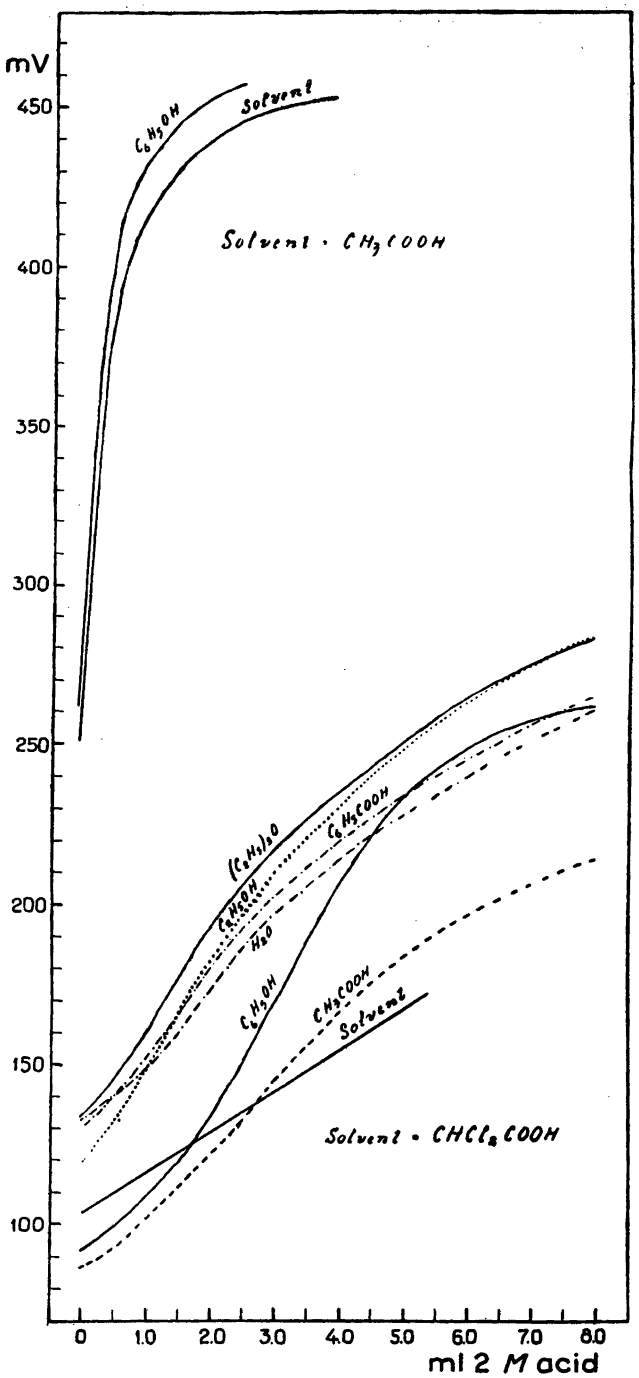
As titrating acid was used 2 *M* $HClO_4$ in CH_3COOH.

In dichloro acetic acid the cells were constituted as follows

Pt	$C_6Cl_4O_2$(sat.) $C_6Cl_4(OH)_2$(sat.) 0.2 *M* $C_6H_5NH_2$ in $CH(_3COOH$ LiCl	$CHCl_2COOH$ LiCl	$C_6Cl_4O_2$(sat.) $C_6Cl_4(OH)_2$(sat.) 0.2 *M* (X,Y.Z) in $CHCl_2COOH$ LiCl	Pt

The readings were taken at room temperature, and the titrating acid was 2 *M* H_2S_4O in $CHCl_2COOH$.

Allthough the substances which have been investigated are usually considered neutral or acid substances, the titration curves shown in the figure


mV
450
400
350
300
250
200
150
100
0
1.0
2.0
3.0
4.0
5.0
6.0
7.0
8.0
ml 2 M acid
C6H5OH
Solvent
Solvent · CH3COOH
(C6H5)2O
C6H5COOH
C6H5OH
H2O
C6H5OH
CH3COOH
Solvent
Solvent · CHCl2COOH

give the typical inflections of neutralization curves. Accordingly they represent the equilibria:

$CHCl_2CCOH + C_6H_5OH \rightleftharpoons CHCl_2COO^- + C_6H_5OH_2^+$
$CHCl_2COOH + CH_3COOH \rightleftharpoons CHCl_2COO^- + CH_3COOH_2^+$
$CHCl_2COOH + C_6H_5COOH \rightleftharpoons CHCl_2COO^- + C_6H_5COOH_2^+$
$CHCl_2COOH + C_2H_5OH \rightleftharpoons CHCl_2COO^- + C_2H_5OH_2^+$
$CHCl_2COOH + (C_2H_5)_2O \rightleftharpoons CHCl_2COO^- + (C_2H_5)_2OH^+$
$CHCl_2COOH + H_2O \rightleftharpoons CHCl_2COO^- + H_2OH^+$

These results demonstrate that even »acids» may act as bases towards more acidic substances.

SUMMARY

No substance is an acid per se; but any given substance may act as an acid towards a substance with less acid strength, or it may act as a base towards a substance with greater tendency to give up protons.

I am indebted to Professor Ölander, Stockholm, for his kind interest and many valuable suggestions and to Dr. Finbak, Oslo, for many interesting discussions on acid-base equilibria.

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Electrophoresis and Ultracentrifugation of Lipid-free Human Serum *

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In an earlier electrophoretic study of sera from which the lipids had been largely removed by precipitation and extraction with organic solvents at low temperature [1], it was *i. a.* found that the migration velocities of the protein components remained unchanged. In the experiments, Tiselius' electrophoretic apparatus was used, employing the *schlieren* method, hence no estimation of the quantitative relations of the different components could be made. It was observed, however, that in the extracted sera the *schlieren* band representing the α-globulin disappeared at pH values below 8. More recently, Zeldis, Alling, McCoord and Kulka [2], using the optical arrangement according to Longsworth, found certain alterations in total electrophoretic area and the relative areas of individual electrophoretic components in the extracted plasmas. Their findings confirmed the observations of Blix, Tiselius and Svensson [3] that the human β-globulin is particularly rich in lipid materials. The present study, which had already been completed when we became aware of the work just mentioned, was undertaken mainly to study the quantitative changes of the protein components brought about by delipidation. — The so called X-protein which is observed on ultracentrifugation of concentrated human sera owes its characteristic properties at least to a great extent to its high content of lipids [4]. Our work also aimed at giving some further elucidation of the question of the relation between the X-protein and other protein components of serum, especially the β-globulin.

* The ultracentrifugations were carried out at the Institute of Physical Chemistry by K. O. P., the rest of the work at the Institute of Medical Chemistry by G. B.

METHODS

Human serum samples were obtained from three normal men and from some patients from the Medical University Clinic in Upsala. Total serum protein was determined both by micro-Kjeldahl analysis and refractometrically with »Eintauch-refractometer». Phospholipid-P of the sera was determined on Bloor extracts using Teorell's method for the phosphorus analysis. Cholesterol was estimated according to the method of Schoenheimer and Sperry [5]. The lipid extraction was performed exactly in the same way as in the earlier work. The extracted sera were also analysed for lipid-P and cholesterol.

Electrophoresis was carried out at + 1° with a potential gradient about 5.5 volt/cm. The optical arrangement was that described by Svensson [6]. All samples were diluted with phosphate buffer of pH 7.68, ionic strength 0.10 plus 0.05 *M* NaCl making the total ionic strength 0.15. They were dialysed against 2 l of the same buffer for two days. In all experiments so much buffer was added to the sera as to make the total protein concentration 1.5 per cent. The electrophoreses were run for 140—145 minutes.

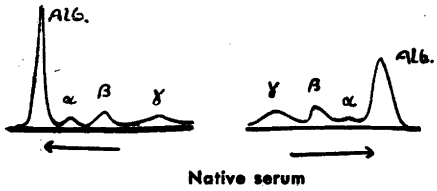
The ultracentrifuge runs were carried out in a Svedberg oilturbine ultracentrifuge at 59 000 R. P. M. and the observations were made with the Lamm scale method. Just before the runs, the sera were diluted with an equal volume of disodium phosphate in order to ensure a suitable separation between the »albumin» an the »X-protein» peak in the sedimentation diagram [4, 7].

EXPERIMENTAL OBSERVATIONS

Figs. 1—5 are reproductions of electrophoretic patterns obtained from some of the samples examined. The quantitative evaluation of these diagrams is beset with considerable errors and only the gross changes observed can be relied upon as real. A large error is encountered in the resolution of the individual peak areas when the separation is incomplete. This is especially the case in some of the extracted sera. The quantitative data obtained are given in Table 1 and 2.

From table 1 it is seen that the total protein, refractometrically determined, regularly decreased after extraction. As the proteins of the extracted sera dissolved completely on resuspension in buffer, this decrease is entirely or almost entirely due to the removal of the lipids. On the whole, both the greatest relative and the greatest absolute loss occurred in the β-globulin (Table 2). The same was found by Zeldis *et al.* in human sera. The reduction is in no case greater than reasonably can be expected from the removal of the lipid part of each fraction. The most marked change in the pattern is usually the

Fig. 1.—5. Electrophoretic patterns before and after extraction of lipids from human serum.



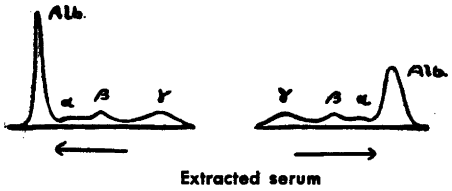
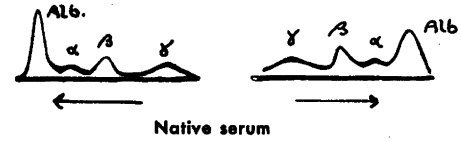


Fig. 1. Normal man (No. 1673/75).



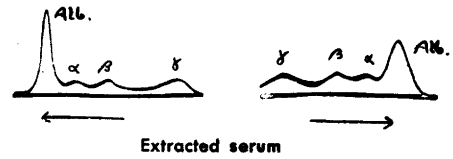


Fig. 2. Case of icterus (No. 1659/61).

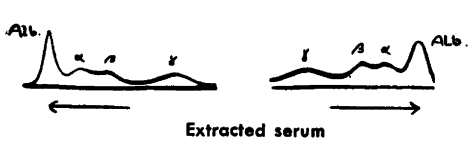
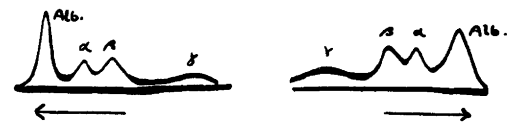


Fig. 3. Case of pregnancy toxemia (No. 1662/64).



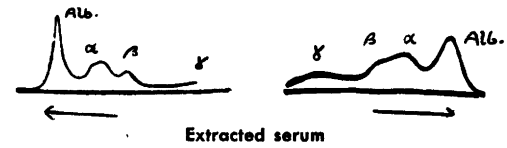
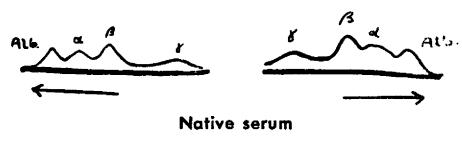


Fig. 4. Case of pregnancy toxemia (No. 1667/71).



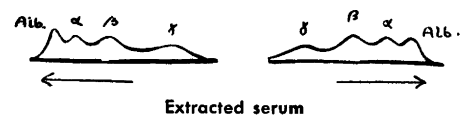


Fig. 5. Case of nephrosis (No. 1669/74).

Table 1. Total protein and lipid content of electrophoretically investigated sera.

		A Total protein Kjeldahl %	B Total protein refract. %	B—A %	Cholesterol + phospholipids * mg %
Normal	Native	7.68	7.75	+ 0.08	320
1653/55	Extracted	7.37	7.40	+ 0.03	42
	Difference	+ 0.31	+ 0.35		
Normal	Native	7.96	7.95	— 0.01	364
1651/54	Extracted	7.66	7.60	— 0.06	53
	Difference	+ 0.30	+ 0.35		
Nephrosis	Native	6.28	6.40	+ 0.12	389
1658/60	Extracted	6.02	6.00	— 0.02	49
	Difference	+ 0.26	+ 0.40		
Icterus	Native	5.79	6.05	+ 0.26	446
1659/61	Extracted	5.68	5.80	+ 0.12	46
	Difference	+ 0.11	+ 0.25		
Normal	Native	7.83	7.95	+ 0.12	450
1673/75	Extracted	7.58	7.35	— 0.23	67
	Difference	+ 0.25	+ 0.60		
Pregnancy	Native	6.63	7.40	+ 0.77	488
toxemia	Extracted	6.64	6.80	+ 0.16	152
1662/64	Difference	+ 0.01	+ 0.60		
Pregnancy	Native	8.11	8.50	+ 0.39	535
toxemia	Extracted	7.87	7.90	+ 0.03	81
1670/72	Difference	+ 0.24	+ 0.60		
Nephrosis	Native	4.55	5.70	+ 1.15	741
1669/74	Extracted	4.32	4.50	+ 0.18	116
	Difference	+ 0.23	+ 1.20		
Pregnancy	Native	5.33	6.05	+ 0.72	765
toxemia	Extracted	5.25	5.50	+ 0.25	127
1667/71	Difference	+ 0.08	+ 0.55		

* The small amounts of lipids remaining in the extracted sera contained no cholesterol.

Table 2. Electrophoretical distribution of α-, β- and γ-globulins in native and extracted sera.

No.	Diagnosis	Serum	Albumin %	α-globulin %	β-globulin %	γ-globulin %
1653/55	Normal	Native	3.79	0.81	1.10	2.05
		Extracted	3.39	0.87	1.05	2.09
		Difference	— 0.40	+ 0.06	— 0.05	+ 0.04
1651/54	Normal	Native	4.09	0.59	0.98	2.28
		Extracted	3.74	0.76	0.94	2.15
		Difference	— 0.35	+ 0.17	— 0.04	— 0.13
1658/60	Nephrosis	Native	1.84	0.65	3.91*	
		Extracted	1.77	0.55	3.69	
		Difference	— 0.07	— 0.10	— 0.22	
1659/61	Icterus	Native	2.46	0.77	1.24	1.59
		Extracted	2.52	0.76	1.08	1.44
		Difference	+ 0.06	— 0.01	— 0.16	— 0.15
1673/75	Normal	Native	4.14	0.60	1.22	1.99
		Extracted	4.05	0.67	1.01	1.62
		Difference	— 0.09	+ 0.07	— 0.21	— 0.37
1662/64	Pregnancy toxemia	Native	2.91	1.31	1.58	1.60
		Extracted	2.38	1.26	1.30	1.85
		Difference	— 0.53	— 0.05	— 0.28	+ 0.25
1670/72	Pregnancy toxemia	Native	— 3.86	0.95	1.58	2.11
		Extracted	— 3.80	0.91	1.29	1.90
		Difference	— 0.08	— 0.04	— 0.29	— 0.21
1669/74	Nephrosis	Native	1.01	1.46	1.68	1.55
		Extracted	0.89	0.95	1.48	1.18
		Difference	— 0.12	— 0.51	— 0.20	— 0.37
1667/71	Pregnancy toxemia	Native	2.27	1.06	1.38	1.34
		Extracted	2.02	1.69	0.70	1.09
		Difference	— 0.25	+ 0.63	— 0.68	— 0.25
Difference, mean			— 0.19	+ 0.03	— 0.24	— 0.15
Decrease in per cent of initial value, average			— 7.2 %	+ 5.3 %	— 15.9 %	— 8.6%

* Hardly any separation between β- and γ-globulin.

decrease in height of the peaks at the same time as the separation becomes incomplete. This means that each component after the extraction appears less uniform than before. Whether or not this is due to an incipient denaturation or to a breaking up of more uniform lipid-protein complexes cannot be decided (the same change is seen in the experiments of Zeldis *et al.* but is not so marked in them as in our cases). The apparent disappearance of the α-component in the earlier study did obviously not depend on an inclusion in some other fraction but on the flattening of the peak.

The figures given in Table 1 show some further facts of interest. The difference in total protein calculated on the Kjeldahl-values before and after extraction are due to removal of the main part of the non-protein nitrogen in the extraction. As could be expected this difference does not vary with the lipid content of the native serum. The Kjeldahl-values and the refractometric values for total protein do not differ considerably in unextracted sera with

Table 3. Relative amounts of components according to ultracentrifuge and electrophoresis experiments.

	Ultracentrifugal distribution				Electrophoretic distribution		
	Total protein concentration	Component	Per cent of total protein		Component	Per cent of total protein	
			Native	Extracted		Native	Extracted
Normal 1673/75	4.0	Albumin	42	88	Albumin	52	55
		Globulin	12	11	α-glob.	8	9
		X-protein	45	0	β-glob.	15	14
		»20-comp.»	1	1	γ-glob.	25	22
Pregnancy toxemia 1670/72	4.3	Albumin	40	86	Albumin	44	48
		Globulin	11	12	α-glob.	11	12
		X-protein	46	0	β-glob.	19	16
		»20-comp.»	3	2	γ-glob.	25	24
	2.8	Albumin	83				
		Globulin	15				
		X-protein	0				
		»20-comp.»	2				
Pregnancy toxemia 1667/71	3.0	Albumin	72	80	Albumin	38	37
		Globulin	11	14	α-glob.	18	31
		X-protein	11	0	β-glob.	23	13
		»20-comp.»	6	6	γ-glob.	22	20

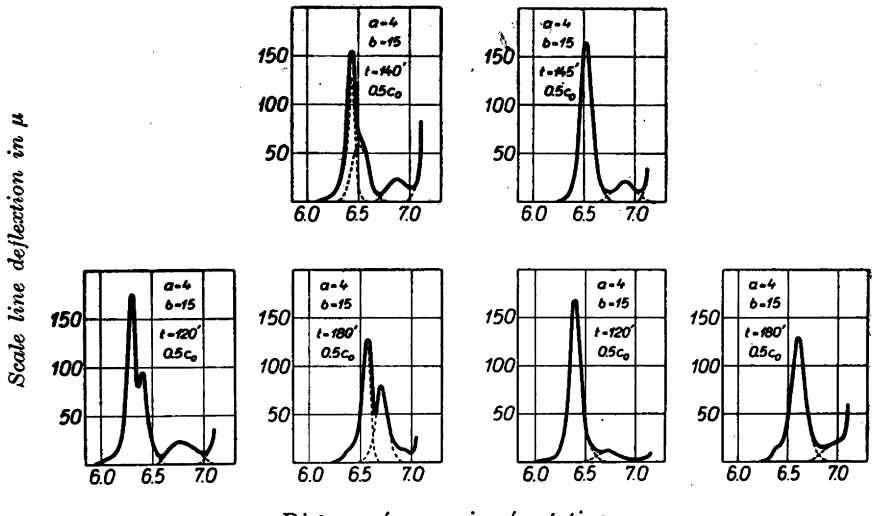


Fig 6. Sedimentation diagrams of human sera before (left hand diagrams) and after delipidation (right hand diagrams).
Upper row: Normal serum (1673/75).
Lower row: Pregnancy toxemia serum (1670/72). The slower sedimenting »X-component» is present in the native samples, but have disappeared from the extracted ones.

normal content of lipids. In hyperlipemic cases the difference increases. The decrease of the refractometric value for total protein on extraction bears also an obvious relation to the lipid content.

Fig. 6 shows the sedimentation diagrams from some of the sera before and after extraction of the lipids. It is quite evident from the diagrams that great changes take place in the sedimentation behaviour of the sera after delipidation. The values calculated for the various components from the diagrams are given in Table 3. The separation of the »X-component» and the »albumin» peak is not sufficiently good, however, to allow an accurate estimation of the amount of the two individual components, but only of their sum. The concentration of the slower sedimenting components will also come out somewhat too high, whereas that for the more rapid sedimenting ones comes out too low [8]. From Table 3 it appears that the »X-component» after extraction disappears into the »albumin» peak, whereas the γ-globulin and the »20-component» show insignificant changes only.

As one of us (K. O. P.) has earlier shown [4, 7] the X-protein, appearing in undiluted or slightly diluted sera, has an s_{20} which varies with the density of

the solution. On dilution the X-protein disappears in the albumin peak of the ultracentrifugal pattern. A mixture of electrophoretically isolated β- and γ-globulin showed in addition to a globulin peak also a component with the characteristics of the X-protein. As pure γ-globulin contained no X-protein, it was concluded that the X-protein was a β-globulin. In a recent work, Oncley, Scatchard and Brown [9] by low temperature ethanol fractionation isolated a β-globulin, designated as a β-lipo-protein which contained about 75 per cent lipids and had the properties of the X-protein. It is evident from their work that only a part of the β-globulin, *viz.* its lipo-protein part, in an isolated state behaves as X-protein. This lipo-protein seems to form only a few per cent of the total serum protein,* whereas the X-protein might amount to 50 per cent or more in concentrated sera. Obviously therefore the X-protein observed in the latter case must in the main be composed of other fractions than the β_1-lipo-protein. It has, indeed, been found earlier [4, 7] that on increasing the total protein concentration of serum solutions the appearance of the X-protein was accompanied by a decrease of the albumin and the globulin peaks. Furthermore, the X-protein, isolated by ultracentrifugation and purified by resuspension and respinning in the centrifuge, split at a suitable density of the solution into two peaks, the one of which sedimented as albumin and the other at a slower rate. Moreover, it was observed that when γ-globulin prepared by salt precipitation was added to a fresh human serum, the X-protein increased, whereas the globulin peak was augmented less than expected. From these observations it was concluded that the X-protein appearing in concentrated sera is a complex which in addition to β-globulin contains considerable amounts of albumin and globulin [4].

The results from the present work give no definite indication that part of the ordinary serum globulin with $s_{20} = 7$ S should enter into the ultracentrifugal X-component. It suggests, therefore, that the X-component of concentrated serum solutions might be a lipo-protein-albumin complex. On the other hand, Petermann [10] has found that when both isolated X-protein and whole serum containing X-component are treated with a lecithinase (from *Clostridium perfringens*), the X-component disappears from the sedimentation diagrams. At the same time the amounts of both the albumin and the glo-

* From the data published by the Harvard group until now, it appears that only 70—80 per cent of the plasma proteins may be accounted for by the products so far isolated by the ethanol technique. As, however, the lipid content of the isolated lipoproteins only amounts to one third of the total lipid content in normal human serum, or even less, it seems reasonable to assume that a considerable part of the remaining 20—30 per cent of the plasma proteins may consist of lipoproteins.

bulin showed a considerable increase. The electrophoretic pattern from isolated X-protein showed a very pronounced decrease for β_1-globulin after treatment with lecithinase, whereas those from whole serum showed only small changes.

It is of course possible that in addition to the β_1-lipo-protein, other serum lipo-proteins enter into the X-component also, *e. g.* the α_1-lipo-protein isolated by the Harvard group and other lipo-proteins not yet isolated.

As there has been some confusion regarding the relation between β-globulin and the X-protein, it must be emphasized that although a certain fraction of the β-globulin in the ultracentrifuge behaves like an X-protein, the total electrophoretic β-globulin should not be identified with the latter. Furthermore, it is evident from the present experiments that the main part of the electrophoretic β-globulin after delipidation retains its original electrophoretic mobility and does not become included in some of the other components. Finally, it must be concluded that the elctrophoretical β-globulin is not a complex of albumin and γ-globulin with lipids, although the X-protein isolated from *concentrated* sera besides the β-lipo-protein contains as well albumin as γ-globulin.

Many of the apparent contradictory results obtained from electrophoresis and sedimentation are evidently due to the fact that serum contains many more components than is generally recognized at present. Several of them have about the same sedimentation constants, but may have different electrophoretic velocities. On the other hand, some of the components show practically the same electrophoretic migration but give rise to several components in the ultracentrifuge.

SUMMARY

Extractions of most of the lipids of serum bring about certain alterations of the electrophoretic pattern, but no component is reduced more than could be reasonably expected from the removal of its lipid part. After delipidation the X-protein of serum entirely disappears; at the same time the albumin increases. The disappearance of the X-protein is accompanied by a corresponding increase of the albumin, whereas the globulin and the »20-component» remained practically unchanged.

We wish to thank Professor Svedberg for the facilities put at our disposal for the ultracentrifugal part of the investigation. The expenses for this part were defrayed from a grant from the Rockefeller Foundation.

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The Molecular Structure of *Cis* and *Trans* Cyclohexanediol-(1,2)

An Electron Diffraction Investigation by the Sector Method

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In previous investigations of the two isomers of cyclohexanediol-(1,2) with m.p. 99° and 104° C the structures have usually been discussed on the assumption that they are *cis/trans* isomers on the basis of a plane six-membered ring. These investigations concerned optical activity and the demonstration of different chemical reactions. The result was that the higher melting form was the *trans*-, and the other the *cis*-compound.

We now know that the cyclohexane ring is not plane. It is therefore necessary to discuss the problem from a somewhat different point of view. As early as 1930 this was pointed out by White [1] who avoided the designations *cis* and *trans*, and introduced Greek letters to denote the two modifications. In this investigation we will use the nomenclature introduced by Hassel for the two types of bonds in the step-formed six-membered ring, ε-bonds for the six bonds from the C-atoms which are parallel to the principal axis of the carbon skeleton, and $\varkappa$-bonds for the six bonds of the other type. In this article the two diols will be referred to by their melting points. The terms *cis* and *trans* will not be used at all.

CHEMICAL PREPARATION

Cyclohexanediol-(1,2) m. p. 104° was prepared by Brunel's method [2]. 1,2-epoxycyclohexane was purified by distillation and heated with water in a sealed glass tube. The diol was purified by recrystallisation from benzene. It is worth taking the trouble to purify the epoxide carefully by distillation during the preparation. By so doing the pure diol is obtained by heating with water.

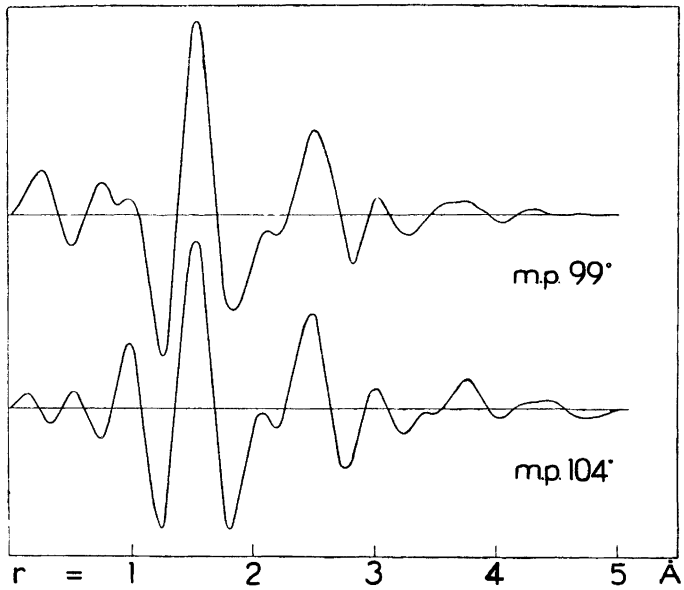


Fig. 1. $\sigma(r)/r$-curves of cyclohexanediol-(1,2) of m. p. 99° C and cyclohexanediol-(1,2) of m. p. 104° C (below).

Cyclohexanediol-(1,2) m. p. 99° was first prepared by Brunel's method [2] by treating 1-iodo-cyclohexane-acetate-2, $C_6H_{10}I.OA$, with silver acetate in glacial acetic acid. Contrary to Brunel the diacetate of cyclohexane was not hydrolised by potassium carbonate. The hydrolysis went much better with hydrochloric acid in absolute methyle alcohol. An attempt to prepare the diol by Markownikow's method [3] by oxidation of cyclohexane with potassium permanganate was very satisfactory, and the greater part of the diol was prepared in this way*.

As a check equal quantities of the two diols were mixed together, giving the equimolecular compound with m. p. 73°.

ELECTRON DIFFRACTION DIAGRAMS AND DETERMINATION OF THE STRUCTURE

Several different electron diffraction diagrams were taken, using the rotating sector method. The microphotometer records revealed intensity curves in close agreement. The Fourier Analysis was based on a mean curve. The evaluated $\sigma(r)/r$-curves are shown in Fig. 1.

* Markownikow's method was slightly modified. The aqueous solution containing potassium carbonate and the diol was heated over the water bath until traces of crystals appeared and was then cooled. When left over night the greater part of the liquid disappeared. The residue was extracted with boiling benzene.

From the chemical preparation of the two diols, we may presume that the two hydroxyl-groups in both isomers are in 1,2-position in relation to the six-membered ring. A number of investigations of cyclohexane and derivates have been carried out by Hassel and his collaborators, proving that the most stable form of the six-membered ring under normal conditions is the step-form. It may especially be mentioned that the investigation of the molecular structure of 1,2-dibrom-cyclohexane [4] reveals the step-formed six-membered ring.

From this starting point we may form four possible structures for 1,2-cyclohexanediol. These are characterized by the connecting of the hydroxyl-groups to the sixmembered ring by the following combinations of the different types of bonds: $\varkappa,\varkappa$, $\varkappa,\varepsilon$, $\varepsilon,\varkappa$, ε,ε. If we take into consideration the ability of the sixmembered ring to transpose, thereby transforming all $\varkappa$-bonds into ε-bonds and vice versa, then only two chemically separable forms are left:

1. $\varkappa,\varepsilon$ which when transposed takes the $\varepsilon,\varkappa$-form, which is the optical antipode of the first one.

2. $\varkappa,\varkappa$ which when transposed takes the ε,ε-form. In this case two separable optically active forms are obtained.

In case 2 we have an equilibrium between two different forms of the molecule. The behaviour of the compound as one single substance is conditional on the equilibrium between the two forms being established so quickly that no chemical separation is possible. These circumstances are more closely discussed in a number of papers [5].

When adjusting these two models to the two diols by use of electron diffraction, the deciding factor is the distances between the O-atoms and the C-atoms and between the two O-atoms. In order to obtain these distances an experimentally determined $\sigma(r)/r$-curve for cyclohexane was subtracted from the experimental $\sigma(r)/r$-curves of the two diols. The two difference-curves produced are shown in Fig. 2.

The distances most conducive to the formation of these curves are the above mentioned C—O and O—O distances. It must then be possible to construct the two difference curves by adding together normal curves for all the appropriate single distances [6]. These distances will vary according to whether the O-atom under consideration is in $\varkappa$- or ε-position. The problem is to find out the contribution of $\varkappa$- and ε-distances to the two curves. It may be mentioned that if the equilibrium in model 2. consists of equal parts of $\varkappa,\varkappa$- and ε,ε-forms, the whole difference between the two experimental curves will be due to the O—O distances (not taking into account the distances between the H-atom in the hydroxylgroups and the remaining atoms).

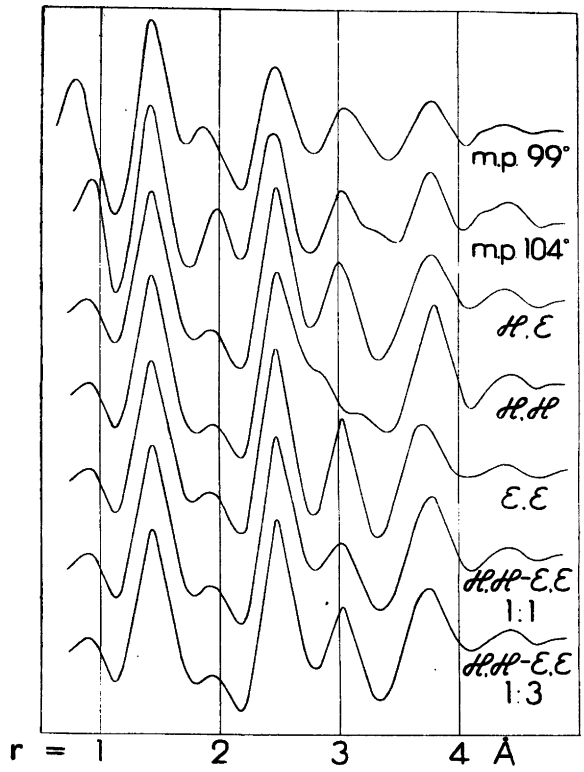


Fig. 2. Experimental difference $\sigma(r)/r$-curves of cyclohexanediol-(1,2) of m. p. 99° and 104° C (above). Below these two corresponding theoretical curves for molecular models with the two O-atoms in κ,ε, κ,κ and ε,ε-positions. The two last are theoretical curves corresponding to the equilibrium mixtures of the κ,κ- and ε,ε-models in proportions 1 : 1 and 1 : 3.

To simplify the following discussion the C-atoms are numbered as shown in Fig. 3.

In the difference curves of Fig. 2 the distinct maxima at 1.43 and 2.47 Å must correspond to the intermolecular distance O—C_1 and O—C_2. From the

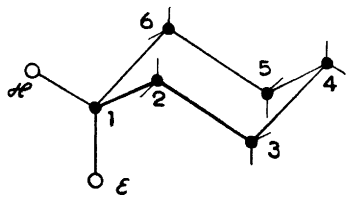


Fig. 3. Numeration of the C-atoms in cyclohexanediol-(1,2).

$\sigma(r)/r$-curves shown in Fig. 1 we may assume the neighbouring C—C-distances to have the usual value of 1.54 Å. Applying this to the sixmembered ring we may calculate all the other distances between the atoms in the possible models of the molecule. In Table 1 the O—C-distances which correspond to the O-atom in $\varkappa$-position and ε-position and the O—O-distances in the different cases are tabulated. Using the experimental values for the distances O—C_1, O—C_2 and C—C given above, calculation of the angle C—C—O gives 112.5°. The angle is a little greater than the tetrahedral value (109.5°) which is quite reasonable when the effects of the Van der Waals forces between the atoms in the molecule are taken into consideration [7].

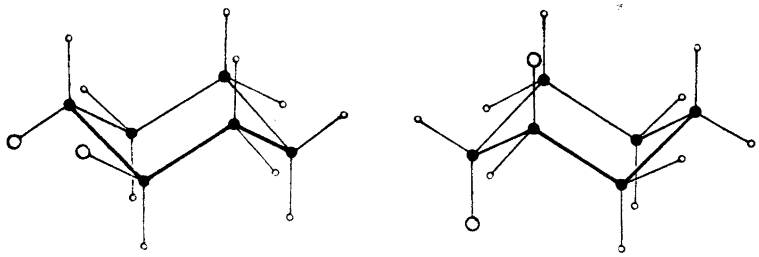
Table 1. Situation of the maxima of the difference $\sigma(r)/r$-curves in Ångstrøm units and the most important corresponding theoretical internuclear distances.

Diol m. p. 99° exp. maxima	Diol m. p. 104° exp. maxima	Theoretical distances		
1.43	1.43	O$\varkappa$—C_1 = 1.43	Oε—C_1 = 1.43	
1.87	1.99	O$\varkappa$—H_1 = 1.98	Oε—H_1 = 1.98	
2.47	2.46	O$\varkappa$—$C_{2,6}$ = 2.47	Oε—$C_{2,6}$ = 2.47	O$\varkappa$—O$\varkappa$ = 2.85
3.06	3.03		Oε—$C_{3,5}$ = 3.03	O$\varkappa$—Oε = 2.96
3.78	3.77	O$\varkappa$—$C_{3,5}$ = 3.85	Oε—C_4 = 3.64	Oε—Oε = 3.73
4.40	4.43	O$\varkappa$—C_4 = 4.31		

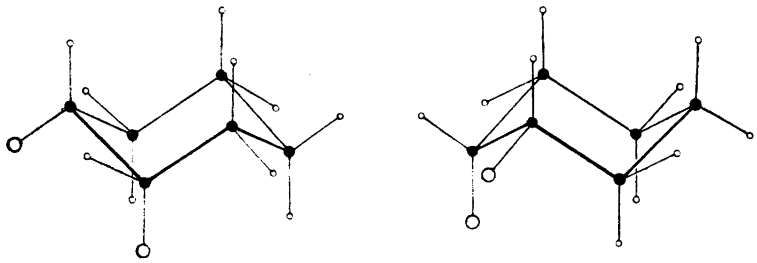
In Table 1 are also shown the r-values corresponding to the maxima of the two difference curves in Fig. 2. It is easy to correlate the experimental maxima to the distances in our molecular models. This confirms our supposition that the sixmembered ring is step-formed and each O-atom placed in either $\varkappa$- or ε-position. It is also observed that all the theoretical maxima are present, but at different heights in both the experimental curves. This means that we may expect the two diols to contain O-atoms in both the $\varkappa$- and ε-position.

In Fig. 2 are shown the theoretical difference $\sigma(r)/r$-curves for the $\varkappa,\varepsilon$-model and mixtures of different composition of the $\varkappa,\varkappa$- and ε,ε-models. These theoretical curves are constructed by using normal curves for the single internuclear distances [6]. It is evident that the model with both the O-atoms in $\varkappa$-position may be excluded. The following two possible structures of the diols are then left: 1) the O-atoms are placed in $\varkappa,\varepsilon$-position, 2) there is an equilibrium between the two models with the O-atoms in $\varkappa,\varkappa$-position and in ε,ε-position respectively, *e. g.* the two models which are transformed into each other by a transposition of the sixmembered ring. In case 2) the ε,ε-form must predominate in the equilibrium mixture.

It is difficult to correlate with any degree of certainty the two experimental difference curves in Fig. 2 and our theoretical curves. Judging from the curves



κ, κ ⇌ ε, ε (m. p. 104°)



κ, ε ⇌ ε, κ (m. p. 99°)

Fig. 4. Models of the two cyclohexanediols-(1,2) of m. p. 99° and 104° C.

it seems most reasonable that the κ,ε-model corresponds to the diol with m.p. 99°. We must bear in mind, however, that the discussion is based on difference $\sigma(r)/r$-curves and the reliability cannot be compared with that of ordinary $\sigma(r)/r$-curves. Small differences in the two curves cannot be given the same significance as in the case of ordinary $\sigma(r)/r$-curves.

A final conclusion, as to which of the two diols corresponds to the κ,ε-model and which corresponds to the κ,κ, ε,ε-mixture, must be based on the fact that the separation into optically active antipodes can be achieved in the case of the diol with m.p. 104° but not in the case of the other diol. (8). The κ,ε-model will consist of two optically active components in equal proportions. The transposing of the sixmembered ring will transform the d-form into the l-form and vice versa. Consequently the separation of the κ,ε-model into optically active antipodes will be impossible. In the case of the κ,κ, ε,ε-model, however, the conversion of the κ,κ-form into the ε,ε-form will not lead to racemisation. Thus the diol of m.p. 99° is the κ,ε-compound and the diol of m.p. 104° is the equilibrium mixture between the κ,κ- and ε,ε-forms. Models of the molecules are shown in Fig. 4.

SUMMARY

An electron diffraction investigation of the vapours of the two forms of cyclohexanediol-(1,2) using the rotating sector method shows that the molecular structures of both substances are based on the symmetrical stepformed sixmembered cyclohexane ring. The diagrams indicate that one of the substances is the $\varkappa,\varepsilon$-form, the other an equilibrium mixture of $\varkappa,\varkappa$- and ε,ε-forms. Taking the fact into consideration that the substance of m.p. 104° is separable into optically active antipodes, the other substance unseparable, it follows that the substance of m.p. 99° is the $\varkappa,\varepsilon$-form and the substance of m.p. 104° is the equilibrium mixture between the $\varkappa,\varkappa$- and ε,ε-forms. Models of the molecules are shown in Fig. 4.

Finally I wish to express my gratitude to Prof. Dr. O. Hassel of the University of Oslo, for giving me the opportunity to carry out the present work in his laboratory and for his helpful criticism and advice.

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Preliminary Communication

Bases, Antibases and Antibase Complexes

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J. N. Brønsted has defined a base as a molecular species, which can combine with a proton to yield the corresponding acid: $B + H^+ \rightleftharpoons BH^+$. The present author agrees, that it hereby has been unequivocally established what should be understood by an acid and a base. However, as pointed out by G. N. Lewis and others, numerous other reactions in chemistry show similarities with acid-base equilibria, and bases may well react *according to the above equation* with many other agents than the proton: so *e. g.* with acid anhydrides, free unsolvated metal ions or unsaturated coordination compounds of any kind.

The author proposes to denote such agents in general as *antibases* and the combination products of base and antibase as *antibase complexes*. We are then immediately led to the fundamental equation:

B	+	A	$\rightleftharpoons$	AB
base		antibase		antibase complex

Depending upon the character of the antibase the AB-complexes are either acids = proton complexes, acid anhydride complexes *e. g.* $CO_3^{2-} = CO_2 + O^{2-}$, or coordination complexes as $HgCl_2$, $Ag(NH_3)^+$, $Ag(NH_3)_2^+$, BF_4^- etc.

G. N. Lewis has developed an electronic theory of acids and bases in which he employs the name base in much the same sense as it is used by Brønsted, and the name acid in a sense close to the here introduced term antibase. But this appears to be an unfortunate choice and not logically correct, for if an extension of the term acid is desired, the antibases *eo ipso* can never be acids, and it is only the reaction products of base and antibase, which show analogy to the ordinary acids.

Just as the proton many other antibases cannot exist in solution without being bound to the solvent, and consequently it is then not possible to measure the absolute stability of the AB-complex, but only the stability relative to the solvated antibase. However, other antibases, *e. g.* CO_2 and BF_3, can exist in a free state in solution or in the vapour phase, and the antibase-base equilibrium constant:

$$k_{A,B} = \frac{a_{AB}}{a_A \cdot a_B}$$

therefore in many cases gives an absolute measure of the stability of the antibase complex. A more detailed analysis of the terms introduced here will follow.

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Studien über Persulfocyansäure

III.* Über die Umlagerung von Persulfocyansäure

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Die Persulfocyansäure $H_2S_3C_2N_2$ ist als Dimercapto-thiodiazol [ab$_1$] zu formulieren. Die Strukturformel wurde von Klason [1] aufgestellt und ist durch spätere Arbeiten namentlich von Hantzsch und Wolvekamp [2] erhärtet worden. Seit den Arbeiten der genannten Forscher galt es als festgestellt, die Säure sei nicht als Substanz isolierbar und zwar wegen ihrer schnellen Umwandlung in den isomeren, nicht sauren Xanthanwasserstoff. Wenn nicht zu verdünnte Lösungen eines Persulfocyanats mit Salzsäure versetzt werden, entstehen Niederschläge, die nach der Meinung von Klason und Hantzsch lediglich aus Xanthanwasserstoff bestehen. Hingegen konnten Lösungen der Säure durch Ausschütteln eisgekühlter, angesäuerter Persulfocyanatlösungen mit Äther dargestellt werden. Diese Lösungen sind aber nicht stabil, sondern setzen bei Stehen Kristalle von Xanthanwasserstoff ab (Klason).

Es ist zu bemerken, dass weder von Klason noch von Hantzsch und Wolvekamp eine nähere Untersuchung der bei Ansäuern von Persulfocyanaten entstehenden Niederschläge vorgenommen wurde, was um so merkwürdiger erscheint, als der eine der Isomeren sauer ist, der andere aber nicht, und eine Separierung deshalb keine allzu grossen Schwierigkeiten darbieten konnte.

Als es mir gelungen war, Bariumpersulfocyanat von grosser Reinheit darzustellen [3], habe ich die Frage von den Existenzbedingungen der Persulfocyansäure aufgenommen.

Es zeigte sich dabei sogleich, dass die Vorstellungen von Klason und Hantzsch über die Unbeständigkeit der Säure stark übertrieben waren.

Wird eine nicht zu verdünnte, nullgradige Lösung von reinem, farblosem $BaS_3C_2N_2$ mit Salzsäure versetzt, so ist der entstehende feinkristallinische Niederschlag von rein weisser Farbe. Er ist ferner im Überschuss von Persul-

* 1. Mitt. *Svensk Kem. Tid.* 56 (1944) 207; 2. Mitt. ebenda 57 (1945) 62.

focyanat löslich, ebenso in Kaliumbicarbonat unter Kohlensäureentwicklung, Eigenschaften, die dem Xanthanwasserstoff nicht zukommen.

Der weisse Niederschlag kann sogar mit eiskaltem Wasser auf einem Glasfilter unter Umrühren ausgewaschen werden, ohne dass der Gehalt an Xanthanwasserstoff einen grösseren Betrag als einen Bruchteil eines Prozents erreicht, und nach Trocknen über Nacht im Vakuum über Schwefelsäure ist er nur auf einige Prozente gestiegen. Auch nach mehrwöchentlichem Aufbewahren des trocknen Präparats in Luft besteht der grösste Teil aus Persulfocyansäure, was durch Digerieren mit Kaliumbicarbonatlösung und Wägen des unlöslichen Rückstands festgestellt wurde.

Der beim Ansäuern reiner Persulfocyanatlösungen bei 0° entstehende Niederschlag besteht also entgegen den Behauptungen von Klason und Hantzsch nicht aus Xanthanwasserstoff, sondern aus dem Isomeren, Persulfocyansäure. Diese Säure lässt sich damit, wenn auch in etwas verunreinigtem Zustande, isolieren, und ihre Eigenschaften können näher untersucht werden. So wurde u. a. die Löslichkeit im Wasser ermittelt. Einer solchen Bestimmung musste jedoch eine Messung der Löslichkeit des Xanthanwasserstoffs in Wasser vorangehen.

Reiner Xanthanwasserstoff wurde aus reinstem Bariumpersulfocyanat-Pentahydrat folgendermassen hergestellt. Zu einer etwa halbmolaren Lösung wurde eine äquivalente Menge 2 *N* Salzsäure gesetzt. Nach zweitägigem Stehen des Reaktionsgemisches unter häufigem Umrühren bei Zimmertemperatur wurde der blassgelbe, aus verfilzten Nadeln bestehende Niederschlag auf ein Glasfilter G 3 genommen, mit Wasser vollständig ausgewaschen und dann im Vakuum über Schwefelsäure getrocknet. Schwefelanalyse nach Carius: 63,71 st. 64,03 % S. 5 g eines solchen Präparats wurden mit 300 ml Wasser bei 20° ± 0,02 während 1,5 Stunden turbiniert, die Lösung durch ein grösseres Glasfilter G 4 filtriert und von der Lösung ein Volumen von 250 ml genau abgemessen. Zwei solche Volumina wurden dann zusammen in einer gewogenen Platinschale auf dem Wasserbade eingetrocknet und nach kurzem Aufbewahren im Vakuum gewogen. Drei solche Versuche lieferten die Zahlen 0,0052, 0,0052 und 0,0053 g in 100 ml Lösung. Als Löslichkeit des Xanthanwasserstoffs in Wasser von 20° wurde 0,0052 g in 100 ml angenommen*.

Für die Ermittlung der Löslichkeit von Persulfocyansäure wurde zunächst die Säure wie oben beschrieben dargestellt und auf einem Glasfilter G 3 mit eiskaltem Wasser ausgewaschen. Dann wurde die feuchte Substanz mit etwa 200 ml auf 20° temperiertes Wasser im Thermostat bei 20° ± 0,02 während einer Stunde lebhaft turbiniert. Die Lösung wurde dann sofort durch ein grösseres Glasfilter G 3 schnell filtriert, die zweite Dezimale des Gewichtes genau bestimmt und dann in einer Porzellanschale auf dem Wasserbade eingetrocknet. Der aus Xanthanwasserstoff bestehende gelbe Rückstand wurde dann mit einer bei 20° gesättigten Xanthanwasserstofflösung in einen gewogenen Glasfiltertiegel G 4 gebracht, einige Mal mit dieser Lösung gewaschen und dann im Vakuum

* Nach Klason [4] löst sich Xanthanwasserstoff in Wasser, Alkohol und Äther etwa im Verhältnis 1 : 400. Diese Löslichkeit ist für Wasser etwa 50 mal zu gross, stimmt aber gut mit der für Persulfocyansäure geltenden überein (s. u.).

über konz. Schwefelsäure getrocknet und gewogen. Die Menge des Lösungsmittels wurde aus dem Gewicht der Lösung nach Abzug des Gewichtes des Eintrockenrückstands berechnet und ferner eine Korrektion für den gelösten Xanthanwasserstoff angebracht. Es wurden Versuche mit stark variierenden Mengen von Persulfocyansäure ausgeführt.

Tab. 1 enthält die Resultate. In der ersten Kolumne sind die ungefähren Mengen von Säure in 100 ml Wasser eingeführt, in der zweiten die entsprechenden Löslichkeiten L in Molarität. Es geht aus der Tabelle hervor, dass die Löslichkeit einen schwachen Gang aufweist. Als Löslichkeit der Persulfocyan in säure in Wasser von 20° kann rund 0,26 g in 100 ml angenommen werden.

Es sind auch Messungen der Löslichkeit in stark verdünnter Salzsäure ausgeführt worden, die erhaltenen Werte sind in der vierten Kolumne der Tabelle eingetragen, die entsprechenden Molaritäten der Salzsäure in der dritten. Da bei diesen Löslichkeitsbestimmungen immer von feuchter Persulfocyansäure ausgegangen wurde, war es für die genaue Ermittlung der Chlorwasserstoffkonzentration notwendig, von einer gewogenen Menge Persulfosäure auszugehen und die dieser entsprechende Menge Trockensubstanz als Xanthanwasserstoff zu bestimmen.*

Tab. 1. Löslichkeit von Persulfocyansäure. $t = 20° \pm 0{,}02$.

	L	C_{HCl}	L	K'_1
4	0,01740	0,05876	0,00675	$4{,}76 \cdot 10^{-2}$
4	0,01728	0,04873	0,00730	4,79 »
3	0,01714	0,01988	0,01066	4,81 »
3	0,01727	0,00989	0,01319	4,74 »
2	0,01717			
2	0,01717	C_{NaAc}		
1	0,01696	0,01003	0,02317	4,77 »
1	0,01700	0,01001	0,02315	4,77 »
		0,0200	0,03070	4,93 »
		0,02004	0,03072	4,91 »
		$C_{[bb]}$		
		0,00998	0,01325	
		0,01001	0,01305	
		0,01997	0,01082	
		0,01997	0,01081	

* Diese betrug bei allen Versuchen ungefähr 2 g, und die Volumina variierten zwischen 125 und 130 ml.

Aus den Löslichkeiten in Salzsäure wurde nach bekannten Formeln eine Berechnung der primären Dissoziationskonstanten (Konzentrationskonstante) der Säure durchgeführt unter der Annahme, dass die sekundäre Dissoziation vernachlässigt werden kann *. Die K_1'-Werte sind in der fünften Kolumne der Tabelle eingeführt. Ähnliche Versuche sind mit variierennen Mengen von Persulfocyansäure-ausgeführt worden. Die aus Versuchsreihen mit je 3, 4 und 5 g Säure (Trochensubstanz) berechneten K_1'-Werte differierten maximal um 6 % von denjenigen der Tabelle 1. Ein gang der Konstantenwerte wurde nicht beobachtet. Zwecks Kontrolle wurde die Löslichkeit in 0,01 und 0,02 *M* Natriumacetatlösungen bestimmt und daraus unter Benutzung der aus den Löslichkeiten in Salzsäure berechneten Konzentration der undissoziierten Persulfocyansäure in gesättigter Lösung 0.0038 *M* K_1' berechnet. Als Dissoziationskonstante der Essigsäure wurde dabei der Wert $1{,}85 \cdot 10^{-5}$ verwendet.

Die Übereinstimmung der Werte beider Versuchsreihen ist gut. Die Persulfocyansäure ist demnach als eine *mittelstarke* Säure zu betrachten.

In der Tabelle sind auch Löslichkeiten der Persulfocyansäure in Lösungen, die in Bezug auf die isomere [bb_1]-Säure 0,01 und 0,02 *M* sind, eingetragen. Die Depressionen durch die [bb_1]-Säure unterscheiden sich nicht wesentlich von denen durch Salzsäure von entsprechender Konzentration, weshalb die [bb_1]-Säure als eine starke Säure hervorsteht, die bei den fraglichen Verdünnungen nur zu wenigen Prozenten undissoziiert ist.

In Methanol und Äthanol ist die Löslichkeit der Persulfocyansäure wesentlich grösser als in Wasser, es lösen sich mehr als 3 g in 100 ml bei Zimmertemperatur. Genaue Messungen sind wegen der schnellen Umlagerung in so konzentrierter Lösung schwierig. Auch Aceton und Äther nehmen bedeutend grössere Mengen der Säure auf als Wasser.

Das soeben geschilderte Verfahren zur Bestimmung der Löslichkeit von Persulfocyansäure in Wasser stellt eine Methode zur quantitativen Bestimmung der Säure als Xanthanwasserstoff dar. Da eine solche auch für andere Zwecke erforderlich ist, sind einige Kontrollversuche über die Zuverlässlichkeit ausgeführt worden.

0,01 Mol $BaS_3C_2N_2.5H_2O$ (reinstes Präparat) wurde in je 200, 300 und 400 ml Wasser gelöst und die Lösungen mit etwas mehr als der äquivalenten Menge 2 *M* Salzsäure versetzt. Nach zweitägigem Stehen unter Umrühren wurde der gelbe Niederschlag von Xanthanwasserstoff auf ein gewogenes Glasfilter G 4 gebracht, viermal unter Umrühren mit Wasser ausgewaschen und dann in Vakuum über konz. H_2SO_4 getrocknet und gewogen. Die Mutterlauge wurde in einer Porzellanschale auf dem Wasserbade eingetrock-

* Die sekundären Alkalisatze reagieren gegen Phenolftalein alkalisch.

net, der Rückstand mit bei 20° gesättigter Xanthanwasserstofflösung digeriert und ebenfalls auf einem Glasfilter G 4 gesammelt, mit Xanthanwasserstofflösung gewaschen, getrocknet und gewogen. Es wurde immer ein kleines Deficit an Xanthanwasserstoff gefunden, in keinem Falle aber grösser als 0,4 %. Das Verfahren kann deshalb als brauchbar betrachtet werden, wenigstens dann, wenn es sich nicht um allzu grosse Genauigkeit handelt.

Das Einengen der Lösung auf dem Wasserbade kann ausgelassen werden, wenn zu der Lösung nach Ausfällen der Persulfocyansäure etwa 0,01 Mol [bb_1]-Säure pro 100 ml gesetzt wird. Wie unten näher entwickelt wird, katalysiert diese Säure die Umlagerung von Persulfocyansäure, wodurch diese praktisch quantitativ als Xanthanwasserstoff ausgefällt wird. Eine Korrektion ist für den gelösten Xanthanwasserstoff anzubringen. Die Genauigkeit ist ungefähr dieselbe wie beim ersten Verfahren.

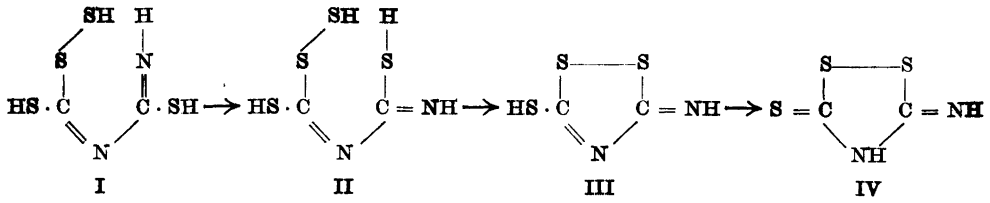
Die Umlagerung von Persulfocyansäure in Xanthanwasserstoff wird durch verschiedene Stoffe, wie schweflige Säure und Schwefelwasserstoff, katalysiert. Von besonderem Interesse ist, dass, wie schon genannt, die mit Persulfocyansäure isomere [bb_1]-Säure katalysierend wirkt, namentlich in salzsaurer Lösung.

Wenn sich z. B. die Umlagerung statt in Wasser in einer 0,01 *M* Lösung dieser Säure abspielt, so ist die pro Stunde umgesetzte Menge ungefähr doppelt so gross wie in reinem Wasser. Ist die Lösung zudem 0,1 *M* an HCl, steigt die Reaktionsgeschwindigkeit auf das 5fache derjenigen in reinem Wasser. Wenn nun die [bb_1]-Säure katalysierend wirkt, so muss der Strukturähnlichkeit halber dies auch für die Persulfocyansäure selbst gelten, d. h. die Umlagerung derselben muss bimolekular sein. Damit steht die Tatsache in bestem Einklang, dass sich in sehr verdünnten wässrigen Lösungen der Säure die Umlagerung äusserst langsam vollzieht.

Der Reaktionsweg bei der Umlagerung ist wahrscheinlich der folgende, wobei wir uns der Einfachkeit halber denken, dass Schwefelwasserstoff als Katalysator wirkt.

Der bei chemischen Eingriffen schwächste Punkt des Thiodiazolringes [ab_1] ist zweifellos die Schwefel-Stickstoffbindung. Unter der Einwirkung von H_2S wird diese gebrochen, wobei gleichzeitig eine Persulfhydryl- und eine Imidogruppe gebildet werden (I)*. Die rechte Hälfte des Moleküls rotiert dann 180° um die einfache N-C-Bindung als Achse, wodurch das Formelbild (II) entsteht. Zwischen den Sulfhydryl- und Persulfhydrylgruppen wird nun ein Mol H_2S abgespalten, wodurch eine Disulfidbindung entsteht und der

* Wenn schweflige Säure als Katalysator wirkt, entsteht intermediär statt Persulfhydryl- eine Thioschwefelsäuregruppe.



I II III IV

Ring wieder geschlossen wird. Formel (III) stellt eine desmotrope Form des Xanthanwasserstoffs dar (IV). Wenn in diesem Schema Schwefelwasserstoff gegen Persulfocyansäure ausgetauscht wird, erhält man die bimolekulare Umlagerung der reinen Säure. Ausser der freien Säure soll auch das eine der primären Anionen nach dem obigen Schema umlagerungsfähig sein, und beide sollen als Katalysatoren wirken können.

Es sind einige orientierende Versuche ausgeführt worden, die Umlagerungsgeschwindigkeit in Wasser und wässrigen Lösungen zu messen, wobei folgendermassen verfahren wurde.

Bariumpersulfocyanat (reinstes Pentahydrat) in halbmolarer wässriger Lösung wird nach Kühlen auf 0° mit einer äquivalenten Menge kalter 1 *M* Salzsäure gefällt. Nach kurzem Umrühren wird der Niederschlag auf einem Glasfilter G 3 von passender Grösse gesammelt, die Mutterlauge scharf abgesaugt und der Niederschlag mit der doppelten der zum Auflösen des Salzes gebrauchten Wassermenge (ebenfalls auf 0° gekühlt und auf vier gleiche Portionen verteilt), unter Umrühren ausgewaschen. Nach scharfem Absaugen des Waschwassers wird die Hauptmenge des Niederschlags auf die zweite Dezimale genau gewogen und sofort in einen gewogenen Turbinierungsbecher aus Jenaglas gebracht. In diesen wird nun auf die fragliche Reaktionstemperatur temperiertes Wasser (100 oder 200 ml) gegossen, der Becher mit Inhalt auf die zweite Dezimale genau gewogen und dann im Wasserthermostat lebhaft turbiniert. Der Turbinierungsbecher, dessen Rand plangeschliffen ist, wird mit einer Glasscheibe gedeckt. Die Wasserverluste während des Turbinierens sind klein und wurden vernachlässigt. Zwecks Abbrechens der Reaktion wird eine 2 *M* Kaliumbicarbonatlösung zugesetzt, das anderthalbe der nach der Gleichung

$$H_2S_3C_2N_2 + KHCO_3 = KHS_3C_2N_2 + H_2O + CO_2$$

erforderlichen Menge und zudem soviel auf die Reaktionstemperatur temperiertes Wasser, dass nach Auflösung der Persulfocyansäure die Lösung höchstens 1 g derselben pro 100 ml enthält. Es wird dann weitere 5 Minuten turbiniert und dann der Niederschlag von Xanthanwasserstoff auf einen gewogenen Glasfiltertiegel G 4 gebracht, mit gesättigter Xanthanwasserstoff-

lösung ausgewaschen und im Vakuum über konz. H_2SO_4 getrocknet und dann gewogen*. Zu der so bestimmten Menge umgelagerter Persulfocyansäure ist der in der Lösungs- und Verdünnungsflüssigkeit gelöste Xanthanwasserstoff zu addieren, bei 20° 0,0052 g pro 100 ml**. Aus dem bicarbonatalkalischen Filtrat ist die nicht umgelagerte Säure mit etwas mehr als der äquivalenten Menge 2 *M* Salzsäure auszufällen und, wie oben beschrieben, als Xanthanwasserstoff zu bestimmen. Da somit die Anfangsquantität von Persulfocyansäure bekannt ist, lässt sich die Wassermenge in der abgewogenen feuchten Säurequantität berechnen und somit eine Korrektion für das Reaktionsvolumen anbringen. Die Menge von Xanthanwasserstoff bei Reaktionsbeginn lässt sich nur schwierig exakt bestimmen, es konnte jedoch durch besondere Versuche festgestellt werden, dass sie nicht 0,5 % des Trockengewichtes übersteigt. Sie wurde beim Berechnen der Versuche vernachlässigt.

Tab. 2. Umlagerung von Persulfocyansäure. $t = 20° \pm 0{,}02$.

Reaktionsz. 4 Std.			Reaktionsz. 6 Std.			Reaktionsz. 4 Std.			
p_o	V	G	p_o	V	G	p_o	V	C_{HCl}	G
1,55	103,0	0,112	1,63	103,5	0,109	1,37	203,8	0,0195	0,079
1,59	102,8	0,110	1,68	102,7	0,107	1,29	203,9	0,0195	0,078
2,54	104,6	0,124	2,42	104,7	0,119	1,78	206,1	0,0193	0,087
2,47	103,9	0,126	2,38	105,0	0,118	1,78	206,0	0,0193	0,087
3,42	105,3	0,144	3,42	106,7	0,137	2,78	208,7	0,0191	0,0103
3,39	105,9	0,145	3,51	107,3	0,136	2,81	208,9	0,0190	0,0104

Reaktionsz. 4 Std.					Reaktionsz. 4 Std.			
p_o	V	C_k	C_{HCl}	G	p_o	V	C_k	G
2,73	104,0	0,0101	0,0096	0,325	1,61	102,5	0,0100	0,244
2,82	103,6	0,0101	0,0579	0,503	1,74	102,6	0,0100	0,242
2,88	104,1	0,0101	0,0960	0,622	2,51	105,0	0,0099	0,282
3,77	106,1	0,0101	0,0942	0,694	3,43	107,1	0,0099	0,325
5,72	108,8	0,0102	0,0919	0,935	5,24	110,5	0,0100	0,404
5,72	108,6	0,0101	0,282	1,437	5,28	110,7	0,0100	0,415

* Das Abfiltrieren des Xanthanwasserstoffs kann und soll im Laufe einer Viertelstunde abgeschlossen sein.

** Wenn der Bicarbonatzusatz wie oben angegeben bemessen wird, gibt das Filtrat mit $BaCl_2$ keinen Niederschlag, weshalb Fehler zufolge unvollständigen Auswaschens der Persulfocyansäure nicht zu befürchten sind.

Wenn die Umlagerung bei Gegenwart von [bb_1]-Säure als Katalysator studiert wird, ist als Lösungs- und Verdünnungsmittel statt Wasser gesättigte Xanthanwasserstofflösung zu verwenden, und die quantitative Bestimmung der nicht umgelagerten Säure geschieht in diesem Fall nach der zweiten Methode, also ohne Eindampfen zur Trockenheit.

Die Reaktionstemperaturen waren 19° und 20° und die Zeiten 4 und 6 Stunden. Der Reaktionsbeginn wurde 5 Minuten nach dem Einsetzen des Turbinierens gesetzt.

Tab. 3. Umlagerung von Persulfocyansäure. $t = 19° \pm 0{,}02$.

Reaktionsz. 4 Std.			Reaktionsz. 4 Std.			
p_o	V	G	p_o	V	C_k	G
1,62	102,4	0,095	1,65	102,4	0,0100	0,222
1,68	102,8	0,097	1,70	102,4	0,0100	0,224
2,45	104,2	0,108	2,51	104,0	0,0100	0,261
2,51	104,1	0,108	2,51	104,3	0,0100	0,259
3,57	106,0	0,122	3,35	106,0	0,0101	0,293
3,56	105,9	0,122	3,43	105,7	0,0101	0,297

Die Tabellen 2 und 3 enthalten die Messresultate von Versuchen in reinem Wasser und von solchen mit [bb_1]-Säure als Katalysator. In Tab. 2 sind auch Messungen, wo statt Wasser stark verdünnte Salzsäure als Lösungsmittel diente, eingetragen. Unter p_o sind die auf 100 ml umgerechneten Mengen von trockner, fester Persulfocyansäure nach Sättigung der Lösung eingeführt, unter V das korrigierte Reaktionsvolumen, unter C_k und C_{HCl} Katalysatorkonzentrationen in Molarität und unter G die auf 100 ml umgerechnete durchschnittliche Umlagerungsgeschwindigkeit in Gramm pro Stunde.

Es geht aus den Tabellen hervor, dass die Umlagerung von Persulfocyansäure in reinem Wasser entgegen dem, was man früher annahm, eine verhältnismässig langsame Reaktion ist und dass das Misslingen der Versuche von Klason und Hantzsch, die Säure zu isolieren, unmöglich deren Unbeständigkeit zugeschrieben werden kann. Ferner wird ersichtlich, dass die Reaktionsgeschwindigkeit mit der Menge der nicht gelösten Säure durchgehend anwächst. Dieses kann nicht durch ein Anwachsen der Löslichkeit mit der Menge fester Persulfocyansäure erklärt werden, da der Gang der Löslichkeit dafür gar zu schwach ist, weshalb diese Tatsache kaum anders zu deuten ist,

als dass sich ausser der homogenen auch eine heterogene Reaktion abspielt. Ein Vergleich zwischen den Messungen bei 19° und 20° ergibt, dass der Temperaturkoefficient rund 10 % beträgt; in diesem ist jedoch auch der durch die Löslichkeitsänderung bedingte Effekt mitwirkend. Besonders auffallend ist das starke Anwachsen der Geschwindigkeit der durch [bb_1]-Säure katalysierten Reaktion mit der Chlorwasserstoffkoncentration, während diejenige der nicht katalysierten unter denselben Bedingungen abnimmt. Dieses Anwachsen ist wahrscheinlich durch das Zurückdrängen der Dissoziation der [bb_1]-Säure durch den Chlorwasserstoff zu erklären, wenn man die an und für sich nicht unwahrscheinliche Annahme macht, dass die undissoziierte Säure einen wesentlich stärkeren katalytischen Effekt hat als das primäre Anion. Die Abnahme der Geschwindigkeit bei Zusatz von reinem Chlorwasserstoff ist auch sicher als ein Aciditätseffekt zu erklären, bedingt durch die Verkleinerung der Konzentration der primären Anionen der Persulfocyansäure.

Xanthanwasserstoff hat eine gewisse Verwendung für Synthesen von sowohl cyklischen wie acyklischen Verbindungen gefunden. In der diesbezüglichen Literatur wird selten angegeben, wie das Ausgangsmaterial dargestellt und gereinigt worden ist. Man bleibt deshalb meistens im Unklaren, ob dieses von reinem Xanthanwasserstoff oder von Gemengen davon mit Persulfocyansäure ausgemacht wurde. Da nicht ohne weiteres angenommen werden kann, dass die Isomeren in allen Fällen gleich reagieren, scheint eine Revision vieler dieser Versuche notwendig zu sein.

Einen mit Persulfocyansäure analogen Bau besitzt Oxy-dithiocyansäure, über deren Darstellung und Eigenschaften der Verf. früher berichtet hat. Sie ist als Hydroxy-mercapto-thiodiazol [ab_1] zu formulieren. Es verdient hervorgehoben zu werden, dass diese Säure, wenn sie aus einem Alkalisalz in Freiheit gesetzt wird, sich in wässriger Lösung so rasch in das isomere cyklische Disulfid Oxo-imido-dithioazolidin (Rhodanhydrat) umwandelt, dass eine Messung der Geschwindigkeit dieser Umwandlung ausgeschlossen scheint [5].

ZUSAMMENFASSUNG.

Es wurde festgestellt, dass Persulfocyansäure entgegen was man früher annahm leicht als feste Substanz isolierbar ist.

Eine Methode zur quantitativen Bestimmung der Säure wurde ausgearbeitet.

Die Löslichkeit der Säure in Wasser wurde ermittelt, ebenso die Löslichkeit in wässrigen Lösungen von Chlorwasserstoff und Natriumacetat verschiedener Konzentration.

Aus den Löslichkeiten in den beiden letzten Medien wurde die primäre Dissoziationskonstante (Konzentrationskonstante) der Säure berechnet.

Die Umlagerung der Persulfocyansäure in Xanthanwasserstoff wurde in Wasser studiert. Es wurde dabei gefunden dass die Geschwindigkeit in einen homogenen und einen heterogenen Teil zerfällt. Die Umlagerung wird durch verschiedene Stoffe katalysiert, namentlich durch Sulfhydrylverbindungen. Besonders untersucht wurde der katalytische Effekt von Dimercapto-thiodiazol [bb_1] eine mit Persulfocyansäure isomere Verbindung. Diese Katalyse wird durch Chlorwasserstoff stark beschleunigt, anderseits bewirkt reiner Chlorwasserstoff eine Herabsetzung der Umlagerungsgeschwindigkeit.

Das Bedürfnis einer Revision älteren Untersuchungen über Umsetzungen von Persulfocyansäure und Xanthanwasserstoff wurde hervorgehoben.

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Löslichkeit und Löslichkeitsbeeinflussung in AgCl-Kristallen als Lösungsmittel bei Bildung heterotyper Mischphasen

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Als heterotype Mischphasen bezeichnen wir im Anschluss an Laves [1] die »anomalen» Mischkristalle zwischen Substanzen mit verschiedenartigem Formeltypus, also z.B. zwischen AgCl und $PbCl_2$ oder zwischen Ag_2S und ZnS. Derartige Mischphasen erscheinen uns von besonderem Interesse für die Fehlordnungszustände in festen Stoffen und die davon wesentlich beeinflussten Eigenschaften der Kristalle, z.B. Leitfähigkeit und Reaktivität. Es ist Aufgabe der vorliegenden Arbeit, die Löslickeit von $PbCl_2$ in AgCl und deren Beeinflussung durch Zusätze von $CdCl_2$ experimentell zu untersuchen und damit die Grenzen der quantitativen Gültigkeit von aus dem Massenwirkungsgesetz abgeleiteten Formeln für die Bildung und das Verhalten dieser heterotypen Mischphasen zu prüfen.

THEORETISCHE BEHANDLUNG

In festem Silberchlorid sind nach Überführungsversuchen von Tubandt [2] praktisch ausschliesslich die Silberionen Träger der elektrischen Leitfähigkeit. Nach der Modellvorstellung von Frenkel [3] ist im thermodynamischen Gleichgewicht eine gewisse Zahl von Silberionen nicht auf normalen Gitterplätzen, sondern stattdessen auf Zwischengitterplätzen eingebaut. Silberionen auf Zwischengitterplätzen und Silberionenleerstellen treten in gleicher Zahl auf. Platzwechsel dieser Fehlordnungszentren ergibt eine Verschiebung elektrischer Ladungen und damit eine elektrische Leitfähigkeit. Weiterhin haben Tubandt und Reinhold [4] sowie Koch und Wagner [5] gefunden, dass die elektrische Leitfähigkeit von festem Silberchlorid im Temperaturbereich von 200 bis

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Ag^+	Cl^-	Ag^+	Cl^-
Cl^-	□	Cl^-	Ag^+
Ag^+	Cl^-	Ag^+	Cl^-
Cl^-	Pb^{2+}	Cl^-	Ag^+

Abb. 1. Modell für die Bildung eines heterotypen Mischkristalls bei Einbau von $PbCl_2$ in das AgCl-Gitter.

350° C durch Zusätze von geringen Mengen Bleichlorid oder Cadmiumchlorid grössenordnungsmässig erhöht wird. Dieser Befund kann modellmässig durch eine Mischkristallbildung zwischen Silberchlorid und den genannten Zusätzen gedeutet werden. Bei Einbau von je einem Pb^{2+}- bzw. Cd^{2+}-Ion an Stelle eines Ag^+-Ions tritt zusätzlich jeweils eine Ag^+-Leerstelle auf, da $PbCl_2$ und $CdCl_2$ auf je zwei Chlorionen nur je ein Kation enthalten (vgl.Abb.1). Die Löslichkeit von $CdCl_2$ in AgCl ist verhältnismässig hoch, da ein angenähert linearer Anstieg der Leitfähigkeit bis zum höchsten Zusatz der Messungsreihe (10 Mol % $CdCl_2$) beobachtet wurde [5]. Die Annahme einer Mischkristallbildung zwischen AgCl und $CdCl_2$ ist ausserdem durch Aufnahme des Erstarrungsdiagramms mittels thermischer Analyse durch Wagner und Hantelmann [6] gesichert. Die Löslichkeit von $PbCl_2$ in AgCl ist geringer. Bei 300° C erfolgt keine Leitfähigkeitszunahme mehr, wenn Versuchskörper mit mehr als 0,8 Mol % $PbCl_2$ hergestellt werden. Hier muss mit der Anwesenheit von $PbCl_2$ als Nachbarphase neben dem Mischkristall gerechnet werden.

Für das Gleichgewicht zwischen festem Bleichlorid als Nachbarphase und dem AgCl-$PbCl_2$-Mischkristall kann folgende Umsetzungsgleichung formuliert werden *:

$$\underline{PbCl_2} = Pb^{2+}(g) + □\,(Ag^+) + 2\,Cl^-(g) \qquad (1)$$

Hierin bezeichnet das Symbol $Pb^{2+}(g)$ ein Bleiion auf einem normalen Kationgitterplatz, $Cl^-(g)$ ein Chlorion auf einem normalen Aniongitterplatz und das Symbol □ (Ag^+) eine Kationleerstelle. Für dieses Gleichgewicht formulieren wir das Massenwirkungsgesetz für ideal verdünnte Lösungen. Die Konzentrationen werden nach Schottky [7] als Gitterkonzentrationen x (mit zugehörigem unteren Stoffindex) gerechnet. Die Gitterkonzentration ist definiert als Quotient aus der Zahl der Zentren des fraglichen Bestandteils

* Die Unterstreichung einer Formel soll bedeuten, dass es sich um eine feste Phase handelt.

und der Zahl der normalen Kationplätze. Unter Weglassung der konstanten Gitterkonzentration $x_{Cl^-(g)} = 1$ sowie unter Beachtung der Konstanz der $PbCl_2$-Konzentration für festes Bleichlorid auf der linken Seite von (1) ergibt sich:

$$x_{Pb^{2+}(g)} \cdot x_{\square(Ag^+)} = K \tag{2}$$

In AgCl-$PbCl_2$-Mischkristallen mit $PbCl_2$ als Nachbarphase ist die Zahl der Ag^+ auf Zwischengitterplätzen gegenüber der Zahl der Ag^+-Leerstellen zu vernachlässigen, da die Leitfähigkeit des Mischkristalls grössenordnungsmässig höher ist als die Leitfähigkeit von reinem Silberchlorid. Wenn aber praktisch lediglich Pb^{2+} auf Kationenplätzen und Ag^+-Leerstellen vorhanden sind, dann müssen diese nach Umsetzungsgleichung (1) sowie aus Gründen der Elektroneutralität in gleicher Konzentration vorhanden sein:

$$x_{Pb^{2+}(g)} = x_{\square(Ag^+)} \tag{3}$$

Kombiniert mit (2) ergibt sich also:

$$x_{Pb^{2+}(g)} = \sqrt{K} \tag{4}$$

Die Massenwirkungsbeziehung (2) entspricht dem Löslichkeitsprodukt für Gleichgewichte mit wässrigen Lösungen. An Stelle einer Anionenkonzentration findet sich hier die Konzentration von Ag^+-Leerstellen, die relativ zur normalen Gitterbesetzung als negativ geladene Zentren aufzufassen sind. Die wichtigste Nutzanwendung des Satzes vom Löslichkeitsprodukt ist die Berechnung der Löslichkeitserniedrigung durch Überschuss an Fremdsalzen mit gleichem Kation oder Anion. Eine analoge Erscheinung ist auch im Falle der Löslichkeit von $PbCl_2$ in festem AgCl vorauszusehen. Bei Zusätzen, die eine Erhöhung der Ag^+-Leerstellenkonzentration bewirken, ist ein Rückgang des Pb^{2+}-Gehaltes der AgCl-Phase im Gleichgewicht mit festem Bleichlorid zu erwarten. Praktisch kann der Gehalt an Ag^+-Leerstellen durch Einbau von $CdCl_2$ in AgCl erhöht werden (vgl. oben).

In einem AgCl-$CdCl_2$-$PbCl_2$-Mischkristall ist an Stelle von (3) im Sinne einer Elektroneutralitätsbedingung anzusetzen:

$$x_{Pb^{2+}(g)} + x_{Cd^{2+}(g)} = x_{\square(Ag^+)} \tag{5}$$

Einsetzen in (2) ergibt:

$$x_{Pb^{2+}(g)} \cdot [x_{Pb^{2+}(g)} + x_{Cd^{2+}(g)}] = K \tag{6}$$

Diese Gleichung gibt uns den gesuchten formelmässigen Zusammenhang zwischen $x_{Cd^{2+}(g)}$, $x_{Pb^{2+}(g)}$ und der Massenwirkungskonstanten K. Aus (6) folgt:

$$x_{Pb^{2+}(g)} = -\tfrac{1}{2}\, x_{Cd^{2+}(g)} + \sqrt{\tfrac{1}{4}\, x^2_{Cd^{2+}(g)} + K} \tag{7}$$

und weiterhin durch Umformung und eine Erweiterung mit $\sqrt{K}$:

$$x_{Pb^{2+}(g)} = \frac{\sqrt{K}}{1 + \left(\frac{x_{Cd^{2+}(g)}}{2\sqrt{K}}\right)^2 + \left(\frac{x_{Cd^{2+}(g)}}{2\sqrt{K}}\right)} \tag{8}$$

Nach Gleichung (4) ist

$$x^{(O)}_{Pb^{2+}(g)} = \sqrt{K} \tag{9}$$

wobei der obere Index (o) zugesetzt ist, um anzugeben, dass diese Beziehung für den Bleigehalt in Silberchlorid ohne $CdCl_2$-Zusatz gilt. Aus (8) folgt mit (9) als Resultat:

$$\frac{x_{Pb^{2+}(g)}}{x^{(O)}_{Pb^{2+}(g)}} = \frac{1}{1 + \left(\frac{x_{Cd^{2+}(g)}}{2x^{(O)}_{Pb^{2+}(g)}}\right)^2 + \left(\frac{x_{Cd^{2+}(g)}}{2\,x^{(O)}_{Pb^{2+}(g)}}\right)} \tag{10}$$

Diese Gleichung ermöglicht eine leichte Übersicht über den zu erwartenden Einfluss eines $CdCl_2$-Zusatzes auf den $PbCl_2$-Gehalt eines AgCl-$PbCl_2$-Mischkristalls und durch Vergleich mit dem Experiment die Prüfung der theoretischen Vorstellungen. Für Zusätze an $CdCl_2$, die klein sind gegenüber der Löslichkeit von $PbCl_2$ in reinem AgCl, also für $x_{Cd^{2+}(g)} \ll x^{(O)}_{Pb^{2+}(g)}$, ist $\frac{x_{Cd^{2+}(g)}}{2\,x^{(O)}_{Pb^{2+}(g)}} \ll 1$, und damit wird:

$$x_{Pb^{2+}(g)} \cong x^{(O)}_{Pb^{2+}(g)} - \tfrac{1}{2}\, x_{Cd^{2+}(g)} \qquad (\text{für } x_{Cd^{2+}(g)} \ll x^{(O)}_{Pb^{2+}(g)}) \tag{11}$$

Mit steigendem $CdCl_2$-Zusatz nimmt hiernach also die Pb-Konzentration im Mischkristall zunächst linear ab.

Für Zusätze an $CdCl_2$, die gross sind gegenüber $x^{(O)}_{Pb^{2+}(g)}$, kann unter Vernachlässigung der 1 im Wurzelausdruck des Nenners von (10) geschrieben werden:

$$x_{Pb^{2+}(g)} \cong \frac{(x^{(O)}_{Pb^{2+}(g)})^2}{x_{Cd^{2+}(g)}} \qquad (\text{für } x_{Cd^{2+}(g)} \gg x^{(O)}_{Pb^{2+}(g)}) \tag{12}$$

Bei Auftragung der Bleikonzentration in AgCl als Funktion des $CdCl_2$-Gehaltes sollte also, ganz analog wie in wässrigen Lösungen, bei grossen Zusätzen die Löslichkeit von $PbCl_2$ umgekehrt proportional dem $CdCl_2$-Gehalt sein, wenn das Massenwirkungsgesetz für ideal verdünnte Lösungen hinreichende Gültigkeit hat.

VERSUCHSMETHODIK UND ERGEBNISSE

Zur Bestimmung der Löslichkeit von $PbCl_2$ in AgCl wurden aus reinem AgCl und einer Mischung von AgCl mit 10 Mol % $PbCl_2$ Tabletten von 1 cm Durchmesser und 0,025 cm Dicke gepresst. Es wurden jeweils 3 AgCl-Tabletten zwischen 2 AgCl-$PbCl_2$-Tabletten gepackt und unter Federdruck in einer ruhenden N_2-Atmosphäre auf 270° C erhitzt. Alsdann diffundieren Pb^{2+} Ionen im Austausch gegen Ag^+-Ionen aus den äusseren Tabletten in die innen liegenden AgCl-Tabletten bis zur Erreichung des Sättigungsgleichgewichts. Zur Analyse wurde jeweils nur die mittelste AgCl-Tablette benutzt, da die beiden andern AgCl-Tabletten durch mechanisch anhaftende Teile der äusseren Tabletten höhere $PbCl_2$-Gehalte aufweisen können, als dem Sättigungsgleichgewicht entspricht. Die Bleibestimmung erfolgte mit Dithizon nach Fischer und Leopoldi [8] durch Photometrieren des roten Pb-Dithizonats in Tetrachlorkohlenstoff mittels Pulfrichphotometer (Grünfilter S 53 entsprechend einem Wellenlängenschwerpunkt von 530 mμ). Die gewogene Tablette wurde in 25 ml 5 procentiger KCN-Lösung + 1 ml gesättigter Kalium-Natrium-Tartratlösung gelöst und auf 100 ml aufgefüllt. Hiervon wurden 5 ml mit Dithizonlösung in Tetrachlorkohlenstoff quantitativ extrahiert. Die Photometrierung wurde baldmöglichst vorgenommen.

In Abb. 2 sind die Pb-Gehalte für verschiedene Erhitzungszeiten von Tablettenpaketen graphisch dargestellt. Es wird ein Grenzwert von 0,63 Mol % $PbCl_2$ erreicht.

In entsprechender Weise wurde die Löslichkeit von $PbCl_2$ in AgCl-$CdCl_2$-Mischkristallen bestimmt. Die Mitteltabletten wurden aus einer Mischung von AgCl + $CdCl_2$ gepresst, während die äusseren Tabletten ausser AgCl und $CdCl_2$ noch überschüssiges Bleichlorid enthielten. Auch hier wurden Versuche mit verschiedener Erhitzungsdauer durchgeführt. Die Zeiten für die praktische Einstellung des Sättigungsgleichgewichtes sind kürzer als für AgCl ohne $CdCl_2$-Zusatz, da die Diffusionskonstante mit der Zahl der Leerstellen wächst. Die Gleichgewichtswerte der $PbCl_2$-Löslichkeit sind in Abb. 3 als Funktion des $CdCl_2$-Gehaltes dargestellt.

Die Löslichkeit von $PbCl_2$ in AgCl wird erwartungsgemäss durch verhältnismässig kleine Zusätze von $CdCl_2$ herabgesetzt. Nach Durchlaufen eines

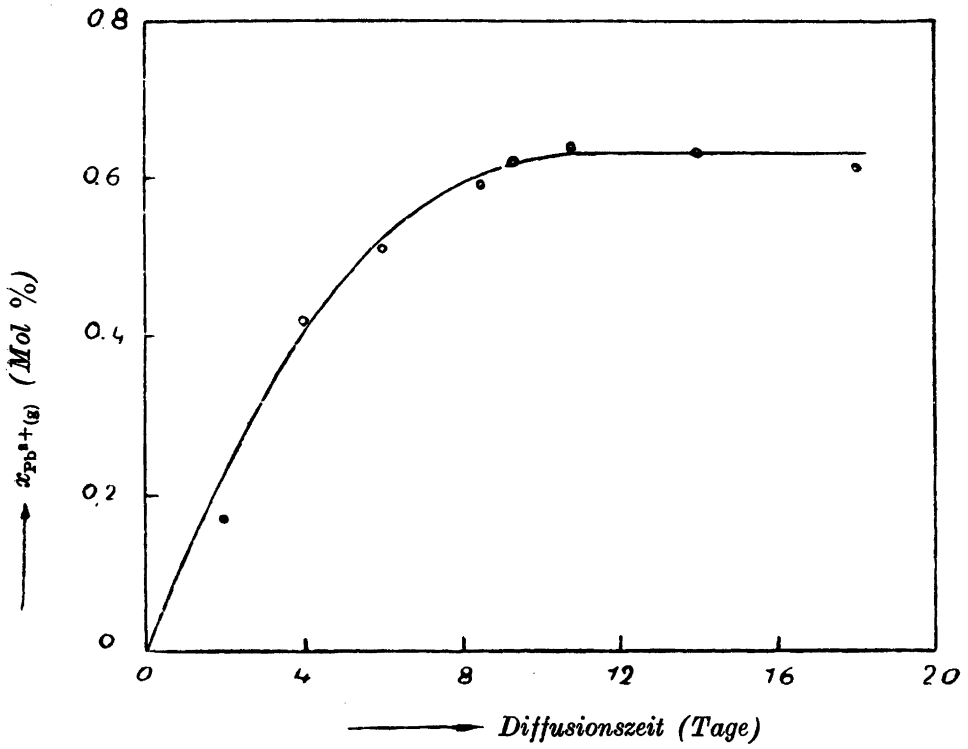

Abb. 2. $PbCl_2$-Gehalt von AgCl-Tabletten als Funktion der Diffusionszeit.

Minimums (etwa 0,32 Mol % $PbCl_2$ für 1,3 Mol % $CdCl_2$) steigt die Löslichkeit des Bleichlorids mit wachsendem $CdCl_2$-Gehalt wieder an. In Abb. 3 ist ausser den Messwerten auch die theoretisch nach Gleichung (7) berechnete Löslichkeitskurve eingezeichnet. Der gemessene Löslichkeitsrückgang für kleine $CdCl_2$-Zusätze entspricht der Berechnung unter Annahme des idealen Massenwirkungsgesetzes. Bei höheren $CdCl_2$-Gehalten sind die gefundenen $PbCl_2$-Gehalte grösser als die berechneten Werte. Derartige Abweichungen sowie das Auftreten eines Minimums mit nachfolgendem Löslichkeitsanstieg findet sich auch bei der Löslichkeitsbeeinflussung von schwerlöslichen Salzen in wässriger Lösung. Beispiele hierfür finden sich u. a. in einer Arbeit von Schärer [9], der einen Vergleich mit den Aussagen der Theorie von Debye und Hückel[10] durchgeführt hat. Auch bei unseren Versuchen dürften die Abweichungen gegenüber dem idealen Massenwirkungsgesetz bei höheren Gehalten durch die elektrostatischen Kräfte zwischen Fehlordnungszentren bedingt sein, deren Ladungszahl vom Normalzustand des Gitters abweicht.

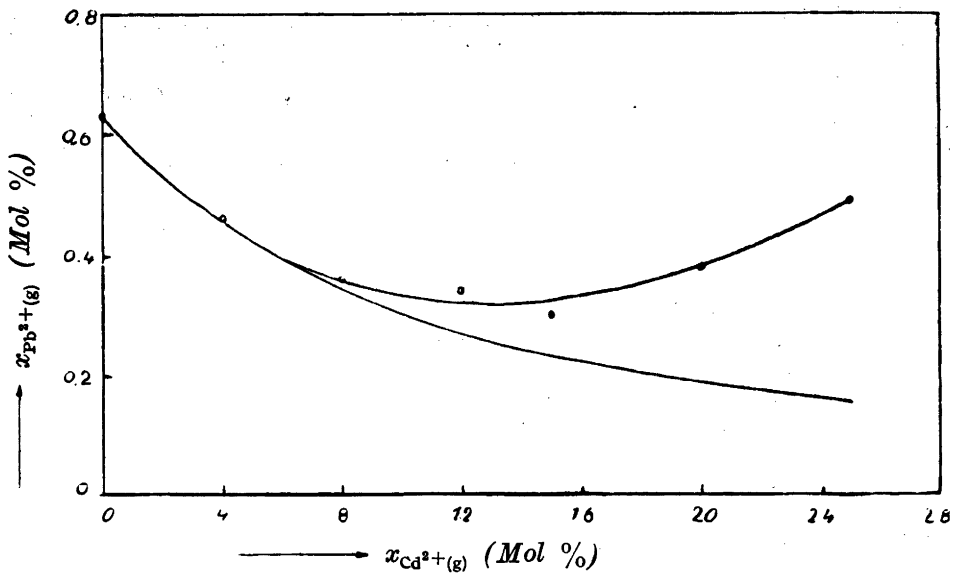

Abb. 3. $PbCl_2$-Sättigungsgehalte in Silberchlorid bei 270° C als Funktion des $CdCl_2$-Zusatzes.

Eine entsprechende quantitative Auswertung unserer Ergebnisse nach dem Vorgang von Schärer ist nicht möglich, da infolge der verhältnismässig hohen Konzentrationen und der niedrigen Dielektrizitätskonstante eine Anwendung des Debye-Hückelschen Grenzgesetzes nicht mehr statthaft ist.

Der hier beschriebene Fall entspricht einem besonders einfachen Typus einer Löslichkeitsbeeinflussung im Kristallgitter einer polaren Verbindung. Weitere Typen sind zu erwarten. Riehl und Ortmann [11] haben eine Zurückdrängung der Löslichkeit von Silbersulfid in Zinksulfid durch Gegenwart von Kupfersulfid gefunden. Diese Beobachtung kann gleichfalls durch die Anwendung des Massenwirkungsgesetzes mit Berücksichtigung der anzunehmenden Fehlordnungszustände gedeutet werden.

ZUSAMMENFASSUNG

Die Löslichkeit von Bleichlorid in festem Silberchlorid bei 270° C beträgt 0,63 Mol %. In einem AgCl-$CdCl_2$-Mischkristall ist die $PbCl_2$-Löslichkeit geringer. Der Rückgang der $PbCl_2$-Löslichkeit durch kleine $CdCl_2$-Zusätze entspricht den Forderungen des idealen Massenwirkungsgesetzes. Der postulierte Mechanismus bei der Bildung heterotyper Mischkristalle wird damit be-

stätigt. Bei 1,3 Mol % $CdCl_2$ wird ein Löslichkeitsminimum von 0,32 Mol % $PbCl_2$ gefunden, und bei höheren $CdCl_2$-Gehalten steigt die Löslichkeit von $PbCl_2$ wieder an. Diese Abweichung vom idealen Massenwirkungsgesetz dürfte analog wie bei wässrigen Lösungen im Sinne der Debye-Hückelschen Theorie durch elektrostatische Kräfte zwischen Fehlordnungszentren zu deuten sein.

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Die Oxydationsgeschwindigkeit von Nickel bei kleinen Zusätzen von Chrom und Mangan

Beitrag zur Theorie des Anlaufvorganges

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Beim Erhitzen von Kupfer, Nickel und verwandten Metallen in oxydieren der Atmosphäre entsteht nach kurzer Zeit eine praktisch porenfreie Deckschicht. In früheren Mitteilungen [1] wurde gezeigt, dass die Diffusion in der Deckschicht als eine Wanderung von einzelnen Ionen und Elektronen beschrieben werden kann. Im besonderen konnte für Cu_2O und NiO gezeigt werden, dass die Bewegung der Kationen als Nachrücken von Gitterionen auf Leerstellen des Kationengitters darzustellen ist.

Durch heterotype Mischkristallbildung ist es möglich, sowohl die Zahl der Leerstellen als auch die Zahl der Ionen auf Zwischengitterplätzen willkürlich zu ändern **. Beim Übergang vom reinen Metall zu einer Legierung mit einem Partner, der in der Oxydphase Besetzung von Zwischengitterplätzen oder Entstehung von Leerstellen verursacht, ist eine Erhöhung der Oxydationsgeschwindigkeit der Legierung gegenüber dem reinen Metall zu erwarten, sofern die Diffusion der Fehlstellen zeitbestimmend ist. Die vorliegende Arbeit enthält systemtatische Beobachtungen an Nickellegierungen. Bei der Oxydation von Nickel entsteht Nickeloxyd, das üblicherweise durch die Formel NiO beschrieben wird. Tatsächlich ist jedoch jeweils ein gewisser Sauerstoffüberschuss vorhanden, der je nach Temperatur und Sauerstoffdruck der umgebenden Gasatmosphäre verschieden ist, wie durch die Arbeiten von Le Blanc und Sachse [3] gezeigt wurde. Wagner und v. Baumbach [4] haben

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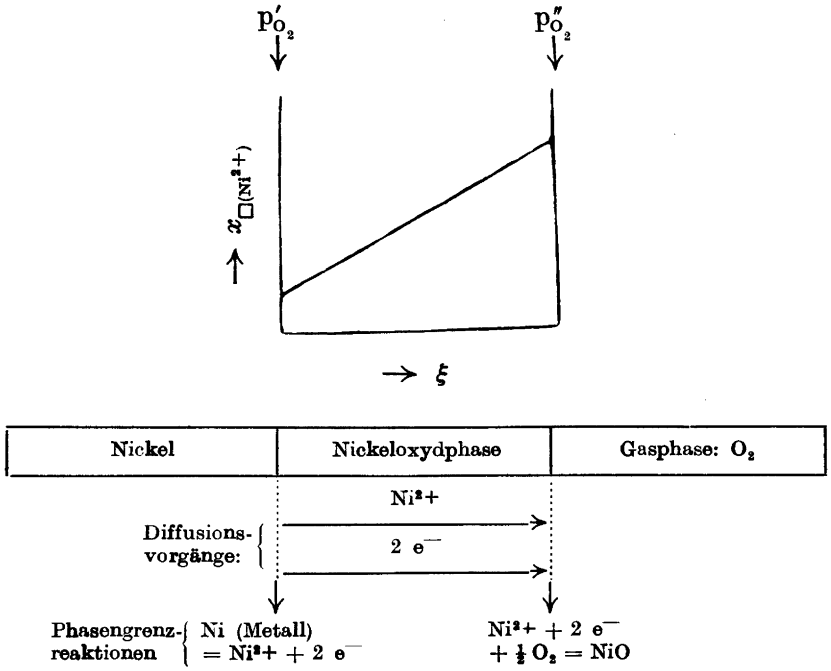
** Vergleiche hierzu unsere voranstehende Arbeit sowie die unter [2] zitierten Arbeiten.

Ni^{2+}	O^{2-}	Ni^{2+}	O^{2-}
O^{2-}	□	O^{2-}	Ni^{3+}
Ni^{2+}	O^{2-}	Ni^{2+}	O^{2-}
O^{2-}	Ni^{3+}	O^{2-}	Ni^{2+}

Figur 1. NiO-Gitter mit Sauerstoffüberschuss = Nickeldefizit (□ = Ni^{2+} Leerstelle).

im besonderen die Existenz eines definierten Gleichgewichtes durch Feststellung einer reversiblen Abhängigkeit der elektrischen Leitfähigkeit vom Sauerstoffdruck der umgebenden Gasatmosphäre gezeigt. Je höher der Sauerstoffüberschussgehalt gegenüber der Formel NiO ist, in desto grösserer Zahl sind Ni^{3+}-Ionen an Stelle von Ni^{2+}-Ionen vorhanden und die Elektroneutralität wird durch die Existenz einer entsprechenden Zahl von Ni-Leerstellen hergestellt (vgl. Abb. 1). Die Ni^{3+}-Ionen enthalten gegenüber den normalen Gitterbestandteilen Ni^{2+} jeweils ein Elektron zu wenig; hierfür



Figur 2. Örtliche Verteilung der Ni^{2+} Leerstellen in Nickeloxyd ohne Fremdoxydzusatz und Erläuterung der Diffusionsvorgänge bei der Oxydation von reinem Nickel.

Ni^{2+}	O^{2-}	Ni^{2+}	O^{2-}
O^{2-}	□	O^{2-}	Cr^{3+}
Ni^{2+}	O^{2-}	Ni^{2+}	O^{2-}
O^{2-}	Cr^{3+}	O^{2-}	Ni^{2+}

Figur 3. Nickeloxyd-Chromoxyd-Mischkristall.

wird auch die Bezeichnung Elektronendefektstelle benutzt. Bei der Bildung von Nickeloxyd auf Nickel in einer Sauerstoffatmosphäre ist der Sauerstoffüberschuss = Nickeldefizit der NiO-Phase am kleinsten an der Phasengrenze Ni/NiO, am grössten an der Phasengrenze NiO/Gas. Dementsprechend wandern Nickelionen und Elektronen von der Phasengrenze Ni/NiO zur Phasengrenze NiO/Gas, d. h. zu Stellen grösserer Leerstellenkonzentration (vgl. Abb. 2).

Ein Oxyd, das sich von einem Metall höherer Wertigkeitsstufe ableitet, kann in das NiO-Gitter unter zusätzlicher Ausbildung von Kationenleerstellen eingebaut werden. Abb. 3 erläutert den Einbau von Cr_2O_3 in NiO ohne Gegenwart eines Sauerstoffüberschusses: Je 2 Cr^{3+}-Ionen ersetzen jeweils 3 Ni^{2+}-Ionen und ergeben somit je 1 Ni^{2+}-Leerstelle. Bei hinreichender Mischkristallbildung ist die Ni^{2+}-Leerstellenkonzentration im Mischkristall wesentlich höher als im Nickeloxyd und dementsprechend ist eine Erhöhung der Oxydationsgeschwindigkeit von Nickel durch kleine Zusätze von Chrom zu erwarten. Entsprechende Überlegungen gelten für Zusätze von anderen Metallen, die bevorzugt dreiwertige (oder höherwertige) Ionen liefern, also z.B. Mangan.

I. VERSUCHSMETHODIK UND ERGEBNISSE

Zur Prüfung der gemachten Voraussagen wurden vergleichende Messungen der Oxydationsgeschwindigkeit von reinem Nickel und von Ni-Cr-Legierungen mit 0,3, 1,3 und 10 Gewichts % Chrom und gleicherweise an Ni-Mn-Legierungen ausgeführt. Die Legierungen lagen in Form von Blechstreifen vor. Die Oxydationsgeschwindigkeit wurde gemessen, indem die Proben in einen senkrecht stehenden Röhrenofen von 1000° C, durch welchen reiner Sauerstoff von 1 Atm. Druck strömte, eingehängt wurden. Nach Versuchszeiten von 1, 4, 9 und 16 Stunden wurde die Gewichtszunahme bestimmt. Für jeden Versuch wurde eine neue Probe eingesetzt. Bei zeitbestimmender

Diffusion und sonst konstanten Bedingungen ist die Oxydationsgeschwindigkeit umgekehrt proportional der Dicke der gebildeten Oxydschicht; vgl. hierzu auch die Gleichungen (46) und (51) in Abschnitt III. Hieraus folgt nach Tammann [5] sowie Pilling und Bedworth [6], dass das Quadrat der Schichtdicke, bzw. das Quadrat der Gewichtszunahme pro cm^2 $(\Delta m/q)$ proportional mit der Versuchszeit t wachsen soll (parabolisches Zeitgesetz). Dementsprechend wurden für die einzelnen Versuche die Quotienten $\frac{1}{t} \cdot \left(\frac{\Delta m}{q}\right)^2$ berechnet, die bei Gültigkeit aller Voraussetzungen unabhängig von der Versuchsdauer sein sollen und die wir als praktische Anlaufkonstanten k'' bezeichnen.

Tabelle 1. Oxydationsgeschwindigkeit von reinem Nickel bei 1000° C.

t (s)	$\Delta m/q$ (g/cm^2)	$k'' = \frac{1}{t} \cdot \left(\frac{\Delta m}{q}\right)^2$ (g · cm^{-4} · s^{-1})
4800	$1{,}6 \cdot 10^{-3}$	$5{,}5 \cdot 10^{-10}$
16080	$2{,}5 \cdot 10^{-3}$	$3{,}8 \cdot 10^{-10}$
44100	$3{,}8 \cdot 10^{-3}$	$3{,}3 \cdot 10^{-10}$
102600	$4{,}9 \cdot 10^{-3}$	$2{,}9 \cdot 10^{-10}$
172800	$5{,}8 \cdot 10^{-3}$	$1{,}9 \cdot 10^{-10}$

In Tabelle 1 sind die Versuchsergebnisse für reines Nickel mitgeteilt. Das parabolische Zeitgesetz ermöglicht keine vollständige Darstellung der Befunde, indem die Zahlenwerte der letzten Spalte von Tabelle 1 einen deutlichen Gang aufweisen. Die Gründe hierfür sind unbekannt.

In Tabelle 2 sind die Messungen für Ni-Cr-Legierungen zusammengestellt. Durch Vergleicht mit Tabelle 1 ist ersichtlich, dass bereits durch Zusätze von 0,3 und 1 % Cr die Oxydationsgeschwindigkeit gegenüber reinem Nickel wesentlich erhöht wird. Damit sind die Voraussagen auf Grund der Modellvorstellungen bestätigt.

Bei Legierungen mit 10 Gewichts % Cr kommt die Oxydation nach einem anfänglichen Induktionsstadium praktisch zum Stillstand, indem die Werte $\Delta m/q$ in Spalte 2 mit wachsender Versuchszeit kaum noch zunehmen. Analog zu Beobachtungen an anderen Legierungssystemen (Fe-Al; Fe-Si; Cu-Al; Cu — Be) [7] dürfte hier die Bildung einer neuen Phase (Ni-Cr-Spinell?)

Tabelle 2. Oxydationsgeschwindigkeit von Ni-Cr-Legierungen bei 1000° C.

Gew. % Cr	t (s)	$\Delta m/q$ (g/cm²)	$k'' = \frac{1}{t} \cdot \left(\frac{\Delta m}{q}\right)^2$ (g · cm^{-4} · s^{-1})
0,3	3 600	$2{,}5 \cdot 10^{-3}$	$17 \cdot 10^{-10}$
»	14 400	$4{,}7 \cdot 10^{-3}$	$15 \cdot 10^{-10}$
»	34 800	$7{,}3 \cdot 10^{-3}$	$16 \cdot 10^{-10}$
»	57 600	$8{,}8 \cdot 10^{-3}$	$14 \cdot 10^{-10}$
1,0	3 600	$3{,}2 \cdot 10^{-3}$	$29 \cdot 10^{-10}$
»	14 400	$6{,}3 \cdot 10^{-3}$	$28 \cdot 10^{-10}$
»	32 400	$8{,}0 \cdot 10^{-3}$	$20 \cdot 10^{-10}$
»	57 600	$12{,}3 \cdot 10^{-3}$	$26 \cdot 10^{-10}$
3,0	3 600	$3{,}1 \cdot 10^{-3}$	$26 \cdot 10^{-10}$
»	14 400	$7{,}2 \cdot 10^{-3}$	$36 \cdot 10^{-10}$
»	32 400	$8{,}0 \cdot 10^{-3}$	$20 \cdot 10^{-10}$
»	57 600	$13{,}4 \cdot 10^{-3}$	$31 \cdot 10^{-10}$
10,0	3 600	$2{,}2 \cdot 10^{-3}$	$13 \cdot 10^{-10}$
»	14 400	$2{,}7 \cdot 10^{-3}$	$5{,}0 \cdot 10^{-10}$
»	32 400	$2{,}9 \cdot 10^{-3}$	$2{,}7 \cdot 10^{-10}$
»	57 600	$2{,}9 \cdot 10^{-3}$	$1{,}5 \cdot 10^{-10}$

mit besonders geringer Ionenbeweglichkeit in Form einer zusammenhängende Schutzchicht erfolgen. Der Befund ist praktisch wichtig, da Ni-Cr-Legierungen als Heizleiter viel benutzt werden und da hierfür eine möglichst hohe Zunderbeständigkeit gefordert wird.

Tabelle 3 enthält die Versuchsergebnisse für Ni-Mn-Legierungen. Auch hier wird die Oxydationsgeschwindigkeit bereits durch Zusätze von 0,3 bzw. 1 % Mn wesentlich gegenüber Reinnickel erhöht. Die Ergebnisse sind eine weitere Bestätigung der modellmässigen Voraussagen.

Die Ergebnisse stehen in Übereinstimmung mit gelegentlichen früheren Beobachtungen. Pilling und Bedworth [6] haben bei 1000°C für möglichst reines Nickel die praktische Anlaufkonstante $k'' = 9{,}5 \cdot 10^{-10}$ bestimmt, während für weniger reines Nickel etwa der doppelte Wert mit $k'' = 19 \cdot 10^{-10}$ gefunden wurde. v. Baumbach und Wagner [4] haben für Nickel, das im wesentlichen Magnesium und nur wenig Eisen als Verunreinigung enthielt, $k'' = 4.9 \cdot 10^{-10}$ bei 1000° C gefunden ($t = 1840$ bis 35600 s), was angenähert mit den Werten in Tabelle 1 übereinstimmt; für Nickel mit etwa 0,5 bis 1 % Mn wurde der etwas höhere Wert $k'' = 15 \cdot 10^{-10}$ gefunden ($t = 9000$ und 21600 s),

Tabelle 3. Oxydationsgeschwindigkeit von Ni-Mn-Legierungen bei 1000° C.

Gew. % Mn	t (s)	$\Delta m/q$ (g/cm²)	$k'' = \frac{1}{t} \cdot \left(\frac{\Delta m}{q}\right)^2$ (g . cm^{-4} . s^{-1})
0,3	3 600	$1{,}4 \cdot 10^{-3}$	$5{,}7 \cdot 10^{-10}$
»	14 400	$3{,}5 \cdot 10^{-3}$	$8{,}6 \cdot 10^{-10}$
»	32 400	$5{,}3 \cdot 10^{-3}$	$8{,}7 \cdot 10^{-10}$
»	57 600	$7{,}6 \cdot 10^{-3}$	$10 \cdot 10^{-10}$
1,0	3 600	$1{,}9 \cdot 10^{-3}$	$10 \cdot 10^{-10}$
»	14 400	$3{,}9 \cdot 10^{-3}$	$11 \cdot 10^{-10}$
»	32 400	$6{,}9 \cdot 10^{-3}$	$12 \cdot 10^{-10}$
»	57 600	$8{,}3 \cdot 10^{-3}$	$17 \cdot 10^{-10}$
3,0	3 600	$2{,}4 \cdot 10^{-3}$	$16 \cdot 10^{-10}$
»	14 400	$4{,}9 \cdot 10^{-3}$	$16 \cdot 10^{-10}$
»	32 400	$8{,}0 \cdot 10^{-3}$	$20 \cdot 10^{-10}$
»	57 600	$9{,}3 \cdot 10^{-3}$	$15 \cdot 10^{-10}$
10,0	3 600	$3{,}4 \cdot 10^{-3}$	$32 \cdot 10^{-10}$
»	14 400	$7{,}1 \cdot 10^{-3}$	$35 \cdot 10^{-10}$
»	32 400	$12{,}2 \cdot 10^{-3}$	$46 \cdot 10^{-10}$
»	57 600	$12{,}6 \cdot 10^{-3}$	$28 \cdot 10^{-10}$

II. THEORETISCHE BEHANDLUNG

Für das Gleichgewicht zwischen Nickeloxyd und der koexistierenden Sauerstoffatmosphäre mit dem Partialdruck p_{O_2} können wir als Umsetzungsgleichung formulieren: O_2-Moleküle der Gasphase reagieren mit 4 Elektronen $e^-(g)$ von normalen Gitterbestandteilen und bilden Sauerstoffionen $O^{2-}(g)$ auf Gitterplätzen einer aussen neu angelegten Netzebene, wozu gleichzeitig 2 Ni^{2+}-Ionen aus dem Inneren des Gitters abgegeben werden. Insgesamt entstehen also auf 1 O_2-Molekül aus der Gasphase je 2 Ni^{2+}-Leerstellen $\square(Ni^{2+})$ und 4 Elektronen-Defektstellen $\square(e^-)$:

$$O_2(\text{Gas}) + 4\ e^-(g) = 2\ O^{2-}(g) + 2\ \square(Ni^{2+}) + 4\ \square(e^-) \qquad (1)$$

Zur Formulierung des Massenwirkungsgesetzes definieren wir nach dem Vorgang von Schottky [8] als »Gitterkonzentration« (Symbol x mit zugehörigem Index) den Quotienten aus Fehlordnungsstellenzahl und der Zahl der normalen Gitterplätze der Ni^{2+}-Ionen bzw. O^{2-}-Ionen. Hiermit lautet das Massenwirkungsgesetz:

$$\frac{x^2_{\square(Ni^{2+})} \cdot x^4_{\square(e^-)}}{p_{O_2}} = K_1 \qquad (2)$$

Ferner ist im Nickeloxyd ohne Zusatz nach Umsetzungsgleichung (1) die Zahl der Elektronendefektstellen doppelt so gross als die Zahl der Ni^{2+}-Leerstellen. Unter Einführung des oberen Index$^{(0)}$ zur Kennzeichnung der Konzentrationen im Nickeloxyd ohne Fremdoxyd bei gegebenem Sauerstoffdruck gilt also:

$$x^{(O)}_{\square(e^-)} = 2\ x^{(O)}_{\square(Ni^{2+})} \tag{3}$$

Somit ergibt sich für Nickeloxyd ohne Fremdoxydzusats nach Gleichung (2) und (3):

$$\frac{[x^{(O)}_{\square(Ni^{2+})}]^2 \cdot [x^{(O)}_{\square(e^-)}]^4}{p_{O_2}} = 16\ \frac{[x^{(O)}_{\square(e^-)}]^6}{p_{O_2}} = K_1 \tag{4}$$

Bei gleichzeitig vorhandenem Chromgehalt in Form von Cr^{3+}-Ionen und Sauerstoffüberschuss = Ni-Defizit ist Gleichung (3) derart zu erweitern, dass die Zahl der Ni-Leerstellen gleich der halben Summe aus Elektronendefektstellen- und Chromkonzentration x_{Cr} ist, wie aus dem Prinzip der Elektroneutralität folgt:

$$x_{\square(Ni^{2+})} = \tfrac{1}{2}\ x_{\square(e^-)} + \tfrac{1}{2}\ x_{Cr} \tag{5}$$

Falls die Chromkonzentration x_{Cr} gross gegen die Fehlordnungskonzentration im Nickeloxyd ohne Chromzusatz ist, kann die Elektronendefektstellen-Konzentration auf der rechten Seite von (5) als additives Glied näherungsweise unberücksichtigt bleiben. Diese Annahme soll bei allen folgenden Überlegungen gelten:

$$x_{\square(Ni^{2+})} \cong \tfrac{1}{2}\ x_{Cr} \qquad (\text{für } x_{Cr} \gg x^{(O)}_{\square(Ni^{2+})}) \tag{6}$$

Einsetzen in (2) ergibt:

$$\frac{\frac{1}{4}\ x^2_{Cr} \cdot x^4_{\square(e^-)}}{p_{O_2}} = K_1 \tag{7}$$

Durch Vergleich mit (4) folgt für das Verhältnis der Elektronendefektstellen-Konzentration $x_{\square(e^-)}$ im Nickeloxyd-Chromoxyd-Mischkristall gegenüber der Konzentration $x^{(O)}_{\square(e^-)}$ im Nickeloxyd ohne Chromzusatz:

$$\frac{x_{\square(e^-)\ (\text{Mischkristall})}}{x^{(O)}_{\square(e^-)}} = \sqrt{\frac{2x^{(O)}_{\square\ (Ni)}}{x_{Cr}}} \qquad \left(\text{für } x_{Cr} \gg x^{(O)}_{\square\ (Ni)}\right) \tag{8}$$

Aus Gleichung (8) ist zu entnehmen, dass der Nickeloxyd-Chromoxyd-Mischkristall eine wesentlich geringere Elektronendefektstellen-Konzentration und damit auch eine kleinere Leitfähigkeit als Nickeloxyd ohne Fremdoxyd be-

sitzen sollte, falls der Chromoxydgehalt gross gegen die Fehlordnungskonzentration im Nickeloxyd ohne Fremdoxydzusatz ist. Diesbezügliche Messungen haben noch nicht zu eindeutigen Ergebnissen geführt. Weitere Untersuchung bleibt vorbehalten.

Wie früher abgeleitet (vgl. Gleichungen (11 a) bis (11 c) in Wagner [1a]) gilt allgemein für die durch einen Querschnitt q hindurchwandernde Stoffmenge N_i einer Teilchenart i in Äquivalenten pro Sekunde:

$$N_i = q \cdot \frac{300}{96500} \cdot \frac{n_i \cdot \varkappa}{|z_i| \cdot \varepsilon \cdot L} \cdot \left(- \frac{d\mu_i}{d\xi} - z_i \cdot L \cdot \varepsilon \cdot \frac{d\varphi}{d\xi} \right) \tag{9}$$

Hierin ist n_i der relative Stromleitungsanteil der Teilchen der Sorte i, N die spezifische elektrische Gesamtleitfähigkeit ($\mathrm{Ohm^{-1} \cdot cm^{-1}}$), μ_i das chemische Potential (erg pro g-Atom), z_i die Wertigkeit der Teilchen der Sorte i, L die Loschmidtsche Zahl, ε die Elementarladung ($= 4{,}80 \cdot 10^{-10}$ abs.el.st.Einh.), φ das elektrische Potential (in abs.el.st.Einheiten) und ξ die Ortskoordinate in der Transportrichtung. Der Zahlenfaktor 300 stammt aus der Umrechnung von absoluten elektrostatischen Spannungseinheiten auf Volt; die Zahl 96500 ist gleich der Zahl der Coulomb pro elektrochemisches Äquivalent.

Unter den hier betrachteten Bedingungen fliesst insgesamt kein elektrischer Strom; die Summe der durch den Querschnitt q transportierten positiven Ladungen muss gleich der entsprechenden Zahl der negativen Ladungen sein. Im Falle von Kationen- und Elektronenwanderung ohne merkliche Anionenbewegung muss gelten:

$$\Sigma N_{i\ (\text{Kationen})} = N_{e^-} \tag{10}$$

Einsetzen der Ausdrücke (9) für jede einzelne Teilchensorte in (10) ergibt für das elektrische Potentialgefälle:

$$\frac{d\varphi}{d\xi} = \frac{1}{L \cdot \varepsilon} \left[- \Sigma \frac{n_i}{z_i} \cdot \frac{d\mu_{i\ (\text{Kationen})}}{d\xi} + n_{e^-} \cdot \frac{d\mu_{e^-}}{d\xi} \right] \tag{11}$$

Entsprechend dem Verhalten der hier betrachteten Systeme wird im folgenden stets mit weitaus überwiegendem Elektronenanteil des Leitungsvermögens gerechnet:

$$n_{e^-} \cong 1 \text{ und } n_{i\ (\text{Kation})} \ll 1 \tag{12}$$

Hiermit folgt aus (11):

$$\frac{d\varphi}{d\xi} = \frac{1}{L \cdot \varepsilon} \cdot \frac{d\mu_{e^-}}{d\xi} \tag{13}$$

Einsetzen in (9) ergibt:

$$N_{i\ (\text{Kation})} = q \cdot \frac{300}{96500} \cdot \frac{n_i \cdot N}{z_i \cdot \varepsilon \cdot L} \cdot \left(- \frac{d\mu_i}{d\xi} - z_i \frac{d\mu_{e^-}}{d\xi} \right) \tag{14}$$

Auf Grund der Gleichgewichtsreaktion:

1 Metallion der Sorte $i + z_i$ Elektronen = 1 Metallatom der Sorte i

ergibt sich für die chemischen Potentiale μ_{Ni} und μ_{Cr} der elektroneutralen Metallatome:

$$\mu_{Ni^{2+}} + 2\mu_{e^-} = \mu_{Ni} \tag{15}$$

$$\mu_{Cr^{3+}} + 3\mu_{e^-} = \mu_{Cr} \tag{16}$$

Zur späteren Verwendung wird die Differenz des chemischen Potentials der Nickelatome gegenüber dem chemischen Potential $\mu^{(Ni)}$ des reinen Nickels gleich $RT \cdot \ln a_{Ni}$ mit a_{Ni} als thermodynamische Aktivität eingeführt. Entsprechendes wird für Chrom angesetzt. Hiermit ergibt sich aus (15) und (16):

$$\mu_{Ni^{2+}} + 2\mu_{e^-} = \mu_{Ni} = \mu^{(Ni)} + RT \ln a_{Ni} \tag{17}$$

$$\mu_{Cr^{3+}} + 3\mu_{e^-} = \mu_{Cr} = \mu^{(Cr)} + RT \ln a_{Cr} \tag{18}$$

Ferner wird in (14) das Produkt $n_i \cdot \varkappa$ durch die Teilleitfähigkeit $\varkappa_i$ der Ionensorte i ersetzt. Hiermit, sowie nach Einsetzen von (17) und (18) folgt aus (14):

$$N_{Ni^{2+}} = - q \cdot \frac{300}{96500} \cdot \frac{\varkappa_{Ni^{2+}}}{2\varepsilon L} \cdot \frac{d\mu_{Ni}}{d\xi}$$

$$= - q \cdot \frac{300}{96500} \cdot \frac{1}{2} \cdot \varkappa_{Ni^{2+}} \cdot \frac{RT}{\varepsilon L} \cdot \frac{d \ln a_{Ni}}{d\xi} \tag{19}$$

$$N_{Cr^{3+}} = - q \cdot \frac{300}{96500} \cdot \frac{\varkappa_{Cr^{3+}}}{3\varepsilon L} \cdot \frac{d\mu_{Cr}}{d\xi}$$

$$= - q \cdot \frac{300}{96500} \cdot \frac{1}{3} \cdot \varkappa_{Cr^{3+}} \cdot \frac{RT}{\varepsilon L} \cdot \frac{d \ln a_{Cr}}{d\xi} \tag{20}$$

Auf Grund der Umsetzungsgleichung:

$$Ni + \frac{1}{2} O_2 = NiO \tag{21}$$

gilt weiterhin:

$$\mu_{Ni} + \frac{1}{2}\mu_{O_2} = \mu_{NiO} \tag{22}$$

In dieser Gleichung ist die rechte Seite praktisch konstant zu setzen, da der Einfluss des Gehaltes an überschüssigem Sauerstoff und Chromoxyd auf das chemische Potential von NiO als Lösungsmittel in weitgehend zutreffender Näherung zu vernachlässigen ist. Unter gleichzeitiger Einführung des Sauerstoffdrucks p_{O_2} der koexistierenden Gasphase an der Stelle ξ ergibt sich:

$$\frac{d\mu_{Ni}}{d\xi} = RT \cdot \frac{d \ln a_{Ni}}{d\xi} = -\frac{1}{2}\frac{d\mu_{O_2}}{d\xi} = -\frac{1}{2} RT \cdot \frac{d \ln p_{O_2}}{d\xi} \tag{23}$$

Einsetzen in (19) ergibt für den Nickeltransport:

$$N_{Ni^{2+}} = q \cdot \frac{300}{96500} \cdot \frac{1}{4} \varkappa_{Ni^{2+}} \cdot \frac{RT}{\varepsilon L} \cdot \frac{d \ln p_{O_2}}{d\xi} \tag{24}$$

Die Teilleitfähigkeit $\varkappa_{Ni^{2+}}$ ist proportional der Ni^{2+}-Leerstellenkonzentration, also im Nickeloxyd-Chromoxyd-Mischkristall nach (6) auch proportional dem Chromgehalt x_{Cr}, sofern dieser gross gegen die Fehlordnungskonzentration im Nickeloxyd ohne Fremdoxyd ist. Folglich gilt für das Verhältnis der Transportgrösse des Mischkristalls gegenüber dem Wert $N^{(O)}_{Ni^{2+}}$ für Nickeloxyd ohne Fremdoxyd:

$$\frac{N_{Ni^{2+}\ (Mischkristall)}}{N^{(O)}_{Ni^{2+}}} = \frac{\varkappa_{Cr}}{x^{(O)}_{\square(Ni)^{2+}}} \qquad (\text{für } x_{Cr} \gg x^{(O)}_{\square(Ni^{2+})}) \tag{25}$$

Für weitere Schlussfolgerungen ist zu beachten, dass der Chromgehalt in einer Anlaufschicht keineswegs gleichmässig verteilt zu sein braucht.

Es erweist sich als zweckmässig, an Stelle der Wanderungsvorgänge in einer ständig dicker werdenden Schicht zunächst die Wanderungsvorgänge in einer Schicht vorgegebener Dicke zu betrachten. Entsprechend Figur 2 soll rechts ein grösserer Sauerstoffdruck (p''_{O_2}) als links (p'_{O_2}) vorgegeben sein. Dann wandert fortlaufend Nickel und Chrom von links nach rechts zu Orten höheren Sauerstoffpotentials unter Sauerstoffaufnahme aus dem Gasraum rechts und unter Anlage von neuen Netzebenen des Oxydgitters auf dieser Seite, während auf der linken Seite Abbau erfolgt. Da die Mengen an Nickel und Chrom vorgegeben sind, ist als Stationaritätsbedingung zu fordern, dass die transportierten Mengen (in Äquivalenten) sich wie die Äquivalentkon-

zentrationen der beiden Metalle verhalten. Da die Äquivalentkonzentrationen z_i mal so gross als die Konzentrationen in g-Atom sind, und da das Verhältnis von Chrom zu Nickel einfach durch den Chromgehalt x_{Cr} als Gitterkonzentration gegeben ist, so gilt:

$$\frac{N_{Cr^{3+}}}{N_{Ni^{2+}}} = \frac{x_{Cr}}{z_{Ni}} \cdot x_{Cr} = \frac{3}{2} \cdot x_{Cr} \quad \text{(unabhängig von } \xi\text{)} \tag{26}$$

Für das Verhältnis der wandernden Mengen folgt ferner durch Division von Gleichung (20) und (19):

$$\frac{N_{Cr^{3+}}}{N_{Ni^{2+}}} = \frac{2}{3} \cdot \frac{\varkappa_{Cr^{3+}}}{\varkappa_{Ni^{2+}}} \cdot \frac{d \ln a_{Cr}}{d \ln a_{Ni}} \tag{27}$$

Wie bereits erwähnt, wandern die Ni^{2+}-Ionen durch Sprung von Ni^{2+}-Ionen auf benachbarte Leerstellen. In gleicher Weise erfolgt auch die Wanderung von Cr^{3+}-Ionen. Das Verhältnis der Teilleitfähigkeiten für Cr^{3+}- und Ni^{2+}-Ionen ist somit zunächst durch das Verhältnis der Ladungszahlen ($= \frac{3}{2}$) und das Verhältnis der Zahl der Einzelsprünge bei vorgegebener elektrischer Feldstärke gegeben.

Bei ideal ungeordneter Verteilung von Cr^{3+}-Ionen und Ni^{2+}-Leerstellen wäre die Zahl der geometrischen Sprungmöglichkeiten von Cr^{3+}- und Ni^{2+}-Ionen auf eine benachbarte Leerstelle einfach durch das Mengenverhältnis der beiden Ionen gegeben. Da Cr^{3+}-Ionen und Ni^{2+}-Leerstellen entsprechend den entgegengesetzten Überschussladungen einander elektrostatisch anziehen, ist das Verhältnis der tatsächlich vorhandenen geometrischen Sprungmöglichkeiten um einen gewissen Faktor β grösser (also gleich $x_{Cr} \cdot \beta$).

Das Zahlenverhältnis der in Richtung der gegebenen Feldstärke wandernden Cr^{3+}- und Ni^{2+}-Ionen ist gleich dem Verhältnis der geometrischen Sprungmöglichkeiten $x_{Cr} \cdot \beta$ mal dem Verhältnis der Kräfte pro Ion (= Verhältnis der Ladungszahlen $= \frac{3}{2}$) mal dem Verhältnis der elementaren Beweglichkeiten B_2 und B_1 für ein Cr^{3+}-Ion bzw. Ni^{2+}-Ion neben einer Kationenleerstelle bei einer Kraft von 1 dyn pro Ion.

Insgesamt ergibt sich für das Verhältnis der Teilleitfähigkeiten:

$$\frac{\varkappa_{Cr^{3+}}}{\varkappa_{Ni^{2+}}} = \frac{3}{2} \cdot x_{Cr} \cdot \beta \cdot \frac{3}{2} \cdot \frac{B_2}{B_1} \tag{28}$$

$$\frac{d \ln a_{Cr}}{d \ln a_{Ni}} = \frac{1}{\beta} \cdot \frac{B_1}{B_2} \tag{29}$$

Die chemischen Aktivitäten a_{Ni} und a_{Cr} sind noch zu den Konzentrationen der Fehlordnungsstellen in Beziehung zu setzen. Einbau eines Nickelatoms in Nickeloxyd erfolgt durch Auffüllung einer Ni^{2+} -Leerstelle und zweier Elektronendefektstellen:

$$Ni + \square\,(Ni^{2+}) + 2\,\square\,(e^-) = Ni^{2+}(g) + 2\,e^-(g) \tag{30}$$

Die Gleichgewichtsbedingung lautet unter der Voraussetzung der Gesetzmässigkeiten verdünnter Lösungen:

$$a_{Ni} \cdot x_{\square\,(Ni^{2+})} \cdot (x_{\square\,(e^-)})^2 = K_2 \tag{31}$$

Einbau eines Chromatoms in Nickeloxyd erfolgt durch Auffüllung einer Ni^{2+} -Leerstelle sowie von 3 Elektronendefektstellen:

$$Cr + \square\,(Ni^{2+}) + 3\,\square(e^-) = Cr^{3+}{}_{(\text{an Stelle von } Ni^{2+})} + 3\,e^-(g) \tag{32}$$

Hierzu gehört die Gleichgewichtsbedingung:

$$a_{Cr} \cdot x_{\square(Ni^{2+})} \cdot (x_{\square(e^-)})^3 = K_3 \cdot x_{Cr} \tag{33}$$

Aus (31) und (33) folgt unter Benutzung von (6):

$$d \ln a_{Ni} = -d \ln x_{Cr} - 2\, d \ln x_{\square(e^-)} \tag{34}$$

$$d \ln a_{Cr} = -3\, d \ln x_{\square(e^-)} \tag{35}$$

Gleichung (34) wird mit $\frac{3}{2}$ multipliziert und von (35) substrahiert:

$$d \ln a_{Cr} = \frac{3}{2}\, d \ln a_{Ni} + \frac{3}{2}\, d \ln x_{Cr} \tag{36}$$

Hieraus ist die linke Seite von Gleichung (29) zu bilden:

$$\frac{d \ln a_{Cr}}{d \ln a_{Ni}} = \frac{3}{2} + \frac{3}{2} \frac{d \ln x_{Cr}}{d \ln a_{Ni}} = \frac{1}{\beta} \cdot \frac{B_1}{B_2} \tag{37}$$

Unter Benutzung der Beziehung $d \ln a_{Ni} = -\frac{1}{2}\, d \ln p_{O_2}$ nach (23) ergibt sich:

$$\frac{d \ln x_{Cr}}{d \ln p_{O_2}} = \frac{1}{2}\left(1 - \frac{2}{3} \cdot \frac{1}{\beta} \cdot \frac{B_1}{B_2}\right) \tag{38}$$

Wegen der höheren Ladung der Cr^{3+}-Ionen kann man ansetzen, dass diese Ionen entsprechend ihrer festeren Bindung weniger beweglich sind (also $B_2 < B_1$). Da jedoch der Faktor β als Maass für die elektrostatisch begünstigte Aufenhaltswahrscheinlichkeit von Cr^{3+}-Ionen neben Ni^{2+}-Leerstellen grösser als 1 ist, erweist sich eine einfache Voraussage über den Zahlenwert der Grösse $\frac{1}{\beta} \cdot \frac{B_1}{B_2}$ in Gleichung (38) als nicht möglich.

Im folgenden wird zur Abkürzung für die rechte Seite der Gleichung (38) das Symbol γ eingeführt:

$$\gamma = \frac{1}{2} \cdot \left(1 - \frac{2}{3} \cdot \frac{1}{\beta} \cdot \frac{B_1}{B_2}\right). \tag{39}$$

Gleichung (38) wird integriert. Als Grenzen werden die Zustandswerte p'_{O_2} und p''_{O_2} and den Phasengrenzflächen links und rechts (vgl. Abb. 2) eingesetzt Die zugehörigen Chromgehalte werden mit x'_{Cr} und x''_{Cr} bezeichnet. Nach Delogarithmierung ergibt sich:

$$\frac{x''_{Cr}}{x'_{Cr}} = \left(\frac{p''_{O_2}}{p'_{O_2}}\right)^{\gamma} \tag{40}$$

Nach Gleichung (38) und (39) kann für $d \ln p_{O_2}$ in (24) die Grösse $\frac{1}{\gamma} \cdot d \ln x_{Cr}$ eingesetzt werden. Gleichzeitig ist gemäss unseren Modellvorstellungen die Ni^{2+}-Teilleitfähigkeit $\varkappa_{Ni^{2+}}$ proportional der Ni^{2+}-Leerstellenkonzentration bzw. nach (6) auch proportional dem Chromgehalt zu setzen:

$$\varkappa_{Ni^{2+}} = K_0 \cdot \varkappa_{\square(Ni^{2+})} = \frac{1}{2} K_0 \cdot x_{Cr} \tag{41}$$

Aus (24), (38) und (41) folgt, dass die Transportgeschwindigkeit $N_{Ni^{2+}}$ unmittelbar proportional dem Konzentrationsgefälle an Chrom wird:

$$N_{Ni^{2+}} = q \cdot \frac{300}{96500} \cdot \frac{RT}{\varepsilon L} \cdot \frac{K_0}{8\gamma} \cdot \frac{dx_{Cr}}{d\xi} \tag{42}$$

Im stationären Zustand einer Schicht mit konstanter Dicke muss aber die Nickeltransportgeschwindigkeit unabhängig von der Ortskoordinate ξ sein. Folglich muss das Konzentrationsgefälle an Chrom konstant sein, also die

Chromkonzentration eine lineare Funktion der Ortskoordinate ξ. Hieraus folgt weiter, dass der mittlere Chromgehalt $\bar{x}_{Cr}$ der Schicht gleich dem arithmetischen Mittel an den Phasengrenzen ist:

$$\bar{x}_{Cr} = \frac{1}{2}(x'_{Cr} + x''_{Cr}) \tag{43}$$

Aus den beiden Gleichungen (40) und (43) sind die Chromgehalte x'_{Cr} und x''_{Cr} an den Phasengrenzen zu berechnen, wenn die zugehörigen Sauerstoffdrucke p'_{O_2} und p''_{O_2}, sowie der mittlere Chromgehalt x_{Cr} der Schicht gegeben ist:

$$x'_{Cr} = \frac{2\bar{x}_{Cr}}{1 + (p''_{O_2} / p'_{O_2})} \tag{44}$$

$$x''_{Cr} = \frac{2\,\bar{x}_{Cr} \cdot (p''_{O_2}/p'_{O_2})^{\gamma}}{1 + (p''_{O_2}/p'_{O_2})} \tag{45}$$

Die Differenz dieser Werte dividiert durch die Dicke $\Delta\xi$ der Oxydschicht ergibt das Konzentrationsgefälle $dx_{Cr}/d\xi$. Nach Einsetzen in (42) ergibt sich für die Transportgeschwindigkeit:

$$N_{Ni^{2+}} = \frac{q}{\Delta\xi} \cdot \frac{300}{96500} \cdot \overline{\varkappa_{Ni^{2+}}} \cdot \frac{1}{4} \cdot \frac{RT}{\varepsilon L} \cdot \frac{2}{\gamma} \cdot \frac{(p''_{O_2}/p'_{O_2})^{\gamma} - 1}{(p''_{O_2}/p'_{O_2})^{\gamma} + 1} \tag{46}$$

Hierin ist $\overline{\varkappa_{Ni^{2+}}}$ die spezifische elektrische Teilleitfähigkeit, die dem mittleren Chromgehalt entspricht; auf Grund von Gleichung (41) gilt:

$$\overline{\varkappa_{Ni^{2+}}} = \frac{1}{2} K_0 \cdot \overline{x_{Cr}} \tag{47}$$

Hierbei sind folgende Fälle zu unterscheiden:

1. Wenn $\gamma = 0$, nach (39) also $\beta \cdot (B_2/B_1) = \frac{2}{3}$ ist, dann ist nach (38) für den Chromgehalt kein Konzentrationsgefälle vorhanden. Gleichung (46) ergibt im Grenzübergang zu $\gamma = 0$:

$$N_{Ni^{2+}} = \frac{q}{\Delta\xi} \cdot \frac{300}{96500} \overline{\varkappa_{Ni^{2+}}} \cdot \frac{1}{4} \cdot \frac{RT}{\varepsilon L} \cdot \ln \frac{p''_{O_2}}{p'_{O_2}} \qquad \text{(für } \gamma = 0\text{)} \tag{48}$$

Die Transportgeschwindigkeit $N_{Ni^{2+}}$ ist nur langsam mit dem Sauerstoffdruck veränderlich, da dieser nur logarithmisch eingeht.

2. Wenn $\gamma > 0$, also $\beta \cdot (B_2/B_1) > \frac{2}{3}$ ist, dann findet nach (38) Chromanreicherung auf der Seite des höheren Sauerstoffpartialdrucks statt. Für $p''_{O_2} >> p'_{O_2}$ ist der letztstehende Bruch in (46) praktisch gleich 1, also die Transportgeschwindigkeit näherungsweise unabhängig von den Sauerstoffdrucken. Dieser Ausdruck ist von gleicher Grössenordnung wie unter Fall 1), da an Stelle des Faktors $\ln \frac{p''_{O_2}}{p'_{O_2}}$ in (48) der Faktor $\frac{2}{\gamma}$ tritt; vgl. Formel (49).

3. Wenn $\gamma < 0$, also $\beta \cdot (B_2/B_1) < \frac{2}{3}$, dann findet nach (38) Chromanreicherung auf der Seite des kleineren Sauerstoffpartialdrucks statt, indem das Chrom dem Nickel nur langsam folgt. Für $p''_{O_2} << p'_{O_2}$ wird der letztstehende Bruch in (46) gleich —1. Für die Transportgeschwindigkeit ergibt sich für Grenzfall 2 und 3 die gleiche Formel:

$$N_{Ni^{2+}} = \frac{q}{\Delta\xi} \cdot \frac{300}{96500} \cdot \overline{\varkappa_{Ni^{2+}}} \cdot \frac{1}{4} \cdot \frac{RT}{\varepsilon L} \cdot \left|\frac{2}{\gamma}\right| \quad (\text{für } \gamma \gtrless 0 \text{ und } p''_{O_2} \ll p'_{O_2}) \tag{49}$$

Vorstehende Ausdrücke sind mit dem entsprechenden Ausdruck für reines Nickeloxyd zu vergleichen. Nach Gleichung (4) ist die Ni^{2+}-Leerstellenkonzentration und damit auch die Ni^{2+}-Teilleitfähigkeit proportional der 6. Wurzel der Sauerstoffdrucks. Im besonderen kann unter Einführung der Ni^{2+}-Teilleitfähigkeit $\varkappa''_{Ni^{2+}}$ für Nickeloxyd unter dem Sauerstoffdruck p''_{O_2} gesetzt werden:

$$\frac{\overline{\varkappa_{Ni^{2+}}}}{\sqrt[6]{p'_{O_2}}} = \frac{\overline{\varkappa''_{Ni^{2+}}}}{\sqrt[6]{p''_{O_2}}} \tag{50}$$

Diese Bezeichung wird in Gleichung (24) eingesetzt, linke und rechte Seite mit $d\xi$ multipliziert und integriert. Hierbei ist die Transportgeschwindigkeit $N_{Ni^{2+}}$ aus Stationäritätsgründen unabhängig von ξ. Nach Division mit $\Delta\xi$ folgt:

$$N_{Ni^{2+}\,(\text{in NiO rein})} = \frac{q}{\Delta\xi} \cdot \frac{300}{96500} \cdot \overline{\varkappa_{Ni^{2+}}} \cdot \frac{6}{4} \frac{RT}{\varepsilon L} \cdot \left[1 - \sqrt[6]{\frac{p'_{O_2}}{p''_{O_2}}}\right] \tag{51}$$

Für $p''_{O_2} \gg p'_{O_2}$ ist die letztstehende Wurzel als substraktives Glied gegen 1 zu vernachlässigen. Aus dem Vergleich von (48) bzw. (49) mit (51) ergibt sich, dass das Verhältnis der Transportgeschwindigkeiten für Ni^{2+}-Ionen im Mischkristall und im reinem NiO im wesentlichen durch das Verhältnis der Ni^{2+}-Teilleitfähigkeiten bzw. Ni^{2+}-Leerstellen im Mischkristall und reinem Nickeloxyd unter dem höheren Druck p''_{O_2} bestimmt ist. Wenn gemäss allgemeiner Voraussetzung der Chromgehalt im Mischkristall gross gegen die Ni^{2+}-Leerstellenkonzentration im reinen Nickeloxyd unter dem Sauerstoffdruck p''_{O_2} ist, dann soll auch die Transportgeschwindigkeit im Mischkristall gross gegenüber dem Wert für reines Nickeloxyd sein.

Aus den vorstehenden Gleichungen ergeben sich noch folgende Einzelaussagen über die treibenden Kräfte der Ionenbewegung:

1. Wenn $\beta \cdot (B_2/B_1) = \frac{2}{3}$, dann ist nach Gleichung (38) der Chromgehalt im Nickeloxyd an verschiedenen Stellen mit verschiedenem Sauerstoffdruck gleich gross. Dann existiert in der Anlaufschicht weder für die Ni^{2+}-Leerstellen noch für den Chromgehalt ein wesentliches Konzentrationsgefälle. Die Wanderung von Ni^{2+}- und Cr^{3+}-Ionen in der Anlaufschicht erfolgt in diesem Falle ausschliesslich unter der Wirkung des Diffusionspotentials (wie z.B. die Wanderung von Ag^+-Ionen in einer αAg_2S-Schicht gemäss den Angaben in Wagner[1b]). Das Diffusionspotential ist nach Gleichung (13) durch das Gefälle des chemischen Potentials der Elektronen bestimmt. Unter Benutzung von Gleichung (13) und (34) ist zu setzen:

$$\frac{d\psi}{d\xi} = \frac{1}{\varepsilon L} \cdot \frac{d\mu_e^-}{d\xi} = -\frac{RT}{\varepsilon L} \cdot \frac{d \ln x_{\square(e')}}{d\xi} = \frac{RT}{\varepsilon L} \cdot \left[\frac{1}{2} \frac{d \ln a_{Ni}}{d\xi} + \frac{1}{2} \frac{d \ln x_{Cr}}{d\xi}\right] \qquad (52)$$

Mit (23) und (38) folgt weiter:

$$\frac{d\varphi}{d\xi} = -\frac{1}{4} \frac{RT}{\varepsilon L} \cdot \frac{2}{3} \cdot \frac{1}{\beta} \cdot \frac{B_1}{B_2} \cdot \frac{d \ln p_{O_2}}{d\xi} \qquad (53)$$

Im speziellen gilt für $\beta \cdot (B_2/B_1) = \frac{2}{3}$:

$$\frac{d\varphi}{d\xi} = -\frac{1}{4} \cdot \frac{RT}{\varepsilon L} \cdot \frac{d \ln p_{O_2}}{d\xi} \qquad \left(\text{für } \beta \cdot \frac{B_2}{B_1} = \frac{2}{3}\right) \qquad (54)$$

Aus Gleichung (54) ist nunmehr quantitativ ersichtlich, um wieviel das elektrische Potential an Orten höheren Sauerstoffdrucks negativer ist, sodass hierdurch Ni^{2+}- und Cr^{3+}-Ionen in dieser Richtung auch ohne Konzentrationsgefälle wandern.

2. Wenn $\beta \cdot (B_2/B_1) < \frac{2}{3}$, dann ist der Chromgehalt und damit nach (6) auch die Ni^{2+}-Leerstellenkonzentration gerade an Orten mit niedrigem Sauerstoffpotential besonders gross. Die Nickelionen wandern auch in diesem Falle nach der allgemeinen Gleichung (24) nach Orten höheren Sauerstoffdrucks, hier mit geringer Ni^{2+}-Leerstellenkonzentration, da $d\psi/d\xi$ nach (53) stärker negativ als nach (54) wird, und infolgedessen erzwingt das Diffusionspotential eine Wanderung entgegen dem Konzentrationsgefälle.

3. Wenn $\beta \cdot (B_2/B_1) > \frac{2}{3}$, dann erfolgt nach (38) eine Chromanreicherung an den Stellen eines höheren Sauerstoffdrucks. Somit wandern Ni^{2+}-Ionen zu Orten mit höherer Ni^{2+}-Leerstellenkonzentration (also zu Orten kleinerer Ni-Konzentration). Das Diffusionspotential liefert hier nach (53) einen kleineren Beitrag als nach Gleichung (54).

Vorstehende Überlegungen gelten zunächst für die stationäre Bewegung in einer Ni-Cr-Oxydschicht von fest vorgegebener Schichtdicke. Für die Vorgänge bei der Oxydation von Ni-Cr-Legierungen mit stetig wachsender Dicke der Oxydschicht kann die bisherige Stationaritätbedingung (26) zur Berechnung der örtlichen Verteilung des Chroms in Oxyd-Mischkristall in Strenge nicht benutzt werden. Wenn $\beta \cdot (B_2/B_1)$ kleiner oder grösser als $\frac{2}{3}$ ist, dann reichert sich Chrom in der wachsenden Schicht entweder auf der Metallseite oder auf der Aussenseite fortlaufend an. Dort ist notwendigerweise die Zudiffusion von Chrom grösser als die Wegdiffusion von Nickel. Bei wachsender Schicht ist anzusetzen, dass die Zusammensetzung der Oxydphase bei gegebenem Verhältnis des Abstandes von der Legierungsoberfläche zur Gesamtschichtdicke zeitlich konstant bleibt. Diese Art der Stationaritätsbedingung lässt sich jedoch nicht nach den sonst bekannten Verfahren zur Berechnung des Konzentrationsgefälles innerhalb der Oxydphase weiterverarbeiten, sodass auf die Formulierung an dieser Stelle verzichtet wird. Es darf jedoch angenommen werden, dass qualitativ die Schlussfolgerungen die gleichen bleiben wie für die Transportgeschwindigkeit in einer Schicht vorgegebener Dicke nach Gleichung (46), (48) und (49).

Eine besondere Erläuterung ist noch für die Höhe des mittleren Chromgehaltes in Anlaufschichten auf Ni-Cr-Legierungen erforderlich. Falls die Platzwechselgeschwindigkeit in der Metallphase klein gegenüber der Platzwechselgeschwindigkeit im Mischkristall angenommen wird, dann ist der mittlere Chromgehalt der Oxydphase gleich dem Chromgehalt der Legierung. Andernfalls geht das Chrom als unedler Bestandteil bevorzugt in die Oxydphase und diffundiert innerhalb der Metallphase in Richtung auf die Phasengrenze Metall/Oxyd, wie an anderen Systemen von verschiedenen Autoren diskutiert [9]. Mangels Unterlagen erübrigt sich eine Untersuchung an dieser Stelle. Zunächst kann man grössenordnungsmässig den mittleren Chromgehalt der

Anlaufschicht gleich dem Chromgehalt der zu oxydierenden Legierung setzen.

Die vorstehenden Ausführungen sind sinngemäss auf andere Systeme zu übertragen. Von allgemeineren Formulierungen wird hier abgesehen, da die einigermassen verwickelten Beziehungen unter stetigem Hinweis auf einen konkreten Einzelfall leichter verständlich sind. Der Umfang der sonstigen Anwendungsmöglichkeiten ist noch nicht abzuschätzen, da zur Zeit die Kenntnis der Fähigkeit von oxydischen und sulfidischen Phasen zur Mischkristallbildung noch ausserordentlig beschränkt ist.

ZUSAMMENFASSUNG

Die Oxydationsgeschwindigkeit von Nickel wird durch kleine Zusätze von Chrom und von Mangan erheblich erhöht. Dieser Befund wird durch Bildung einer heterotypen Mischoxydphase mit erhöhter Ni^{2+} -Leerstellenkonzentration und dementsprechend grösserer Platzwechselhäufigkeit der Ni^{2+} -Ionen im Vergleich zu reinem Nickeloxyd gedeutet. Die treibenden Kräfte für Wanderungsvorgänge in derartigen Mischozydphasen werden im einzelnen analysiert. Bei Ni-Cr-Legierungen mit höherem Cr-Gehalt sinkt die Oxydationsgeschwindigkeit wieder ab; in Analogie zu anderen Systemen ist die Bildung einer neuen Phase mit geringerer Ionen-Beweglichkeit anzunehmen.

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Neue Versuche mit beweglicher calorimetrischer Bombe

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In der Svedberg-Festschrift berichteten Smith und Sunner über Bestimmungen von Verbrennungswärmen mit schaukelnder, bzw. rotierender Bombe. Es wurde gezeigt, dass die Verbrennungswärmen von chlorhaltigen Substanzen durch Schaukeln der Bombe und von bromhaltigen durch Rotieren derselben mit genügender Genauigkeit bestimmt werden konnten.

Die Schaukelversuche schienen indessen insofern nicht zufriedenstellend, als die benutzte, ziemlich komplizierte mechanische Vorrichtung nur eine verhältnismässig kleine Schaukelamplitude gestattete: nur etwa 50° von der vertikalen Stellung. Infolgedessen war die Reduktion des Chlors durch die arsenige Säure der Bombenlösung, wahrscheinlich eben wegen unzureichender Bewegung der Bombe, bei sehr chlorreichen Substanzen unvollständig, wenn auch der Fehler in den Verbrennungswärmen zu vernachlässigen war. Daneben war die Korrektur für die Friktionswärme bei dieser Vorrichtung nicht unbedeutend: 0,001° bei einer Temperaturerhöhung von etwa 1°.

Die Erfinder der ersten Schaukelbombe (Popoff und Schirokich [1]) erwähnen, dass ihre Bombe mit Hilfe von zwei Schnüren bewegt wurde, die an einem auf der Bombenachse befindlichem Rad befestigt waren. Weitere Einzelkeiten werden nicht angegeben.

Nun schien es zunächst möglich, mit Hilfe von Rad und Schnüren die *vertikal* aufgehängte Bombe *rotieren* zu lassen, wodurch ja eine ebenso vollkommene Berührung von Gas- und Flüssigkeitsphase erzielt werden könnte wie durch Schaukeln. — Die in der Mitteilung von Smith und Sunner erwähnte rotierende Bombe beschreibt eine Bewegung in *horisontaler* Lage. — Wir hatten ursprünglich beabsichtigt, die schaukelnde Bewegung einfach durch Handkraft unter Zuhilfenahme eines Metronoms zu bewirken. Durch Wegnahme der komplizierten Schaukelvorrichtung von Smith und Sunner könnten auch viel grössere Amplituden erreicht werden. Da man erwarten konnte, dass eine Bewegung durch Handkraft nicht so gut reproduzierbar

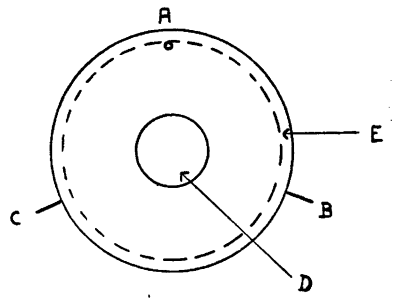

Fig. 1. Die zu den Versuchen benutzte Schaukelvorrichtung.

wäre wie die durch eine mechanische Vorrichtung bewirkte, haben wir zu den Versuchen mit Schaukelbewegung die in Fig. 1 wiedergegebenen Konstruktion* benutzt:

In der Figur stellt E das auf der Aufhängungsachse (D) befestigte Rad dar, B und C sind Zapfen am Rade. Bei A hat ein Kupferdraht (Diameter 0,3 mm) seinen Endpunkt. Von A aus geht der Draht in einer Spur bis B, wendet um den Zapfen und geht über A weiter nach C, nach Wendung weiter nach B, u. s. w. Beim Aufwinden des Drahtes (durch ein Loch in Kalorimeterdeckel), welches mit konstanter Geschwindigkeit geschieht, erhält die Bombe eine schaukelnde Bewegung, und zwar können Amplituden bis 100° in jeder Richtung erzielt werden. Betreffs möglicher Wasserverluste durch den Draht und Friktionswärme gilt, was Smith und Sunner hinsichtlich der horizontalen Rotationsbombe angeführt haben. Besondere Bestimmungen dieser Grössen brauchen nicht vorgenommen zu werden.

Man kann nach Belieben Verbrennungen entweder mit schaukelnder oder mit rotierender Bombe ausführen. Das letztgenannte Verfahren ist aus dem Grunde am vorteilhaftesten, da bei vollständiger Umdrehung die Reduktion des freien Halogens am schnellsten vor sich geht und alle Konzentrationsunterschiede sicher ausgeglichen werden. Indessen hat es sich herausgestellt, dass beim Rotieren Tiegel und Schild bisweilen in der festen Armatur der Bombe hängen bleiben, was ein Misslingen der Verbrennung zur Folge haben kann. Am vorteilhaftesten scheint es zu sein, in einem Versuche die Bombe zuerst schaukeln, dann rotieren zu lassen (s. u. bei Äthylenbromid).

Die Methode wurde mit Hilfe von Bromverbindungen ausgearbeitet, die ja in höherem Grade als Chlorverbindungen geeignet sind, zur Prüfung auf die Verwendbarkeit der Methode zu dienen. Es wurden α-Bromnaphthalin, Brombenzol und Äthylenbromid verbrannt.

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* Konstruktion von Bjellerup.

Bei Verbrennung von *α-Bromnaphthalin* führte die Bombe 20 halbe Schaukelbewegungen (Zeit 75 ± 3 s) aus, bei *Brombenzol* 12 vollständige Umdrehungen (Zeit 82 ± 4 s) und schliesslich bei *Äthylenbromid* 6 halbe Schaukelbewegungen nebst 9 Umdrehungen (Zeit 86 ± 4 s). Die Bewegungen wurden 30 s nach der Zündung in Gang gesetzt. Zu dieser Zeit ist die Verbrennung der Substanz vollständig.

Die Vollständigkeit der Bromreduktion wurde zweimal in der Weise geprüft, dass unmittelbar nach dem Abschlusse einer Verbrennung die Bombengase durch Natronlauge in einer Effektivwaschflasche geleitet wurden. Es konnte in der Lauge kein Bromid nachgewiesen werden. Die Armatur ist beim Öffnen der Bombe oft von einer braungefärbten Flüssigkeit befeuchtet (wahrscheinlich freies Brom in Bromid gelöst). Wir fanden indessen, dass die von diesem Fehler herrührende Korrektur sehr klein ist. Auch wenn angenommen wird, dass alles Brom (auch das Bromid) in der Armaturflüssigkeit als freies Element vorliegt, betrüge die Korrektur nur etwa −0,4 cal.

Die Substanzen (alle drei flüssig) wurden nach Verkade und Coops [2, 3] zur Verbrennung in kleinen Glaskölbchen zusammen mit Paraffin eingewägt. Einige Einzelheiten wurden verbessert zur Vermeidung von zufälliger Russbildung, wie später von Bjellerup beschrieben wird. Übrige experimentelle Anordnungen und Versuchsbedingungen nach Smith und Sunner, deren Präparate auch benutzt wurden: sie wurden durch Destillation gereinigt und ihre Reinheit durch Bestimmung von Dichte und Brechung kontrolliert. Wir finden es überflüssig, diesbezügliche Ziffern hier anzuführen. Die gefundenen Konstanten stimmten gut mit den Angaben der Literatur überein.

Für die wahrscheinlich sehr kleine Friktionswärme wurde in der Weise korrigiert, dass die Bestimmung des Wasserwertes (mit Benzoesäure) in genau derselben Weise wie die Bestimmung der Verbrennungswärme geschah, für α-Bromnaphthalin also mit Schaukelbewegung, für Brombenzol mit Umdrehen. Da zwischen diesen beiden Bestimmungen einige Änderungen in der Bombe vorgenommen wurden, die den Wasserwert veränderten, können wir auf Grund des vorliegenden Ziffermaterials nicht behaupten, dass der »Schaukelwasserwert» und der »Umdrehungswasserwert» gleich gross sind. Dass dies der Fall ist, halten wir für sehr wahrscheinlich. Eine Bestimmung unter Schaukeln + Umdrehen ergab den Wasserwert 4709 cal, während bei Umdrehen 4710 ± 2 cal gefunden wurde. Für Äthylenbromid (verbrannt mit Schaukeln + Umdrehen) wurde mit dem Schaukel-Wasserwert gerechnet.

Als Beispiel für die Genauigkeit einer Wasserwertbestimmung (Umdrehen) führen wir an (Benzoesäure 0,9 g, Temp.-Erhöh. 1,1 —1,2°):

4692 4694 4697 4694 4692 M = 4694 ± 1,5 cal

Beim Schaukeln ist die Genauigkeit mindestens ebenso gut. Verbrennungswärme des Paraffinöls 10990 ± 4 cal; Bombenlösung 30,00 ml etwa 0,25 *N* arsenige Säure; Sauerstoffdruck 30 Atm.

In den Tabellen bedeuten: m = mg Substanz; m_1 = mg Paraffinöl; Q = Verbrennungswert in cal/g; red. = reduziertes Brom in %; Br = wiedergefundenes Brom in %.

Tabelle 1. α-Bromnaphthalin.

m	m_1	Q	red.	Br
441,10	296,80	5800	89	98,6
437,90	298,90	5801	90	99,8
440,60	301,80	5802	91	99,2
457,55	294,40	*5798*	91	99,4

M = 5800 ± 1 (Smith und Sunner: 5800,5 ± 1,5)

Tabelle 2. Brombenzol.

m	m_1	Q	red.
491,30	294,90	4737	91
455,00	224,30	4738	92
466,95	315,05	*4736*	90

M = 4737 (Smith und Sunner: 4743 ± 1)

Tabelle 3. Äthylenbromid.

m	m_1	Q	red.
473,00	431,00	1576	94
537,85	432,30	1578	95
423,00	460,20	*1580*	92

M = 1578 (Smith und Sunner: 1588 ± 1,5)

Einige Werte, die unter Russbildung verliefen, wurden verworfen. Die Abweichung bei Äthylenbromid liegt noch innerhalb der Versuchsfehler, kann andererseits auch davon herrühren, dass dieses Präparat nicht so rein gewesen ist wie dasjenige von Smith und Sunner.

Washburn-Korrektur ist nicht angebracht worden.

ZUSAMMENFASSUNG

Die Untersuchung zeigt, dass die hier benutzte vereinfachte Methode für Schaukeln und Umdrehen einer calorimetrischen Bombe für die Bestimmung der Verbrennungswärmen von bromhaltigen (und auch von chlorhaltigen) Verbindungen gut verwendbar ist.

Für Unterstützung von Seiten des *Kemiska Nobelfonden* spricht der eine von uns (Sm.) seinen herzlichen Dank aus.

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Crystalline Lipoxidase

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The purification of soy bean lipoxidase, an enzyme capable of promoting the oxidation of linoleic acid and related substances, has been attempted by several workers. Süllman [1] prepared an acetone powder of the enzyme containing full activity but only 27 % of the dry substance of the water extract. Cosby and Sumner [2] have reported a 60-fold purification of the enzyme, and Balls, Axelrod, and Kies [3] succeeded in effecting a purification 115 times that of a 2.5 % water extract of soy bean meal. Theorell, Bergström and Åkeson [4] obtained a preparation electrophoretically homogeneous having an activity of about 130 units per milligram. Later these authors [5] reported a preparation which was again homogeneous electrophoretically, but which this time showed an activity of 221 units per milligram.

The present investigation was begun in the attempt to improve the method of isolation of lipoxidase so as to obtain larger yields of pure enzyme intended to be used in the studies of the properties and activity of lipoxidase. A preliminary account of this work has appeared previously [6].

EXPERIMENTAL

The lipoxidase activity determinations were carried out using the spectrophotometric method described previously [5]. The only modification was the use of side-arm test tubes with rubber stoppers rather than the Thunberg tubes used previously. These were filled under a stream of oxygen, thus avoiding the danger of mixing by foaming encountered when evacuating the Thunberg tubes. The activity unit used is defined as the amount of enzyme that produces one micro-equivalent of peroxide (or conjugated diene) in one minute. It was often found convenient to express the purity of the enzyme preparations as units per density unit per cm at 280 mμ.

* National Research Council Fellow 1946—47.

RESULTS AND DISCUSSION

Preliminary experiments to determine the effect of pH of the extraction fluid upon the degree of extraction of lipoxidase were made using 6.6 ml fluid per gram of fat-free soy meal. The results shown in Table 1 indicated that an extraction with acetate buffer at pH 4.5 resulted in only a slight loss in yield, but a two-fold increase in purity over the water extraction was gained.

Table 1. The effect of pH of the extraction fluid upon the degree of extraction of lipoxidase.

	pH of the extraction fluid				
	4.0	4.5	5.0	5.5	Water
Units/ml	156	240	189	213	276
Units/mg N	95	104	60	52	48

Attempts to precipitate the enzyme from this extract with basic lead acetate, as had been done in previous preparations in this laboratory, indicated that excessive quantities of lead were necessary to cause any precipitation of the enzyme. In attempts to find another precipitant it was found that barium acetate precipitated non-active material, and that acetone reduced the amount of barium necessary. It was also found that small quantities of lead removed further inactive material. A comparison of the purity of the original extract itself with those after various treatments showed that the combined treatment with barium acetate, acetone, and basic lead acetate gave the largest increase in purity without loss in yield. However, attempts at ammonium sulfate fractionation of the extract thus obtained showed that the activity could not be recovered; but if the pH were raised to 6.7 with ammonia prior to the addition of barium acetate, acetone, and lead acetate, the activity could be recovered by precipitation with ammonium sulfate.

The use of buffer at pH 4.5 as extraction medium and the use of barium acetate as a precipitant of inactive material were prompted by the suspicion that the substance, which had been so difficult to separate from the enzyme in previous preparations, may have been a carbohydrate, perhaps phytic acid. The work of Fontaine, *et al.*[7], shows that the solubility of the phosphorus present in fat-extracted soy bean flour is greatly reduced at pH 4.0—4.5, and thus an extraction of soy flour in this pH range should probably reduce the amount of phytin extractable. Barium acetate was used in the belief that it would remove most of the remaining phytic acid. Preparations in which these procedures were not used always showed the presence of brown, sirupy material in the later stages of the purification, and this substance could not be separated

from the enzyme by any means tried. However, when the initial extraction was made at pH 4.5 followed by barium-acetone-lead precipitation at pH 6.7, this gummy material was no longer encountered.

Using the above preliminary experiments as a guide, a large preparation was made as follows: 6 kg of de-fatted soy meal were suspended in 40 liters $M/10$ acetate buffer at pH 4.5 and passed through a basket centrifuge to remove undissolved material. Yield — 4.2 million units. The supernatant fluid was adjusted to pH 6.7 with ammonia, and 5 volumes of 20 % barium acetate solution, 10 volumes of acetone and 2 volumes of 20 % basic lead acetate were added per 100 volumes of extract. The inactive precipitate was removed in a large separator. Yield 2.9 million units. The inactive slight precipitate formed upon the addition of 25 g of ammonium sulfate per 100 ml of extract was removed by sedimentation and decantation. The active precipitate formed upon the addition of ammonium sulfate to bring the concentration to 40 g per 100 ml of extract was removed in the same manner and redissolved in a minimum of water. Yield — 2.8 million units. After heating the solution at 63° for 5 minutes, a bulky, inactive precipitate was centrifuged off, and the remaining preparation containing 2.9 million units had an activity of 90 units per mg. Refractionation between 37 and 50 % saturation with ammonium sulfate reduced the yield to 2.4 million units but increased the activity to 230 units per milligram. This preparation was equal in activity to the best previously-reported preparation, but was still obviously not pure because of its intense, brown color.

The preparation was then dialysed against $M/50$ phosphate buffer at pH 5.5 and the precipitate which formed was discarded. Ninety per cent alcohol was added at 0° to give an alcohol concentration of 3.0 %. The active precipitate was centrifuged off and redissolved in $M/50$ phosphate buffer at pH 7.5. The preparation at this stage contained 0.85 million units at 502 units per milligram. Electrophoresis in the small Tiselius apparatus showed the presence of three components, of which lipoxidase was 63.5 %. Thus the pure enzyme should have an activity of about 790 units/mg. At the end of the electrophoresis a fraction having an activity of 748 units/mg was separated. The bulk of the preparation (502 U/mg) was subjected to electrophoresis in the large Tiselius apparatus, and a fraction containing 0.12 million units at 610 U/mg was obtained.

A larger-scale repetition of this procedure was made with 15 kg of soy flour and 100 l of $M/10$ acetate buffer at pH 4.5. At the stage after heating to 63° for 5 minutes 11.4 million units remained, but in the subsequent fractionation between 35 and 50 % saturation with ammonium sulfate, only 7.8 million units of enzyme at an activity of 45 U/D_{230} could be removed. Frac-

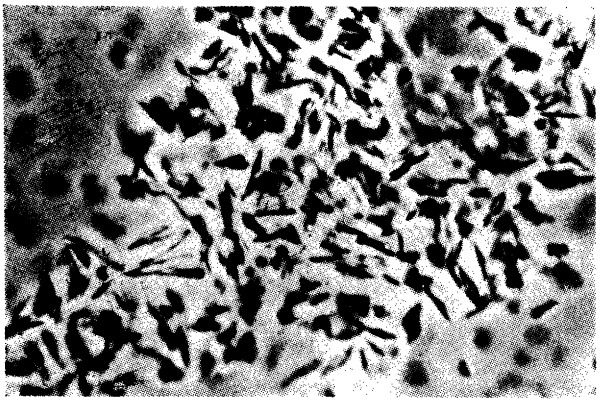

Figure 1. Crystalline lipoxidase (1200 ×).

tionation with alcohol this time required 12 % alcohol to precipitate the enzyme, of which 7.6 million units were recovered, having an activity of 257 U/D_{280}. In the fractionation of this preparation with ammonium sulfate, the fraction between 35 and 50 % saturation yielded 2.9 million units at 231 U/D_{280}, and the fraction between 50 and 60 % saturation yielded 1.8 million units at 358 U/D_{280}. Electrophoresis of this latter fraction showed it to contain two minor components and 58 % lipoxidase. The small fraction of supposedly pure enzyme obtained at the end of the electrophoresis had an activity of 725 U/mg and had a D_{280} of 1.75 per mg per ml per cm. The bulk of this fraction (50—60) was subjected to electrophoresis in the large Tiselius apparatus, and 0.34 million units of lipoxidase, having 471 U/D_{280}, or approximately 820 U/mg, were separated.

Crystallization of this latter preparation was accomplished by slowly dialysing a concentrated solution of the enzyme against ammonium sulfate of slowly increasing concentration. Microscopic, colorless plates or sheaves shown in Figure 1, crystallized out. The small amount of amorphous material remaining in the preparation was dissolved away by washing the preparation at a slightly lower ammonium sulfate concentration. The crystals, entirely free from amorphous material, assayed 479 U/D_{280} or 850 U/mg, and the washings assayed 457 U/D_{280}, or approximately 810 U/mg. The pure enzyme represents a purification of about 70 times that of the original extract, or about 150 times that of a water extract.

The fraction from which the enzyme was crystallized was shown to be homogeneous electrophoretically at pH 6.0 in the small Tiselius apparatus. The crystalline sample was found to be homogeneous according to sedimentation and diffusion patterns. The sedimentation constant, determined on the

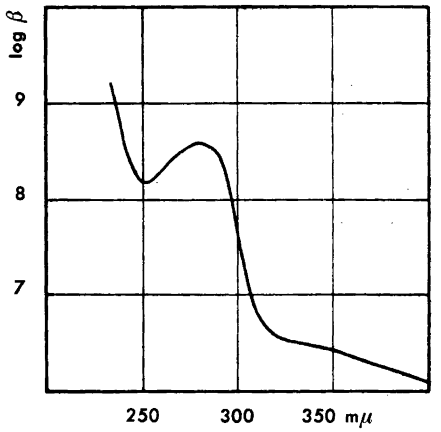

Figure 2. Absorption spectrum of crystalline lipoxidase.
($\beta = ln \frac{I_0}{I} \cdot \frac{l}{c} \cdot \frac{l}{d}$ when c = moles/ml).

crystalline material, was found to be 5.62×10^{-13}, the diffusion constant 5.59×10^{-7}, and the partial specific volume 0.750. The molecular weight was thus found to be 102,000. At pH 5.92 lipoxidase migrates anodically at a velocity equal to $u = 1.81 \times 10^{-5}$, and at pH 3.97 it migrates cathodically at a velocity of $u = 4.63 \times 10^{-5}$. Thus the isoelectric point is approximately pH 5.4. A mobility curve made with more impure material agreed very well with these values.

The absorption spectrum (Figure 2) is that of a common protein with a somewhat greater than average absorption at 280 mμ, indicating the probability that lipoxidase has a comparatively large amount of tyrosine or tryptophane in its structure. No evidence of a prosthetic group being present was indicated by the light absorption curve.

It was previously reported from this laboratory that lipoxidase contained nearly one atom of iron per molecule. In a series of determinations of iron on impure samples of lipoxidase obtained during the course of this investigation, the iron bore no relationship to the degree of purity. The iron content of a sample 94 % pure was found to be sufficient for one atom iron per molecule if its molecular weight were 370,000. It is thus clear that the iron must be present only as an impurity. This is in agreement with the observations that the crystalline lipoxidase is not inhibited by fluoride, azide, cyanide, pyrophosphate, or diethyl dithiocarbamate.

It should be pointed out that the procedure as outlined can not be rigidly applied without the use of pilot experiments. The two accounts of large preparations which have been given serve to illustrate the variation in method that can be expected from two batches of the same soy meal. Application of the same procedure to a sample of finely-ground soy meal from the Archer Daniels

Midland Company of Minneapolis gave a low yield of very impure enzyme. It is clear that the method of isolation has to be adapted to the soy meal available.

It is interesting to notice that this homogeneous enzyme preparation does not need any activitor under our assay conditions. The turnover number for lipoxidase acting upon linoleic acid at 20° is of the magnitude of 330 molecules per second per enzyme molecule. It is rather striking that this enzyme promoting the same reactions as heavy metal ions, does not use any heavy metal or detectable prosthetic group for its activity. This puts lipoxidase into a unique position among the oxidation enzymes known so far.

SUMMARY

The preparation of crystalline homogeneous soy bean lipoxidase is described. Lipoxidase is a colorless protein containing no iron or detectable prothetic group. The molecular weight is 102,000 and its isoelectric point is pH 5.4. The activity of the pure enzyme is of the magnitude of 330 moles of linoleic acid oxidized per mole enzyme per second under the conditions of the assay used.

The costs of this investigation were met by *Statens Tekniska Forskningsråd*. We are indebted to Docent K. O. Pedersen, Institute of Physical Chemistry, Upsala, for the measurements of diffusion and sedimentation constants.

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Die Bildungsweise der Chlorhydrine

VII. Darstellung von β-Propylenchlorhydrin durch Addition von Chlorwasserstoff an Propylenoxyd

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Der Verlauf der Addition von Chlorwasserstoff an Propylenoxyd ist von verschiedenen Forschern untersucht worden. Markownikoff[2] und Nef[3] meinten, es werde ausschliesslich α-Chlorhydrin ($CH_3CH(OH)CH_2Cl$) gebildet, Michael[4] war dagegen der Auffassung, dass im Reaktionsprodukte auch die β-Verbindung ($CH_3CHClCH_2OH$) anwesend sein könnte, eine Annahme, die ihm nicht möglich war zu bestätigen.

Der eine von uns (Sm.) zeigte vor vielen Jahren[5] durch Bestimmung der alkalischen Zersetzungsgeschwindigkeit des Additionsproduktes, dass bei der Addition beide Isomeren gebildet werden. Auf Grund einer approximativen kinetischen Analyse werden bei seinen Additionsversuchen (gasförmiger Chlorwasserstoff und stark gekühltes Propylenoxyd) die entstehende α-Verbindung auf 90 %, die β-Verbindung auf 10 % geschätzt*). Versuche, die beiden Isomeren von einander durch Destillation in einer Kolonne nach Linnemann zu trennen, scheiterten. Im Zusammenhang mit neuen Versuchen, die von Forsberg unternommen worden sind, besonders die Addition von Chlorwasserstoff an Alkylenoxyde quantitativ zu untersuchen, fanden wir es wünschenswert, mit verbesserten Hilfsmitteln diese Fraktionierungsversuche zu wiederholen.

Forsberg hat gezeigt, dass bei der Reaktion zwischen Propylenoxyd und Chlorwasserstoff eine Erhöhung der Reaktionstemperatur eine Vermehrung des Gehaltes an β-Verbindung zu Folge hat. Dasselbe ist bei der analogen

* Die tatsächlich gefundenen Geschwindigkeitskoeffizienten[5] gaben einen höheren Gehalt an dem β-Isomeren (etwa 20 %); die geschätzte Zersetzungsgeschwindigkeit dieser wurde doch als wenig zuverlässig (und zwar zu hoch) angesehen.

Addition an Glyceringlycid der Fall[6]. Die Addition wurde daher bei etwa 80° durchgeführt, bei welcher Temperatur etwa 25 % β-Verbindung entsteht. Das erhaltene Additionsprodukt wurde zuerst in einem Aufsatz mit innerer Kühlung[7] (Höhe etwa 120 cm) bei etwa 60 mm fraktioniert. Die zuerst übergehenden α-chlorhydrinreichen Anteile (etwa 75 % der Gesamtmenge) wurden mit Alkali in Propylenoxyd umgewandelt, zu diesem Chlorwasserstoff wie oben addiert, das Additionsprodukt von neuem fraktioniert u. s. w. Wiederholte kinetische Bestimmungen gaben uns Auskunft über die Veränderungen in der Zusammensetzung des Isomerengemisches durch die Fraktionierungen.

Aus einer Reihe solcher Versuche erhielten wir zuletzt etwa 250 g Gemisch mit etwa 45 % β-Verbindung. Dieses Gemisch wurde dann — zweckmässig in kleinen Mengen — einer langsamen Destillation in einer Podbielniakkolonne unterworfen[8]; Druck etwa 50 mm, Verhältniss Rückfluss: Destillat etwa; 8 : 1. Es wurden 4 Fraktionen aufgefangen: zwischen der ersten und der letzten war der Siedepunktsunterschied (bei 52 mm) 4°; Siedepunkte 58°, bzw. 62°. Die höchste Fraktion wurde in Bezug auf ihre alkalische Zersetzungsgeschwindigkeit untersucht, dann nochmals in derselben Kolonne destilliert, wodurch eine sehr kleine, aber doch sicher nachweisbare »Verbesserung« der Geschwindigkeitskonstante erzielt wurde. Die Untersuchung dieses Produktes ergab:

$n_D^{20} = 1{,}4390$; $d_4^{20} = 1{,}1092$; $MR_D = 22{,}42$ (ber. 22,48)

C_3H_7OCl (94,54) Ber. Cl 37,51

Gef. » 37,65, 37,63 (durch alkalische Hydrolyse)

Die alkalische Hydrolyse bei 20,00° wird in Tab. 1 wiedergegeben. In dieser bedeuten: a = Chlorhydrinkonz.; b = Barytkonz. in Äqviv.; t = Min.; x = zersetzte Menge (in Äqviv.); k = bimol. Geschw.-koeff.; % = zersetzte Menge.

Tabelle 1. Alkalische Hydrolysegeschwindigkeit des β-Propylenchlorhydrins (bei 20,00°).

	$a = 0{,}01712$		$b = 0{,}00971$
t	x	k	%
6,00	0,00219	2,68	22,6
13,00	393	2,68	40,5
21,00	523	2,62	53,8
30,00	627	2,62	64,6
65,00	832	2,65	85,7
		M = 2,65	

Der Geschwindigkeitskoeffizient ist ohne jeden Gang. Es muss eine reine Verbindung vorliegen.

Der flüchtigste Anteil des erhaltenen Additionsproduktes hatte eine Konstante, die sehr nahe (Abweichung etwa 1 %) mit derjenigen übereinstimmte, die durch Zersetzung von aus Allylchlorid durch Wasseraddition dargestelltem Chlorhydrin (reine α-Verbindung) erhalten worden war. Die alkalische Hydrolyse dieses Präparates ist in Tabelle 2 wiedergegeben. Seine physikalischen Eigenschaften waren:

$$n_D^{20} = 1{,}4387;\ d_4^{20} = 1{,}1120;\ MR_D = 22{,}35 \text{ (ber. } 22{,}48)$$

C_3H_7OCl Ber. Cl 37,51
Gef. » 37,52, 37,41 (durch alkalische Hydrolyse)

Tabelle 2. Alkalische Hydrolysegeschwindigkeit des α-Propylenchlorhydrins (bei 20,00°).

$a = 0{,}01527$		$b = 0{,}01021$	
t	x	k	%
2,00	0,00201	7,76	19,9
3,00	281	7,90	27,9
5,00	402	7,78	39,8
7,00	498	7,86	49,4
11,00	634	7,94	62,9
15,00	720	7,85	72,0
22,00	819	7,89	81,2

Die kinetische Analyse zeigt auch hier ein vollkommen isomerenfreies Produkt. Dass die Addition von Wasser an Allylchlorid eindeutig verläuft, hatte auch Smith *(l. c.)* gezeigt; seine Geschwindigkeitskoeffizienten zeigten indessen einen kleinen von Versuchsfehlern herrührenden Gang.

Es ist durch die oben angeführte Versuche somit von neuem bewiesen worden, dass durch Addition von Chlorwasserstoff an Propylenoxyd sowohl α- wie β-Propylenchlorhydrin gebildet werden. Die Mengenverhältnisse dieser Isomeren ändern sich u. a. mit der Temperatur, bei welcher die Addition vorgenommen wird, worüber Forsberg bald Bericht erstatten wird.

Schliesslich ist zu erwähnen, dass Forsberg die Synthese der β-Verbindung in anderer Weise durchgeführt hat. Das von ihm erhaltene β-Chlorhydrin war in jeder Hinsicht mit dem hier beschriebenen identisch.

ZUSAMMENFASSUNG

Eine Mischung von α-Propylenchlorhydrin ($CH_3CH(OH)CH_2Cl$) und β-Propylenchlorhydrin ($CH_3CHClCH_2OH$) wurde durch Addition von Chlorwasserstoff an Propylenoxyd dargestellt. In dieser in Bezug auf β-Chlorhydrin etwa

10-prozentigen Mischung wurde durch eine Kombination von Alkalibehandlung und Destillation den Gehalt an β-Chlorhydrin auf etwa 45 % gebracht. Aus dieser Mischung wurde β-Propylenchlorhydrin durch fraktionierte Destillation rein dargestellt.

Durch Bestimmung der alkalischen Hydrolysegeschwindigkeit des erhaltenen Produktes wurde gezeigt, dass dasselbe in reiner Form ohne Einmischung von α-Verbindung erhalten worden war.

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The Effect of Hydrothermal Denaturation of Collagen upon its Reactive Groups

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The denaturation of water-soluble proteins is generally considered to involve the unfolding of the coiled chains of the globular protein. Thereby the reactivity of various protein groups will be altered [1]. Denaturation of the fibrous protein collagen (mammalian) by the shrinkage of the fibres in water of 60—70° C leads to disorganization of the parallelly aligned chains, forming the native protein [2]. The fibrous state is intimately connected with the function of restraining forces between elementary chains and units. By the application of heat, the kinetic energy of the chains will be increased. Hence the cohesive forces will be weakened. The chains will tend to revert to the thermodynamically stable state of a random distribution. The temperature at which shrinkage of collagen takes place is the point at which the disruptive forces exceed the intermolecular cohesion [3].

The mechanism of the shrinkage of collagen fibres has been comprehensively investigated by Wöhlisch [4] from the thermodynamic point of view. Further, the important contributions of Meyer [5], Fauré-Fremiet [6], Küntzel [7] and Grassmann [8] are noted. In earlier papers [7,9] extensive literature references are given.

It would be expected that the reactivity of collagen would be changed by the deep-going alteration of its micellary structure. It is known that the inertness of native collagen fibres toward trypsin is destroyed by denaturation [8]. The elementary composition of the protein, its content of basic groups, more particularly amino ones and its acid binding capacity are not affected [8]. However, it has been indicated in earlier investigations [9] that the reactivity of collagen towards high-molecular compounds, reacting partly by means of co-ordinate valency forces is increased by denaturation.

In a recent critical review of the influence of denaturation of globular proteins upon the properties of the protein groups, Anson [10] points out that it is not possible at the present stage of knowledge of protein structure to define denaturation by means of description of the change in structure of the protein molecule. Anson further remarks that the increase in reactivity of protein groups when a protein is denatured must be explained by some general structural theory. This change is probably connected in some way with the breaking of the bonds between polypeptide chains, although, as Anson points out, our present limited knowledge does not justify further theorization.

The influence of hydrothermal denaturation on the reactivity of mammalian collagen has been investigated, with a view to ascertaining which groups are altered.

These groups functioning as connecting links between the units of the collagen lattice are: 1) *Salt-like links*, due to the attraction of oppositely charged side groups in juxtaposition. This type of cross-link apparently is of secondary importance for the cohesion of the collagen structure compared to: 2) *co-ordination bonds* between H- and O-atoms of the imino- and carbonyl-groups of peptide groups on adjacent protein chains (hydrogen bonds). These links seem to be the principal stabilizing agent of the collagen lattice [11].

MATERIALS AND METHODS

Collagen in the form of calf skin (delimed after regular light liming and made isoelectric (pH 5.5)) was used for the main experiments. Its content of ash was less than 0.05 %. Further, hide powder (Freiberg) was employed for experiments with systems containing reacting substances of high-molecular weight, in order to minimize the topochemical factor. The hide substrate was employed in: 1. Native state. 2. Denatured state. Collagen was denatured by holding the specimens for 5 min. in water of 72° C. All specimens were then dehydrated in two changes of acetone, air-dried and kept so. Further, specimens not acetone-dehydrated were used in some runs. No difference in the behaviour of hydrated and dehydrated specimens was found.

Both native and denatured collagen in the form of calf skin contained identical amounts of protein N; 17.97 %. The figures for hide powder were 17.93 and 17.90 % N respectively.

The reagents used were of analytical grade. The special compounds and extracts employed have been described in earlier papers [12]. The high-molecular fraction of lignosulphonic acid was prepared by the Erdtman »bis»-method [13].

The H-ion concentration was measured at 20° C by means of the hydrogen electrode; checked by the glass electrode. The titration curves plotted in the graphs are the average of at least six separate runs.

EXPERIMENTAL AND RESULTS

Titration curves

Any alteration in the reactivity of the basic protein groups induced by the collapse of the orderly fibre structure will be detected by means of the titration curve. The maximum acid binding capacity of collagen for which equilibrium pH values of ca. 1 are required is not changed by denaturation; this only proves that denaturation does not lead to fission of peptide groups with the formation of new amino and carboxylic groups. From considerations of the disorganization of the collagen chains, taking into account possible steric changes affecting the strength of the polar groups present in internal compensation, the comparison of the acid binding power of native and denatured collagen in a higher pH range, *e. g.*, 3—5, should be expected to be more informative. The technique used was similar to the one described by Harris [14], also found satisfactory in the work of Booth [15] on the influence of denaturation of albumin on its titration curve and employed by Atkin [16] in collagen investigations.

Quantities of native and denatured skin in ca. 1 mm cubes, and of hide powder equivalent to 1.00 g collagen were treated in resistance glass flasks with 100 ml of solutions made up as follows: 20 ml 10 % aqueous solution of sodium chloride, x ml 0.1 N HCl-solution and $80 - x$ ml of destilled water. The solutions were shaken at intervals during 24 h and the equilibrium pH values of the filtered solutions were determined. It will be noted that each solution contains two volume percent sodium chloride which is added to equalise the internal pH of the substrate and that of the external solution in order to avoid unequal ion distribution, rapidly set up in salt-free solutions of acid. Corresponding solutions of hydrochloric acid containing salt but *without* added protein were measured as blanks. The difference between the curve showing the effect of added acid on the final pH of the series containing protein and the curve for the blank solution gives directly the acid binding power of collagen at any pH value between 2 and 5. It may be remarked that some difficulty was encountered in the determination of the H-ion concentration of the solutions in the pH range 4—5.5.

Further complementary series were run with a constant amount of hydrochloric acid (10 ml 0.1 N HCl on 1 g collagen) but varying the total volume from 50 ml to 1000 ml. The results of these series showed the same trend as these of the main runs. In the series with denatured hide powder quite large amounts of protein (up to 5 % of the original weight) were brought into solution, accordingly affecting the pH values, especially in the higher pH range. Parallel runs with denatured skin showed average losses of 1 % collagen. Titration curves for the native and denatured calf skin collagen are given in Fig. 1. The curves of Fig. 2 show the effect of denaturation on the acid binding capacity of the specimens.

The fixation of hydrochloric acid by collagen from solutions with final pH values less than 3 is evidently not affected by denaturation. In the systems

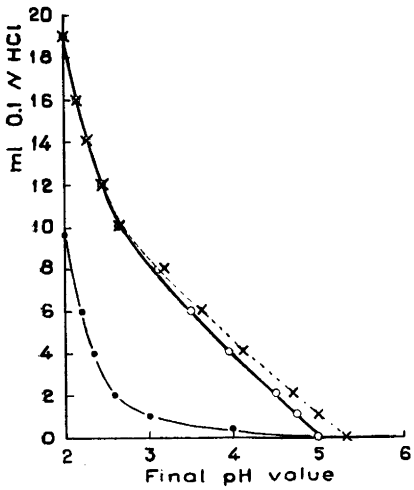

Fig. 1. Titration curves of native and denatured collagen.
—●— *Correction blank for HCl.*
—○— *Native collagen.*
– – × – – *Denatured collagen.*

with equilibrium pH values in the range 3—5, the denatured collagen possesses a slightly greater affinity for H-ions than the native type. However, the increase is not more than 5 % of the value of the maximum acid fixation capacity of collagen at the most. Since the titration of the system: collagen—solutions of sodium hydroxide containing 4 volume percent sodium sulphate, gave irregular values, these curves are not included. However, at pH values > 11, a common curve represents the removal of OH-ions from the solutions by native and denatured collagen, whereas in the pH range < 11 the denatured protein shows somewhat greater affinity for OH-ions;

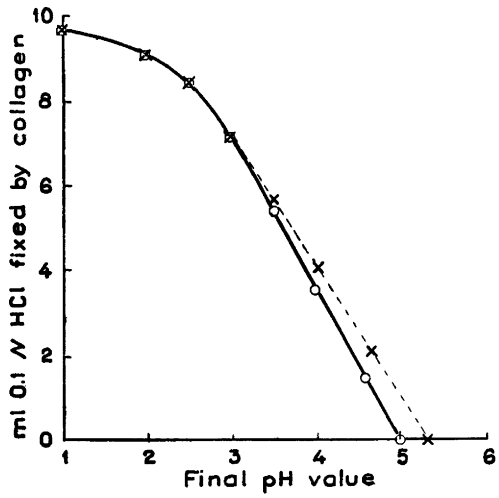

Fig. 2. Fixation of hydrochloric acid by native and denatured collagen.
—○— *Native collagen.*
– – × – – *Denatured collagen.*

the average increase being of the order of 5—10 percent of the total alkali-binding capacity of collagen.

Reactivity towards certain complex compounds

Since the titration curves prove that denaturation only slightly modifies the reactivity of the acid binding protein groups, it was deemed of interest to ascertain the behavior of the two types of mammalian collagen towards reactants predominantly reacting with hide protein by means of electro-valent forces. For that purpose a formaldehyde condensed naphthalene sulfonic acid consisting of a mixture of about equal parts of the di- and tri-nuclear disulfonic compounds was employed [17]. On account of the absence of phenolic and other co-ordination active groups in this compound, complications due to secondary reactions of nonionic nature should be eliminated as far as possible. The second type of sulpho-acid used was the high-molecular fraction of lignosulphonic acid.

Portions of 2 g of collagen in the form of calf skin cubes were shaken in 100 ml portions of 5 % solutions of these acids for 48 h, this being sufficient to attain equilibrium. The final pH values were 1.4 and 1.2 for the solutions of naphthalene- and ligno-sulphonic acids respectively.

A great number of basic chromic salts have been investigated. Two typical runs are given. A solution of 67 % acid chromic sulphate* with composition corresponding to the empirical formula $Cr_2(OH)_2(SO_4)_2 \cdot Na_2SO_4$, and a solution of 67 % acid chromic chloride corresponding to the formula $Cr_2(OH)_2Cl_4 \cdot 2NaCl$ were employed at a concentration of 1 eq. Cr per l.

The ratio of solution to substrate was 100 to 2 and the time of interaction 144 h. All series were run at room temperature (ca. 20° C). The treated stock was washed free from water-soluble matter, dried and analyzed by the usual methods for determination of the agents irreversibly fixed by collagen. The sulfur content of the sulfo-acid treated specimens was further determined, by the method of Grote-Krekeler. This determination is important for the following series in which the fixation of vegetable tannins by sulpho-acid saturated collagen is investigated. For the differentiation of irreversibly fixed vegetable tannins and sulpho acids in the resulting product, accurate S-figures are required [17]. The findings are shown in Tables 1 and 2.

Table 1. Fixation of chromic salts by collagen.

Type of compound	meq. Cr fixed by 1 g collagen in the form of:	
	Native collagen	Denatured collagen
67 % acid chromic chloride	3.3	3.3
67 % acid chromic sulphate	4.2	4.15

* % acidity = valencies of Cr in combination with acid groups (SO_4; Cl), expressed in per cent.

Table 2. Fixation of sulpho acids by collagen.

Type of sulpho-acid	% Sulpho-acid irreversibly fixed by: Native collagen	Denatured collagen
Condensed naphthalene sulphonic acid (Final pH 1.4)	29.8	29.3
High-molecular fraction of lignosulphonic acid (Final pH 1.2)	50.6	50.3

According to these data, denaturation of collagen does not alter the ionic protein groups involved in the fixation of low-molecular chromic salts and sulpho-acids.

Some types of chromium compounds are for their fixation by collagen dependent upon its co-ordinate function beside the availability of electrovalent protein groups. The fixation is a function of the state of the co-ordinate valency centres of hide protein which are probably located on the peptide groups. The afore-mentioned type of chromic salt brought to a high degree of basicity, leading to increased molecular size of the chromium complexes, was applied as previously described. The 40 % acid chromic sulphate and the 33 % acid chromic chloride, with a composition corresponding to the empirical formula: $Cr_2(OH)_4Cl_2 \cdot 2NaCl$ were employed in solutions containing 1 eq. Cr per l.

Further, the interaction between collagen and a highly aggregated sulphato-sulphito chromiate, mainly consisting of uncharged and negatively charged chromium complexes, corresponding to the formula: $Na_2(Cr_2(OH)_2(SO_4)_2(SO_3))$ was investigated. In reactions of solid proteins with solutions containing highly aggregated solutes, the topochemical influence of the protein substrate is a complicating factor particularly marked in denatured skin. The shrinkage and twisting of the micelles should lead to a reduction of interfibrillar and intermicellar space. The diffusion of the large molecule into the interior of the substrate will be retarded and impede the further progress of the reaction. By the use of finely divided hide powder, this topochemical complication may be largely eliminated. Table 3 contains the data.

Table 3. Fixation of aggregated chromic salts by collagen.

Type of compound	meq. Cr fixed by 1 g collagen in the form of: Native collagen	Denatured collagen
33 % acid chromic chloride	5.6	7.8
40 % acid chromic sulphate	6.4	8.0
Sulphito-sulphato-chromiate	7.1	10.9

Evidently the denatured collagen fixes greater amounts of aggregated chromium compounds than the native protein. An exceptionally large effect

is evident in the fixation of sulphito compound which according to experimental information available mainly reacts by means of co-ordinate valency. The increased fixation of these types of compounds is evidently caused by structural alterations induced by the shrinkage of collagen. Since agents predominantly attached to collagen by means of its acidic groups (carboxyl), for example, simple chromic salts, or by means of its basic groups, for example, simple sulpho-acids, are unaffected by denaturation it follows that the increased fixation of highly aggregated substances by denatured collagen is indicative of activation of non-ionic valency forces in the denaturing process.

The fixation of vegetable tannins

These complicated polyphenols are generally conceived to react both by means of the electro-valent basic groups and the co-ordinate *loci* of collagen. Methods for the differentiation of the main types of fixation have been proposed [17, 18]. The increased reactivity of co-ordinate peptide bonds resulting from the disorganization of the protein chains in hydro-thermal denaturation is strikingly demonstrated by data from the fixation of vegetable tannins by collagen in the native and the denatured state.

Portions of hide powder equal to 2 g collagen were treated for 3 days in 50 ml portions of solutions of vegetable tanning extracts containing 1 % tannin. The tanned hide powder was sucked free from solution and further treated for 3 days in 50 ml of solutions containing 2 % tannin. Further treatments of 4 days each were carried out in solutions of 3, 5 and 10 % strength in tannin. The tanned hide powder was washed free from water-soluble matter (negative gelatin-salt test). The content of irreversibly fixed tannin was obtained by analysis of the dried hide powder for ash and collagen (N × 5.62). The difference between 100 and the percentages of these constituents being taken to be irreversibly combined tannins. Table 4 contains some typical data.

Table 4. Fixation of vegetable tannins.

Type of vegetable extract	Final pH	% irreversibly fixed tannin on collagen basis by:	
		Native collagen	Denatured collagen
Wattle bark	4.6	42.1	67.3
Quebracho wood	5.5	46.2	70.5
Sumach	4.0	34.4	46.8
Tannic acid	3.4	65.2	88.0
Myrobalan	3.0	66.7	100.4

These data demonstrate the far-going influence of the denaturating pretreatment on the fixation of vegetable tannins by hide protein. A differentiation between the effect of the ionic protein groups (basic) and the co-ordi-

nate ones (peptide) was attempted in series with collagen containing its basic groups completely blocked by irreversibly fixed condensed sulpho-acids.

Native and denatured hide powder were treated in solutions of vegetable tanning extracts as described in the foregoing series. Further, both types of hide powder were in two consecutive treatments saturated with the high molecular fraction of lignosulfonic acid at final pH values of 1.4 in order to block the electro-valent groups. The pretreated hide powder contained 51.2 and 50.4 % sulpho-acid fixed by collagen in native and denatured state respectively. The sulpho-acid hide powder did not change the pH value of 0.1 *N* HCl-solution, proving the complete absence of free basic groups. The specimens were then treated with vegetable tannins in the same manner as described for the blank hide powder. After removal of reversibly attached matter from the stock by washing, it was dried and analyzed. From complete analysis of the stock including the content of S, the percentage of lignosulpho-acid fixed by protein was obtained (S × 14.7 = fixed lignosulpho-acid). The rest of the irreversibly fixed matter represented the vegetable tannins fixed by the non-ionic protein groups; the »peptide fraction». Table 5 contains the data.

Table 5. Fixation of tannins by hide powder and hide powder with inactivated basic groups, in native and denatured state.

Type of tannin	Substrate	In % on collagen basis: Total matter irreversibly fixed by collagen	fixed sulpho-acid	fixed vegetable tannin
Sulphited quebracho	Native collagen	54.8	0	54.8
	Sulpho-acid »	88.7	51.2	37.5
	Denatured »	84.1	0	84.1
	Sulpho-acid saturated denatured collagen	108.3	49.1	59.2
Myrobalan	Native collagen	76.7	0	76.7
	Sulpho-acid »	101.6	51.2	50.4
	Denatured »	113.1	0	113.1
	Sulpho-acid saturated denatured collagen	126.7	49.6	77.1

Table 6. Allotment of vegetable tannins to the electro-valent and co-ordinate groups of collagen.

Type of tannin	State of collagen	% tannin on collagen basis fixed by: Electrovalent groups	Co-ordinate groups
Sulphited quebracho	Denatured	24.9	59.2
	Native	17.3	37.5
	Additional fixation due to denaturation	7.6	21.7
Myrobalan	Denatured	36.0	77.1
	Native	26.3	50.4
	Additional fixation due to denaturation	9.7	26.7

In Table 6 the allotment of the fixed vegetable tannins to the two main types of protein groups is given. The figures show that the parts of the tannins which are fixed by non-ionic protein groups mainly account for the additional fixation due to the denaturative treatment. However, with some vegetable tannins both types of tannin fixation are proportionally increased by denaturation. Evidently the reactivity of the tannins is too complex to be formulated in a simple manner [19].

DISCUSSION

From the plausible assumption that the main cohesive forces in the collagen super-structure consist of: 1. salt-like links between oppositely charged acidic and basic groups on adjacent protein chains, and: 2. cross-links due to co-ordinate valency forces between $\overset{|}{\underset{|}{CO}}\cdots\cdots\overset{|}{\underset{|}{HN}}$ groups in juxtaposition, the following explanation of the reported findings may be advanced. The hydrothermal denaturation of collagen fibres involves a folding of the collagen chains. By the distortion of the structure, the distance between the chains may in certain points be so greatly enlarged that the force does not suffice for bridging the chains. Interlocking links located on the protein backbone (hydrogen bonds) are primarily affected and ruptured. The free valency forces on the liberated groups (hydrated), no longer internally compensated, will then be available for reaction and the heat denatured collagen will hence possess greater potency of co-ordinate reactivity of the peptide bonds than the original type of collagen.

The electrostatic intermolecular forces are not affected or only slightly so, by the shrinkage of the chains. The slightly increased H-ion fixation in the pH range in the vicinity of the iso-electric zone of collagen is probably due to a weakening of the salt-like cross-links by the widening of the gap between chains in the coiling. Diminished internal compensation of the electro-static force should connote increased reactivity of the polar protein groups towards external ions, as, *e. g.*, hydrogen ions. In systems of high ionic concentration, as, for example, acid solutions of low pH values, the greater reactivity of these easier available protein groups is not apparent for obvious reasons.

Collagen of fish skin (teleost) does not show increased affinity for the high-molecular compounds investigated in denatured state [19]. This finding as well as other differences between mammalian and teleost collagen, *e. g.*, the latter's low degrees of hydrothermal and tryptic resistance, are considered to indicate the non-participation of co-ordinate links on peptide bonds in this type of collagen [19].

It was found in earlier investigations [9] that by heat denaturation of globular proteins, as, *e. g.*, albumins, their affinity for substances, reacting electrovalently as well as co-ordinately, is greatly impaired. This finding may indicate that by the »opening up» of the globular structure and the setting of the denatured protein into structures of higher degree of orientation of elementary units, certain reactive protein groups are inactivated. Their function as interlockers of individual chains should then explain the decreased reactivity of globular proteins resulting from denaturation. It is noteworthy that the reactivity of casein which is not denaturable is not affected by hydrothermal treatment.

The data are in accord with the views of the mechanism of denaturing advanced by Astbury and Lomax [20], assuming interrelationship between the native and denatured forms of the globular and fibrous proteins.

SUMMARY

The hydrothermal denaturation (shrinkage) of collagenous fibres does not alter the maximum acid binding capacity of collagen. However, a comparison of the titration curves of native and denatured fibres shows that the H^+-fixation of collagen in the pH range 3—5 is slightly increased by denaturation, as is also the uptake of OH-ions from solutions of final pH values < 11.

The irreversible fixation of simple condensed sulpho acids is not affected by the treatment, neither is the uptake of basic chromic salts of low degree of aggregation. Highly aggregated chromium compounds show preferred affinity for denatured collagen. The vegetable tannins show the same trend. The last two mentioned types of compounds are for their reaction with proteins partly dependent upon co-ordinate valency forces, probably located on peptide groups. The increased fixation of these compounds by denatured collagen is probably a direct result of the rupture of intermolecularly co-ordinated cross-links on the peptide groups (hydrogen bonds), caused by the distortion of the fibre structure in the denaturation process. Evidence is presented in support of the view that in hydrothermal denaturation of collagen links of the hydrogen bond type are ruptured and the peptide bond made accessible for co-ordination of external reactants.

The data are in accord with the views of Astbury and Lomax on the mechanism of denaturation of globular and fibrous proteins.

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The Acidic and Basic Properties of Oxides

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I. ACID BASE DEFINITIONS

Since the days of Berzelius oxides have been connected with acid base character, *e. g.* CaO, MgO, FeO are commonly known as basic, SiO_2, CO_2 as acidic oxides. The acidic and basic properties of these compounds were characterised by their ability to react mutually forming salts:

$$\text{Acid} + \text{Base} \rightarrow \text{Salt}$$
$$\textit{e. g.}\quad SiO_2 + CaO \rightarrow CaO.SiO_2$$

Recently Lux [1] has announced a more precise acid base definition suitable for compounds like these, writing:

$$\text{Base} = \text{Acid} + O^{2-} \qquad (1)$$
$$\textit{e. g.}\quad SiO_4^{4-} = SiO_3^{2-} + O^{2-}$$
$$SiO_3^{2-} = SiO_2 + O^{2-}$$
$$CaO = Ca^{2+} + O^{2-}$$

In this way there is obtained an analogy to the acid base functions of Brönsted for the protolytic systems.

$$\text{Acid} = \text{Base} + H^+$$

The analogy is not only a formal one. There exists at least a qualitative parallellism between the proton affinity and the tendency to split off an oxygen ion, as will be seen on comparing the acid strength of the compounds:

$$H_2SO_4 > H_2CO_3 \text{ and } SO_3 > CO_2$$
$$H_3PO_4 > H_3BO_3 \text{ and } P_2O_5 > B_2O_3$$

(A stronger bond between the oxygen and the central atom is usually connected with a weaker binding tendency for a proton to the oxygen atom.)

Other acid-base definitions have also been advanced in order to formulate a wider acid base conception. Especially to be mentioned are the theories of Ussanowich [2] and Lewis [3]. According to the definition of Ussanowich the red-ox as well as the protolytic reactions are special cases of the general acid base reactions, which also comprise all reactions which involve ion exchanges, dissociations or associations of ions. This concept is so extensive, that according to it there would apparently be few reactions that are not to be considered as acid base reactions.

Lewis connects the acid base reactions with the formation of a coordinative bond:

$$: \underset{\cdot\cdot}{\overset{\cdot\cdot}{S}} + :B \rightarrow : \underset{\cdot\cdot}{\overset{\cdot\cdot}{S}} : B$$

The difficulties connected with the use of this acid base criterion in inorganic chemistry have become evident as the progress during the later years has led to an understanding of the limited validity of the Lewis-Langmuir octet [4]. Applied to oxides, the definition would give only one single base, the O^{2-} ion; on the other hand oxides as CaO, Na_2O are not bases, but Na^+ and Ca^{2+} are acids.

This should be sufficient to demonstrate the advantage of the definition of Lux, where the acid and base formulation is in agreement with the older, well known acid base terminology. In addition to the already mentioned analogy with the Brönsted acid base function, the definition of Lux also has the great advantage that it does not imply any theoretical assumption as to the way in which the oxygen ion is bound to the cation. On the other hand it excludes the possibility to regard the analogous reactions of the sulphide and fluoride ions as acid base reactions. However this limitation of Lux' definition seems to be of less importance than the difficulties one will meet on applying the mentioned extended acid base concepts to oxides.

Using the formulation:

$$\text{Base} = \text{Acid} + O^{2-}$$

the constant:

$$K = \frac{a_{\text{acid}} \cdot a_{O^{2-}}}{a_{\text{base}}} \qquad (2)$$

will be a measure of the strength of the acid base pair.

The characteristic point of the protolytic acid base reaction is the proton exchange between an acid and a base molecule. It would be interesting to

consider to what extent we may treat the role of the oxygen ion from the same point of view.

The systems we are considering, are built up of great oxygen ions and relatively small cations. The oxygen ions are, however, always more or less polarized by the adjacent cations. Even in a melt of alkali oxides we have to take into account a polarization of the oxygen ion.

[In this connection it is important to remember that oxides of cations with only weak polarizing character are unstable at room temperatures. The weakly polarized oxygen ions show a marked tendency to combine to O_2^{2-} or even to O_2^-. According to older data Cs_2O_2 is stable up to 1100° C in oxygen atmosphere, while K_2O_2 gives off oxygen already at 600° C.]

Consequently we have to assume that in a system of oxides or salts of oxy-acids the oxygen ions present are in a more or less polarized state dependent on the adjacent positive ions. The characteristic process of acid base reactions will then be the transfer of an oxygen ion from one state of polarisation to another. For example the reaction between carbonate and silicon dioxide to silicate and carbon dioxide may be written:

or $$O_{CO_3^{2-}} [+ SiO_2] \rightarrow O_{SiO_3^{2-}} [+ CO_2]$$

[It should be noticed that the oxygen ions within a silicate group cannot be split off independent of each other. Each group acts as a reactive unity.]

In systems built up of oxygen ions we meet three important types of acid base transitions:

a) Atoms with a high ionisation energy (S, C, etc.) frequently give acid base transitions accompanied by an alteration of their coordination number for oxygen,

$$\textit{e. g.} \quad SO_3 + O^{2-} \rightarrow SO_4^{2-}$$
$$CO_2 + O^{2-} \rightarrow CO_3^{2-}$$

b) For atoms with a medium ionisation energy the coordination number may be unchanged,

$$\textit{e. g.} \quad B_2O_3 + 3O^{2-} \rightarrow 2BO_3^{3-}$$
$$SiO_2 + 2O^{2-} \rightarrow SiO_4^{4-}$$

In these cases the acid base transition consists in the breaking of an oxygen bridge between two SiO_4 or BO_3 groups:

$$\left[\rangle Si - O^{2-} - Si \langle\right] + O^{2-} \rightarrow 2\left[-\overset{|}{\underset{|}{Si}} - O^{2-}\right]$$

The transition of the acid to the corresponding base may therefore be connected with a breaking down of macromolecule structures.

c) For atoms with a low ionisation energy (the typical cation forming ones) the acid base transition may be connected with a formation of an oxide phase:

$$Mg^{2+} + O^{2-} \rightarrow MgO_{(s)}$$

In general the heterogeneous reactions are of considerable importance in these acid base systems. (Reactions of this kind offer a simple method of studying activity coefficients in melts [5].)

II. EXPERIMENTAL METHODS FOR THE INVESTIGATION OF ACID-BASE EQUILIBRIUMS IN SYSTEMS OF OXIDES AND SALTS OF OXY-ACIDS

According to the Lux' acid base definition (1), which has been introduced in the foregoing chapter, the determination of an acid base equilibrium may in the principle be regarded as an examination of oxygen ion activities.

Several experimental methods for examinations of this kind are already proposed. Of these the following may be mentioned:

a. The oxygen electrode

Electrometric determination of oxygen ion activities should be possible by means of an electrode where a reaction like

$$O^{2-} = \tfrac{1}{2}\, O_{2(g)} + 2\, e$$

is potential determining. There are still very few details known about the use of the oxygen electrode for this purpose [1, 6, 7, 8] and the results published are not very satisfying.

Experiments carried out at temperatures of about 1000° C show a variation in the potential with the composition of the melt which is difficult to explain theoretically. It is interesting, however, to notice that measurements of potentials with electrodes of C and SiC in slags at 1600° C have given interesting results which indicate the possibility of obtaining well repeatable potentials at these temperatures [9].

In spite of the difficulties hitherto encountered, there is still reason to believe that this method will find its use in the present problem.

b. Partition of solutes between metal and oxide phases

To illustrate this type of equilibrium we may mention the metallurgically important reaction:

$$FeO_{(metal)} = FeO_{(slagg)} = Fe^{2+} + O^{--}$$

which already has been the object of several examinations. Even the solubility of metals in melts in the presence of oxygen should presumably be useful for this purpose, *e. g.* a reaction like:

$$4Ag_{(metal)} + O_{2(g)} = \left[4Ag^{+} + 2O^{2-}\right]_{(melt)}$$

c. Indicators

The use of indicators for the purpose of examining the acidity of glasses was proposed more than 10 years ago [8, 10]. The principle is demonstrated by the function of the chromate indicator:

$$\underset{\text{yellow}}{2\ CrO_4^{2-}} = \underset{\text{green}}{2\ Cr^{3+}} + 5\,O^{2-} + {}^3/_2\ O_2$$

At a constant oxygen pressure the colour of the melt will be a function of the oxygen ion activity. The colour has to be determined after the melt has been cooled, and therefore indicates a frozen equilibrium. As, however, the equilibrium of the indicator system probably is slowly adjusted, due to the participation of gaseous oxygen in the equilibrium — the cooling of the melt should not be any serious source of errors.

d. Measurements of equilibriums involving volatile acids or bases

The hitherto most important equilibriums suitable for the examination of acid base properties are the decompositions of carbonates. The relative acidity of acids may be determined by means of reactions like:

$$Ca^{2+} + CO_3^{2-} = CaO + CO_2$$

or

$$SiO_2 + CO_3^{2-} = SiO_3^{2-} + CO_2$$

by examination of the composition of the melt in equilibrium with CO_2 gas at constant pressure.

In addition to the system CO_3^{2-}-CO_2, other systems may be usable and of interest in order to extend the pO-range outside the limits of the carbonate system, *e. g.* SO_4^{2-}-SO_3, in some cases SO_3^{2-}-SO_2, OH^--H_2O and the corresponding systems with volatile oxyhalogenides as $POCl_3$, $VOCl_3$.

REVIEW OF THE PRESENT EXPERIMENTAL MATERIAL

The experimental results hitherto are rather qualitative in nature. It seems justified, however, briefly to consider them as disclosing some general traits of the acid base equilibria in these systems which have no analogies in the protolytic acid base systems. This applies especially to the case of the polyacids.

Before discussing these, however, we shall briefly consider the base scale of some of the common basic oxides.

A qualitative picture of the differences in base strength of a number of basic oxides may be obtained by comparing reversible thermic decomposition reactions as:

$$MCO_{3(s)} \rightleftharpoons MO_{(s)} + CO_2 \qquad (3)$$

$$MSO_{4(s)} \rightleftharpoons MO_{(s)} + SO_3 \qquad (4)$$

Table 1. The standard free energy change by thermal decomposition of carbonates and sulphates.

M	$\Delta F_{\text{carbonate}}$	$\Delta F_{\text{sulphate}}$	Diff.
Ba	52	108	56
Na			57
Li	40	94	54
Ca	31	86	55
Mg	16	61	45
Mn	15	51 (61)	36 (46)
Cd	12	50	38
Pb	10	62	42
Co	9	44	35
Ag	7	58	51
Fe^{II}	7		
Zn	4	46	42
Ni	— 2	46	48
Cu	— 2	42	44
Fe^{III}		31	
Be		29	

The picture was strictly correct only if the carbonate and sulphate ions might be considered as quite unpolarized and if effects arising from differences in crystal structure were negligible. We may, however, presume that the base strength is the deciding factor determining the decomposition equilibrium.

In Table 1 values of free energi ΔF_{298} in kcal are compared for a number of reactions of type (1) and (2). We find with only few inversions the same succession in the two sequences, starting with Ba, Sr, Li and Ca, and ending with Zn, Ni and Cu^{II}. The carbonates of the higher alkali metals melt before perceptible decomposition and cannot be directly compared.

It also appears that ions with inert gas structure are exceptionally weak acids (the oxides are strong bases) and that the sequence of the acid strength of these coincides with that of the ionic potentials.

Ions without inert gas structure are distinctly stronger acids (the oxides weaker bases). In this case it is not so easy to point out a simple relation between ionic potensial and acidity (*cf.* Mn^{II}, Co^{II}, Fe^{II}, Ni^{II}, Ag etc). Most of the values in the table are taken from Kelleys data [11]. The ranges of error are in some cases rather wide, especially in ΔF_2 (*cf.* the two values for Mn). It is therefore uncertain whether the inversions in the sulphate series as compared with the carbonate series are real ones, or if they are merely due to errors in the quoted values of ΔF. The values of the carbonate series are generally more reliable than those of the sulphate series and should therefore at present be regarded as the best measure of the relative acid base strengths of oxides.

Table 2. The free energy change by thermal decomposition of carbonates at 25, 1000 and 1600°C.

M	$\Delta F_{carbonate}$ (25° C)	$\Delta F_{carbonate}$ (1000° C)	$\Delta F_{carbonate}$ (1600° C)
Ba	52	15	— 4
Li	40	— 1	— 23
Ca	31	— 4	— 24
Mg	16	— 22	— 39
Mn	15	— 24	— 41
Cd	12	— 22	— 37
Pb	10	— 22	— 35
Co	9	— 31	— 54
Ag	7	— 25	— 43
Fe	7	— 33	— 52
Zn	4	— 31	— 45

The extrapolation of ΔF to higher temperatures gives an acid-base scale not very different from the one at 298° K. Table 2 represents ΔF at 25°, 1000° and 1600° C for some common oxides.

The difference

$\Delta F_1 - \Delta F_2 = \Delta F'$ represents ΔF_{298} for the reaction

$$MSO_{4(s)} + CO_{2(g)} = MCO_{3(s)} + SO_{3(g)}$$

For the stronger bases (BaO etc) $\Delta F'$ is approximately equal to ΔF_{298} for the reaction:

$$SO_4^{-2}{}_{(aq)} + CO_{2(g)} = CO_3^{-2}{}_{(aq)} + SO_{3(g)}$$
$$\Delta F_{298} = 59.1 \text{ kcal}$$

$\Delta F'$ shows a slightly decreasing tendency with decreasing base strength of the oxides.

The acidity of more acid oxides may in the principle be measured in relation to the acidity of the carbon dioxide by examining equilibriums like

$$SiO_2 + (Alk)_2CO_3 = (Alk)_2SiO_3 + CO_2$$

Niggli [12] has investigated the systems $K_2CO_3 - SiO_2$, $K_2CO_3 - TiO_2$, $Na_2CO_3 - SiO_2$, $Na_2CO_3 - TiO_2$ and $Li_2CO_3 - SiO_2$ by examining the composition of melts in equilibrium with carbon dioxide gas of 1 atm.

Flood, Förland and Roald have investigated carbonate systems including $K_2CO_3 - B_2O_3$, $Na_2CO_3 - B_2O_3$ and $Li_2CO_3 - B_2O_3$. The results of these investigations will be reported in a subsequent communication [13]. In the present paper only a few general traits will be mentioned.

In a melt of K_2CO_3 at temperatures about 1000° C the following sequence of the acid strengths is found

$$PO_3^- > BO_2^- \geqq SiO_3^{2-} > TiO_3^{2-}$$

This is in accordance with general metallurgical experience.

If we, however, compare these systems with the corresponding Na_2CO_3 and Li_2CO_3-systems we observe the interesting effect that the polyacids appear to behave as much stronger acids in the Li than in the Na systems and here again much stronger than in the K systems.

In a melt of K_2CO_3 a relatively small addition of B_2O_3 expels an amount of CO_2 corresponding to the formation of metaborate (BO_2^-). In the corresponding Na system the reaction comes to an end approximately midway

between the meta and ortho compositions (near $BO_{2,5}^{2-}$), while in Li_2CO_3 all B_2O_3 is converted to orthoborate (BO_3^{3-}).

The corresponding effect is even more pronounced in the silicate systems, somewhat less in the titanate systems.

As we have seen, only a small effect of this kind was demonstrated in connection with the relative acidity of SO_3 and CO_2.

It is therefore difficult to explain this effect except by assuming that the stability of the oxygen bridges of the polyacid depends upon the cations in such a way that the tendency

$$\left[X - O^{2-} - X\right] + O^{2-} \rightarrow 2\left[X - O^{2-}\right]$$

increases in the sequence K^+, Na^+, Li^+.

This opinion is supported by observations from different fields. Lux and Rogler [10] have by means of the chromate indicator examined the acidbase character of different oxides when these are added to a sodiumborate melt. They found that a number of oxides usually regarded as typical basic (among others BaO and Li_2O) at low concentrations behave as strong acids. This they attempted to explain as a special property of the boron atom by assuming that the boron, which normally coordinates 3 oxygen in a planar configuration may under the influence of a polarizing cation be forced to coordinate 4 oxygen in a tetrahedral configuration. Hence, they suppose, the boron trioxide should appear as a substantially stronger acid.

The assumption of 4 coordination in these systems must, however, be regarded as a rather questionable hypothesis. 4 coordination of oxygen to boron in anhydrous compounds is known in borosilicates and borowolframates only. Even by means of Na_2O Zintl and Morawietz [14] was unable to produce stronger basic borate than corresponding to the BO_3^{3-} ion. It has been claimed that 4 coordinated boron exists in acidic sodium borate glasses [15] but other investigations deny this [16]. Besides, according to this theory, the 4 coordination structure at the sodium oxide content used by Lux and Rogler should be complete even before the addition of the second oxide.

As we have seen these effects are by no means special for the borate systems. On the contrary they seem to be still more pronounced in the silicate systems.

Effects similar in kind also seem to be determining for the thermic stability of the pyrosulphates. They decompose according to

$$O_3S - O - SO_3^{2-} = SO_4^{2-} + SO_3$$

The essential accordingly is the breaking of an oxygen bridge. Experiments (which will be described in a subsequent paper [17] show a distinctly decreasing stability in the sequence

$$K > Na,\ Tl^{I} > Li > Ag$$

Hence it may be regarded as a general effect that the stability of the oxygen bridges depends on the cations present. The simplest explanation of this would be that pure geometrical relations are decisive. By breaking down the macrochains and nets connected by the oxygen bridges, a closer packing of the ions may be the result. The tendency of the bridges to break up should then depend on the gain in energy which may be larger the less the cation radius is.

The decomposition of the pyrosulphates, however, clearly demonstrates that the cation radius is not the only determining factor. As a matter of fact Tl-pyrosulphate is less stable than K, and Ag less than Na, despite the fact that the relation between the ionic radii in this case is opposite.

This indicates that a polarizing effect as well has to be taken into account. As is known the polarizing power of ions of the transition elements are stronger than that of the inert gas ions (with equal radius).

Observations from other fields also demonstrate the existence of these effects. The viscosity in acid silicate melts shows a distinct dependence of the cation in such a way that the viscosity decreases in the succession

K, Na, Li

The simplest explanation of this is that the size of the macromolecules decreases in the same succession.

Effects similar in kind have also been noted by the geochemists. It has been stated as a general rule for the minerals formed by cooling the magma that the larger the cation and the less its charge, the more will its silicate type tend towards a low excess in charge per oxygen atom in the Si-O structure. (Compare also Paulings second rule determining ionic crystal structures.)

The same rule is also demonstrated by number and stability of existing polysilicates. This is evident from Table 3.

Bowen and Morey [18] have characterized the alkalisilicates by stating that the »dissociation of disilicate» ($Si_2O_5^{2-}$) decreases rapidly with increasing atomic weight of the alkali metal. Comparing the silicates of the transition elements with those of the alkali and alkaline earth metals the existence of polarizing

Table 3. Polysilicates of some common elements.

System	Compound		Cation radius
Li_2O—SiO_2	Li_2SiO_3		0.60
	$Li_2Si_2O_5$	Diss. before melting	
Na_2O—SiO_2	Na_2SiO_3		0.95
	$Na_2Si_2O_5$	M. p. ca 960° C	
K_2O—SiO_2	K_2SiO_3		1.33
	$K_2Si_2O_5$	» ca 1045° C	
	$K_2Si_4O_9$		
MgO—SiO_2	$MgSiO_3$	Diss. before melting	0.65
CaO—SiO_2	$CaSiO_3$	M. p. 1540° C	0.99
SrO—SiO_2	$SrSiO_3$	» 1580° C	1.13
BaO—SiO_2	$BaSiO_3$	» 1600° C	1.35
	$2BaO3SiO_2$		
	$BaO2SiO_2$		
FeO—SiO_2	no metasilicate		0.75
ZnO—SiO_2	» »		0.74
PbO—SiO_2	$PbSiO_3$	» 780° C	1.32

effects is evident besides the purely geometrical one. Fe^{2+}, Zn^{2+} and Pb^{2+} behave, when compared with the alkaline earth metals, as if they had a smaller ionic radius than corresponding to the space they actually occupy in the crystals.

The general conclusion from this review of the present experimental material is that the stability of the polybridges depends largely on the cation present in such a way that the stability decreases with increasing ionic potential and increasing polarizing power of the cation.

In molten systems this is manifested as a distinct increase in the acidity of the polyacids, and as a decrease in the viscosity, while in the solid systems the effect is manifested as a decrease in number and stability of the existing polycompounds.

These effects partly correspond to the well known rule that polarizable anions are able to form salts with moderately polarizing cations only (compare the stability of salty hydrides, peroxides, halogenides etc).

The difference is that in the last cases the polarizing power is the deciding factor in determining the stability, while in the case of polybridges, the stability seems to be quite as much determined by purely geometrical relations.

In this last case both effects must be taken into account.

SUMMARY

The acid base properties of the oxides can, according to Lux be formulated in analogy to Brönsted's acid base function by the scheme of reaction

$$\text{acid} + O^{2-} = \text{base}$$

The general types and the characteristic traits of reactions of this kind are discussed, as well as experimental methods for investigating them.

The most important results are so far obtained in equilibria of the type

$$CO_3^{2-} + \text{acid} \rightleftharpoons \text{base} + CO_2$$

In the case of polyacids, where the acid base transition consists in a breaking of oxygen bridges, as schematically represented by

$$X - O^{2-} - X + O^{2-} \rightleftharpoons 2\ X - O^{2-}$$

it appears that the acid strength is largely dependent on the cations present. The tendency of the reaction to go towards the right is stronger the less the cation, and the stronger its polarizing power.

These phenomena are also displayed by the viscosity of melts, by the thermic stability of pyrobridges and by the stability relations of the solid silicates.

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The Solubilities of Separate Sulphanilamides and Their Mixtures („Sulpha-Combination")

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The sulphanilamides most effective in clinical practice are all liable to cause injuries to the kidneys owing to the fact that these compounds or their acetyl derivatives are excreted as supersaturated solutions from which concrements may be formed in the renal tubules. Provided that the sulphanilamides do not affect each other's solubility but dissolve independently, the risk of concrement formation might be almost eliminated if, for the attainment of a certain blood concentration or effect, not merely one compound but a mixture of several equivalent sulphanilamides were employed. From the clinical standpoint it cannot make any difference if several compounds are used instead of one when all the drugs are equally effective. The risk of concrement formation will, however, be reduced in this case, since each drug in the mixture is excreted in a considerably lower degree of saturation than when a single compound is employed.

This new principle of treatment has been called »sulpha-combination». It was first developed and used in Sweden. At an early date Hagerman started his clinical tests with a combination of two compounds since we had stated that the sulphanilamides do not exert any mutual solubility effects [1]. Later Frisk, Hagerman, Helander and Sjögren published extensive experimental proofs [2]. In these investigations the solubilities of the most suitable sulphanilamides and their acetyl derivatives separately and in mixtures were determined. Also experiments on the concrement formation in animals and determinations of absorption and excretion were carried out. The formula of the most suitable mixture, »sulphadital», was given and its actions and uses described. This mixture contains sulphathiazole, sulphadiazine, and sulpha-

* The authors are indebted to Mr. Yngve Ekhamn for technical assistance.

merazine in certain proportions. Independently of these authors the same idea was developed by Lehr in U. S. A., who has also demonstrated the practicability of the method [3].

In our earlier publications data on the solubility of some important sulphanilamides and their acetyl derivatives, separately and in mixtures, have been given. However, it was not possible to publish all details before. A more complete account may be of twofold interest. The results constitute the main basis of the »sulpha-combination» principle but they are also of interest from a chemical point of view. Since the results of earlier determinations of the solubilities of single sulphanilamides are very divergent and the cause of these differences had to be cleared up. To the compounds earlier investigated, we added elkosin and its acetyl derivative, as they are said to have particularly suitable solubility properties [4, 5].

We have also used our rather extensive data to calculate the acid dissociation constants (pK_a) and the solubilities of the undissociated compounds (S_0). These calculations are based on the simple laws of solubility following the procedure of Krebs and Speakman [6]. The majority of the sulphanilamides are ampholytes. However, within the physiological pH range, the basic functions have been disregarded and the compounds considered as weak monobasic acids. With knowledge of pK_a and S_0 the solubility may be calculated at various pH values. By comparing these values with the experimental ones it is possible to test the validity of this conception.

METHODS

The solubilities of the following compounds have been determined separately and in mixtures: sulphathiazole (2-sulphanilamido-thiazole), sulphadiazine (2-sulphanilamido-pyrimidine), sulphamerazine (4-methyl-2-sulphanilamido-pyrimidine) and their N^4-acetyl derivatives; also the separate solubilities of elkosin (6-sulphanilamido-2,4-dimethyl-pyrimidine) and its N^4-acetyl derivative. Fresh urine (a mixture from 11 men) was used as solvent for all compounds and mixtures of compounds. For the free sulphanilamides and for certain of the acetyl derivatives a phosphate buffer (usually $M/30$) were also used and in some cases diluted sodium hydroxide. The determinations of the solubility were carried out at pH 5.9—7.9 and in a few cases also outside this range. The desired pH value of the urine was obtained by the addition of the necessary amount of hydrochloric acid, sodium carbonate, or sodium hydroxide. A small amount of toluene was added to the urine as a preservative. This does not influence the results. The solubilities are given in mg of dissolved substance per 100 ml of solvent (mg %).

In order to determine the concentration of the dissolved substance, the solutions were suitably diluted, the acetyl derivatives hydrolysed and the compound determined according to the method of Bratton and Marshall [7]. Small or negligible corrections were made for the extinction of the reagents and the solvent. From the values of extinction, the amount of substance per 100 ml solution was found by interpolation of standard curves of known solutions.

This method of calculation is more complicated when dealing with the mixtures. The wave length of the maximum colour intensity is not the same for all three sulphanilamides used in the mixtures. The difference, however, is so slight that the curves almost coincide. Thus it is impossible to distinguish between the sulphanilamides in the mixtures. If sulphadiazine is read as sulphathiazole an error of only — 3 % is made. Similarly using sulphamerazine, the error is + 3 %. Thus the errors of the determination of a mixture of sulphadiazine, sulphathiazole and sulphamerazine mutually compensate each other provided the concentrations of sulphadiazine and sulphamerazine are about the same.

In working with mixtures of the free sulphanilamides and their acetyl derivatives, the free sulphanilamides are determined first and calculated as sulphathiazole (f mg %). Then the acetyl derivatives are hydrolysed and the compounds all together determined as sulphathiazole (s mg %). The difference ($s - f$) is converted into acetyl sulphathiazole (a mg %). The total content of dissolved substance is then given by the equation $t = a + f$.

When determining solubility values, special care must be taken to obtain saturation but to avoid supersaturation of the solution. This can be done by working at constant temperature. Either the solid substance may be dissolved in the solvent or a solution may be supersaturated and allowed to become stabilized. In most cases we used an excess of the finely powdered substance.

The solutions were made in Erlenmeyer flasks with glass stoppers, shaken mechanically and kept at 37° C in a water bath for at least 24 hours. Samples were filtered and analysed for concentration and pH.

In the case of sulphathiazole, elkosin and their acetyl derivatives the solubility was also determined after the crystallisation from a supersaturated solution in 1/30 M phosphate buffer. These solutions were made by saturation at 100° C followed by mechanical shaking at 37° C until the excess of substance had crystallized out. At different times samples of the clear solution were analysed for concentration and pH. — Some determinations of concentrations were also made of supersaturated solutions of acetyl sulphathiazole and acetyl elkosin. In these cases the solutions were cooled down in 2 min. to 37—38° C, then shaken at 37° C in the same manner as mentioned above. Analyses were made immediately after cooling down, and also after 15 and 30 minutes. This was done in order to compare the ability of the compounds to form supersaturated solutions.

The pH determinations were made with a glass electrode using a standard buffer solution. To simplify the procedure, an aliquot was withdrawn for the determination of the concentrations and the solutions were then allowed to cool to 20° C before the pH was measured. No precipitate was formed during this short period of cooling. The pH did not vary appreciably between 37 and 20° C. With the pH-meter used the error did not exceed ± 0.03 units.

The measurements of extinction values have been carried out with a single cell photoelectric colorimeter according to Brunius*. The errors in these measurements, the errors

* Constructed in the State Institute for Public Health, Stockholm, Sweden.

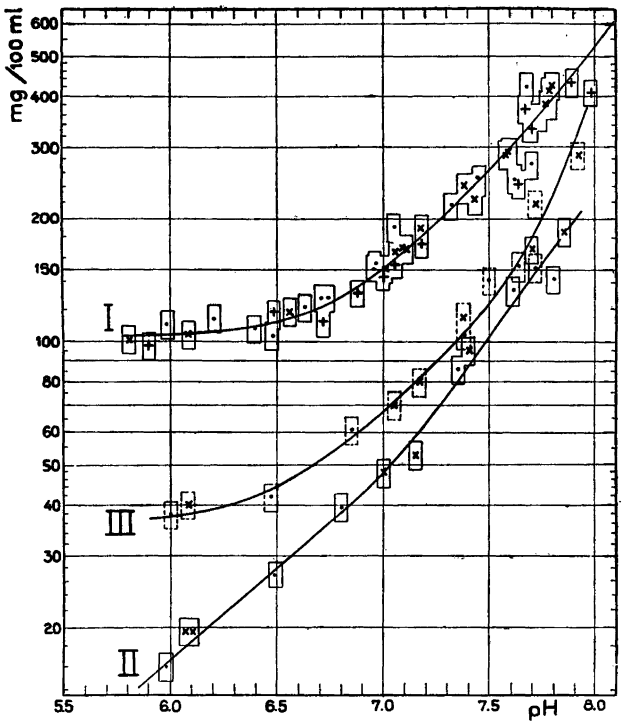

Fig. 1. The solubility at 37° C of:
I Sulphathiazole.
II Sulphadiazine.
III Sulphamerazine.
Solvents: Phosphate buffer (x), urine (·) and diluted sodium hydroxide (+).

in the dilutions necessary for the determination, and other possible errors will give a rather great total error. Quadruple determinations of sulphathiazole at pH 6.10 were, for example, 107, 100, 107 and 101 mg %. It may be concluded that in the case of a single free sulphanilamide, the possible maximal error can amount to ± 8 %, while in a mixture of three substances it amounts to ± 10 %. The same values for the acetyl derivatives would be ± 10 % and ± 12 % respectively. The last figure is also applicable to mixtures of all six compounds.

No consideration was given to the accuracy of the pH measurements when calculating the above errors. If only two determinations are made, a difference of 0.06 units cannot be distinguished with any certainty with the pH-meter used. At a pH of 6 or less this is of negligible importance. With an increasing pH the slope of the solubility curves grows steeper and a difference of 0.06 units may be of great influence on the results. For example, a change of the pH from 7.94 to 8.00 results in an increase of the solubility of acetyl sulphathiazole from 78 to 98 mg %, the difference being about 20 %.

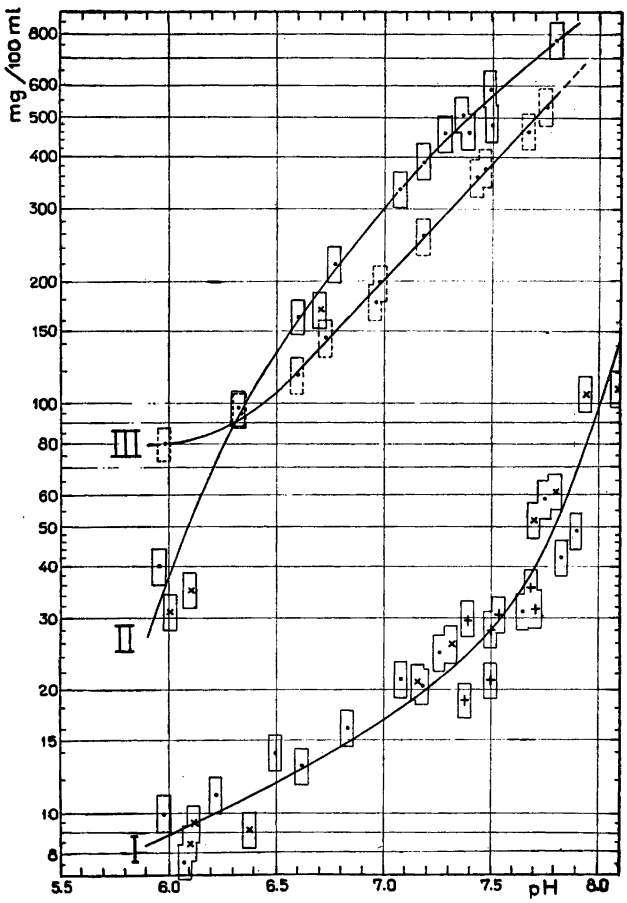

Fig. 2. Solubility at 37° C of:
I Acetylsulphathiazole.
II Acetylsulphadiazine.
III Acetylsulphamerazine.
Solvents: Phosphate buffer (x), urine (·) and diluted sodium hydroxide (+).

RESULTS

The experimental values of the solubility are plotted against the pH on a logarithmic scale. Each point is surrounded by a rectangle graphically showing the maximal error of the determination. The values for each substance are plotted in the same system, this being possible due to the fact that the solubilities at a certain value of pH seem to be independent of the three solvents used. The different solvents are indicated by different signs. From

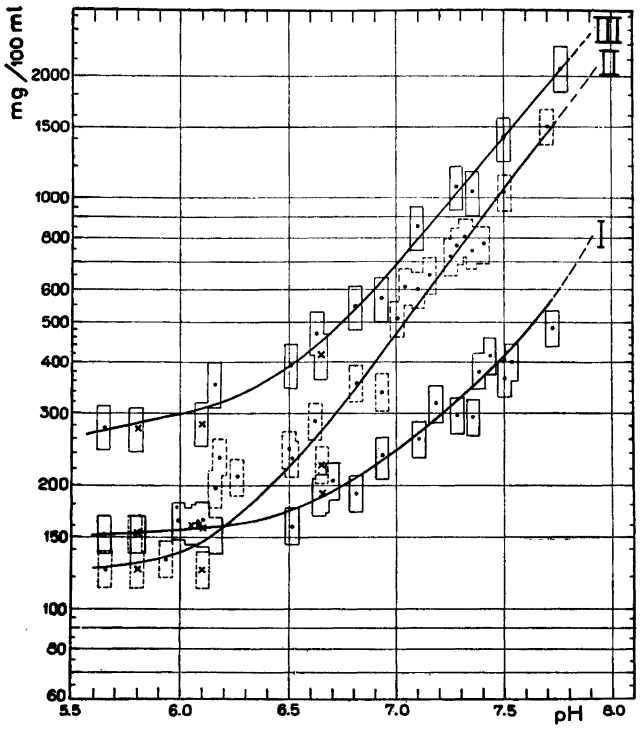

Fig. 3. The solubility at 37° C of the sulphanilamides and their acetyl derivatives in mixtures:
I Sulphathiazole + Sulphadiazine + Sulphamerazine.
II Acetylsulphathiazole + Acetyl sulphadiazine + Acetyl sulphamerazine.
III Sulphathiazole + Sulphadiazine + Sulphamerazine and their acetyl derivatives.
Solvents: Phosphate buffer (x) and urine (·).

the curves the solubility at various pH values can be read. The solubilities so obtained are to be considered as averages.

Fig. 1 shows the solubilities of sulphathiazole, sulphadiazine and sulphamerazine at pH 5.9—7.9 and 37° C, Fig. 2 the corresponding values for the acetyl derivatives. The curves illustrate, as is already known, that sulphathiazole is slightly more soluble than the two sulpha-pyrimidine compounds. They also show that the solubility increases strongly with increase in pH. Sulphathiazole is more soluble than its acetyl derivative. In the case of sulphadiazine and sulphamerazine the opposite is true.

The most important question is, however, to ascertain the extent to which the compounds influence each other's solubility. Fig. 3, Tables 1 a and 1 b show the results of these determinations at pH 5.5—7.9. It is quite evident

Table 1 a. The solubility, in mg/100 ml at 37° C, of sulphathiazole, sulphadiazine, sulphamerazine, and their acetyl derivatives, separately and in mixtures.

Substances	Source of values		Solubility at pH					
			5.5	6.0	6.5	7.0	7.5	7.9
Sulphathiazole	Fig. 1		103	105	111	151	263	450
	Krebs' and Speakman's nomogram and	pK_a and S_0 from our determinations	105	110	120	150	250	450
		pK_a and S_0 according to Krebs and Speakman	95	100	115	170	320	690
Acetyl sulphathiazole	Fig. 2		7.1	8.8	11.5	17	28	69
	Krebs' and Speakman's nomogram and	pK_a and S_0 from our determinations	7.5	8.0	10.3	17	37	85
		pK_a and S_0 according to Krebs and Speakman	7.5	8.2	10.5	18	41	95
Sulphadiazine	Fig. 1		10	17	28	48	104	200
	Krebs' and Speakman's nomogram and	pK_a and S_0 from our determinations	13	15	23	49	130	320
		pK_a and S_0 according to Krebs and Speakman	12	15	27	63	185	440
Acetyl sulphadiazine	Fig. 2		10	3	34	305	565	850
	Krebs' and Speakman's nomogram and	pK_a and S_0 from our determinations	20	37	95	290	900	—
		pK_a and S_0 according to Krebs and Speakman	27	45	100	285	850	—
Sulphamerazine	Fig. 1		37	38	44	68	127	310
	Krebs' and Speakman's nomogram and	pK_a and S_0 from our determinations	36	38	45	65	135	290
		pK_a and S_0 according to Krebs and Speakman	43	46	56	88	190	410
Acetyl sulphamerazine	Fig. 2		79	80	105	202	388	650
	Krebs' and Speakman's nomogram and	pK_a and S_0 from our determinations	75	82	105	180	400	900
		pK_a and S_0 according to Krebs and Speakman	89	105	140	310	800	1950

Table 1 a (continued)

(Sulphathiazole +sulphadiazine +sulphamerazine)	Fig. 3	153	158	173	245	420	800
	Fig. 1	150	160	183	267	494	960
Acetyl- (sulphathiazole + sulphadiazine + + sulphamerazine)	Fig. 3	126	138	222	470	1070	2000
	Fig. 2	96	127	251	524	981	1569
(Sulphanilamides + acetyl derivatives)	Fig. 3	265	300	390	695	1420	2550
	Figs. 1 and 2	246	286	434	791	1475	2529
	Fig. 3, curves I and II	279	296	395	715	1490	2800

that the three compounds of sulphadital dissolve independently of one another. This is the case, at least within this pH range, regarding a mixture of the three sulfa drugs, a mixture of their acetyl derivatives, and a mixture of all six compounds. The calculated and experimental values are in good agreement (Table 1 b). The results agree with the theoretical expectations, because the substances even in saturated solutions are present in very low concentrations and a decrease of solubility by means of influencing the activity coefficients is hardly possible. With the pH constant and only small changes of the concentration of neutral salts the magnitude of the total concentration of the common ions is always the same.

When making a good mixture for clinical use, the choice of constituents is of primary importance. From various points of view, the mixture used in our experiments has been found to be the most suitable one. Another important point is the proportions of the components. Besides the solubility of the free sulphanilamides and their acetyl derivatives, many pharmacological questions have to be considered. These have been discussed in a previous paper [2]. The most ideal composition hitherto has been found to be: sulphathiazole 37 %, sulphadiazine 37 % and sulphamerazine 26 %. With normal doses of this mixture none of the excreted products in the urine exceed their solubility limits, if the reaction is approximately neutral [2].

Our investigations on elkosin show that this compound has a high solubility while its acetyl derivative has a low one, the solubility of the latter being about the same as that of acetyl sulphathiazole (Fig. 4, Table 2). Meier, Allemann and v. Meyenburg have found much higher solubilities for these compounds [4]. They determined the solubility of the acetyl derivative in the usual ways *i. e.*, by dissolving the substance or by crystallizing the substance

Table 1 b. Ratio between found and calculated values for the solubility of mixtures over the pH-range 5.5—7.9. The quotients in parenteses include extrapolated values.

Substances	The quotient calculated	pH					
		5.5	6.0	6.5	7.0	7.5	7.9
Sulphathiazole + sulphadiazine + sulphamerazine	Solubility according to Fig. 3 / Solubility calculated from Fig. 1	(1.02)	0.99	0.95	0.92	0.85	(0.83)
Acetyl sulphathiazole + acetyl sulphadiazine + acetyl sulphamerazine	Solubility according to Fig. 3 / Solubility calculated from Fig. 2	(1.31)	1.09	0.88	0.90	1.09	(1.27)
Sulphathiazole + sulphadiazine + sulphamerazine + their acetyl derivatives	Solubility according to Fig. 3 / Solubility calculated from Fig. 1 and 2	(1.08)	1.05	0.90	0.88	0.96	(1.01)
	Solubility according to Fig. 3 / Solubility calculated from Fig. 3, curves I and II	(0.95)	1.01	0.99	0.97	0.95	(0.91)

from a supersaturated solution. In the latter case very high values were obtained (Table 2). Evidently these authors believe that the acetyl derivative of elkosin has an especially high ability to remain in a supersaturated solution. From a practical point of view this would be important. However, we could not verify these findings. As is seen from Tables 3 and 4 this property is not more pronounced than in the case of acetyl sulphathiazole.

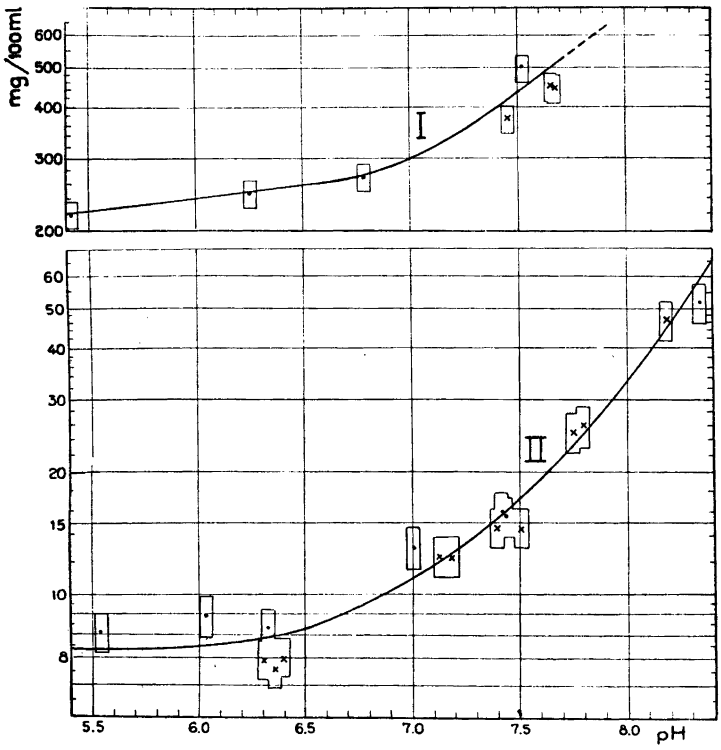

Fig. 4. The solubility at 37° C of:
I Elkosin.
II Acetyl elkosin.
Solvents: Phosphate buffer (x) and urine (·).

Table 2. The solubilities, in mg/100 ml at 37° C, of elkosin and acetyl elkosin.

Substance	Source of values		Solubility at pH					
			5.5	6.0	6.5	7.0	7.5	7.9
Elkosin	Fig. 4		222	238	256	300	435	620
	Krebs' and Speakman's nomogram and our pK_a and S_0		220	225	245	290	440	800
	Meier, Alleman, and v. Meyenburg		362	—	600	—	1100	—
Acetyl elkosin	Fig. 4		7.3	7.5	8.4	11.0	17.5	29
	Krebs' and Speakman's nomogram and our pK_a and S_0		7.5	7.8	8.5	11.0	18.5	35
	Meier, Alleman, and v. Meyenburg	from supersaturated solution	190	—	224	—	485	—
		by direct dissolving substance	24	—	24	—	39	—

Table 3. The solubility, in mg/100 ml at 37° C, of sulphathiazole, elkosin, and their acetyl derivatives determined by crystallizing the substance from supersaturated solutions or by direct dissolving the substance.

Substance	Method of preparing saturated solutions	Time for stabilizing of solutions, days	pH at 20° C	Solubility	
				Found	Calculated from solubility curve
Sulphathiazole	From supersaturated solution	1	7.12	186	170
	» » »	2	7.11	169	170
	» » »	1	7.48	280	258
	» » »	2	7.45	250	248
	» » »	1	7.73	360	355
	» » »	2	7.74	352	360
	By direct dissolving substance	1	7.78	380	380
	» » » »	2	7.78	380	380
	From supersaturated solution	1	7.89	436	445
	» » »	2	7.89	428	445
	By direct dissolving substance	1	8.08	588	600
	» » » »	2	8.07	616	590
Acetyl sulphathiazole	From supersaturated solution	1	7.50	27	28
	» » »	2	7.48	28	27
	By direct dissolving substance	1	7.55	35	30
	» » » »	2	7.59	36	32
Elkosin	From supersaturated solution	1	7.45	402	420
	» » »	2	7.45	370	420
Acetyl elkosin	From supersaturated solution	1	6.29	7.5	7.8
	» » »	2	6.35	6.9	8.0
	By direct dissolving substance	1	6.39	7.0	8.1
	From supersaturated solution	1	7.16	14.5	12.5
	» » »	2	7.12	12.3	12.2
	By direct dissolving substance	1	7.00	13.0	11.0
	From supersaturated solution	1	7.71	28	22
	» » »	2	7.74	25	23

According to Gsell, the concentration of elkosin in the urine after a dose of 3 grams daily is 100—300 mg % of the free compound and 150—400 mg % of the total compound (*i. e.* free plus acetylated compound)[5]. After 5 grams daily, the corresponding concentrations are 300—500 mg %, and 400—700 mg % respectively. The average degree of acetylation in the urine is said to be about 20 % (12—35 %). If these concentrations are compared with the solubilities at various pH values found by us, it is evident that after a dose of 3 grams daily free elkosin does not necessarily appear in a supersaturated state.

Table 4. Concentrations of acetyl sulphathiazole and acetyl elkosin in supersaturated solutions. Parallel determinations have same numbers.

Determination	Substance	Supersaturated solutions kept at 37°, minutes	pH	Concentration of substance		Solubility from curve
				mg/100 ml	% of zero value	
1	Acetyl sulphathiazole	0	6.28	26	100	10.8
	»	15	6.28	18	69	10.8
	»	30	6.28	14	54	10.8
2	»	0	6.78	111	100	14.3
	»	15	6.78	63	57	14.3
	»	30	6.78	48	44	14.3
3	»	0	7.58	116	100	32
	»	15	7.62	102	88	34
	»	30	7.64	82	71	35
1	Acetyl elkosin	0	6.28	39	100	7.8
	»	15	6.29	27	69	7.8
	»	30	6.30	21	54	7.9
2	»	0	6.83	67	100	9.9
	»	15	6.87	52	78	10.1
	»	30	6.82	43	64	9.8
3	»	0	7.68	204	100	21.5
	»	15	7.70	137	67	22.0
	»	30	7.74	106	57	23.0

After a dose of 5 grams daily, the concentrations of free elkosin in the urine is always higher than its solubility up to a pH value of about 7. Acetyl elkosin, however, is always excreted as a strongly supersaturated solution. If 20 % of the lowest dose and the lowest total concentration (150 mg %) were acetylated, then the acetyl derivative would appear in a concentration of 30 mg %. Even this value is about 3 times as high as the solubility at pH 7. At a lower pH value, the difference is still greater. As the acetyl derivative has such a low solubility, it is probable that elkosin will give the same renal complications as other single sulphanilamides.

From the solubility curves for the single compounds (Figs 1 and 2) we have obtained the solubilities at various pH values between 5.9 and 7.9 in order to calculate pK_a and S_0 according to Krebs and Speakman. It is assumed that the compounds appear as sparingly soluble mono-basic acids. If the solu-

Table 5. Average pK_a and S_0 values. The standard errors are obtained in the usual manner $\left(E = \sqrt{\frac{\Sigma \Delta^2}{n\ (n-1)}}\right)$.

Substance	pK_a according to		S_0 according to	
	our determinations	Krebs and Speakman	our determinations	Krebs and Speakman
Sulphathiazole	7.37 ± 0.03	7.10	103 ± 2	92
Acetyl sulphathiazole	6.87 ± 0.06	6.81	7.1 ± 0.4	7.1
Sulphadiazine	6.47 ± 0.07	6.28	11 ± 1	9.9
Acetyl sulphadiazine	5.6 ± 0.3	5.86	10.5 ± 1	18.6
Sulphamerazine	7.05 ± 0.03	6.95	35 ± 0.5	41
Acetyl sulphamerazine	6.84 ± 0.04	6.55	71 ± 3	80
Elkosin	7.48 ± 0.04	—	218 ± 6	—
Acetyl elkosin	7.30 ± 0.03	—	7.0 ± 0.2	—

bility at pH 5.9 is S' and the other ten solubilities in turn are S'', K_a values for each substance may be calculated from the equation:

$$K_a = \frac{S'' - S'}{\frac{S'}{[H]''} - \frac{S''}{[H]'}}$$

The single values showed a certain trend indicating that the compounds did not exactly follow the theoretical solubility curve for mono-basic acids. Especially in the case of acetyl sulphadiazine was this pronounced. From the average value of pK_a and the solubilities from our curves *(S)* at various pH a series of S_a values were calculated according to the following equation:

$$S_0 = \frac{S}{1 + 10^{pH-pKa}}$$

The average pK_a and S_0 values are given in Table 5. The solubilities calculated in this way agree rather well with those obtained from the experiments reported in Tables 1 and 2. From this it may be concluded that, for practical purposes, it is possible to consider the sulphanilamides as mono-basic acids, although this may not be exactly true from a chemical point of view. We

thus are able to confirm the opinion of Krebs and Speakman that knowing the values of pK_a and S_0 the solubilities may be calculated with satisfactory accuracy within the physiological range of pH.

SUMMARY

The solubilities of sulphathiazole, sulphadiazine, sulphamerazine and the corresponding N^4-acetyl derivatives were determined at the pH-range 5.9—7.9, in different solvents saturated with the compounds separately and with their mixtures. The compounds do not affect each other's solubility. This is the main basis for the »sulpha-combination» principle.

The solubilities of elkosin and N^4-acetylelkosin were also determined and discussed.

The acid dissociation constants (pK_a) and the solubility of the undissociated compounds (S_0) were calculated.

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Nuclear Oxidation of Silvan and Furfuryl Alcohol

Preparation of 3-Methylpyridazine and 3-Hydroxymethylpyridazine

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The nuclear oxidation of furfural has recently been investigated with special regard to the structure of addition products formed by the oxidation[1]. In this communication the oxidation of silvan and furfuryl alcohol is discussed and the preparation of stable addition compounds of these furans described.

By acid hydrolysis the addition products yield yellow unsaturated 1,4-dicarbonyl compounds which proves the former to be derivatives of 2,5-dihydrofuran.

In analogy with the synthesis of pyridazines introduced by Paal and Schultze [2] 3-methylpyridazine and 3-hydroxymethylpyridazine, respectively, are formed by the addition of hydrazine to the dicarbonyl compounds.

SILVAN

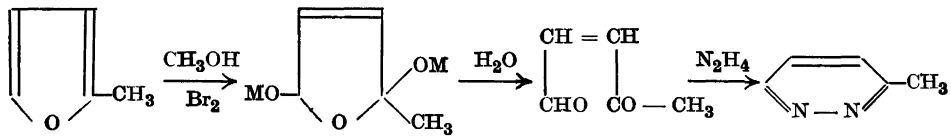
The formation of an addition compound by oxidation of silvan (I) has so far not been reported. Meinel [3] mentions, that silvan is not methoxylated in the usual manner with bromophthalimide in methanol but yields a product which contains a considerable amount of bromine. However, we have prepared, a dimethoxydihydrosilvan in a 65 per cent yield by methoxylation with bromine in methanol. The acetal is a colourless liquid with properties similar to the corresponding dihydrofuran derivative described earlier [4]. The analysis, a methoxy and a molecular weight determination demonstrate that two methoxy groups have been added to silvan.

By acid hydrolysis and distillation of the reaction product a yellow liquid is collected boiling at 55—75°/10 mm. No doubt it consists mainly of β-acetylacrolein (III) but did not give the correct analytical values. The best determinaton was obtained of a fraction boiling at 68°/10 mm. which contained

59.9 % carbon and 6.58 % hydrogen (calc. for $C_5H_6O_2$: 61.2 % and 6.14 %). It is an established fact that carbonyl compounds of this type may retain water or alcohol firmly. We therefore believe that our product also contains a small amount of water or methanol which is sufficient to account for the diverging analysis.

The β-acetylacrolein was not subjugated to a closer study but it may be said, that this ketoaldehyde is more stable than malealdehyde (Wohl and Mylo [5]).

When dimethoxydihydrosilvan is hydrolyzed and hydrazine added to the reaction mixture, 3-methylpyridazine (IV) is formed in analogy with the synthesis of pyridazines introduced by Paal and Schulze [2]. The base was obtained in a 56 per cent yield. It has previously been prepared by Poppenberg [6] and his description of the properties of this compound is in perfect agreement with our observations. The synthesis of 3-methylpyridazine proves that the dihydrosilvan is 2,5-dimethoxy-2,5-dihydrosilvan (II).



I II III IV

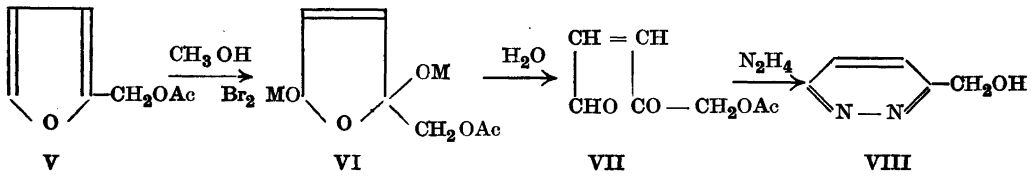
FURFURYL ALCOHOL

Meinel [3] has prepared a dimethoxydihydrofurfuryl alcohol by the action of methanolic bromine on furfuryl alcohol. He believed this addition product to be 4,5-dimethoxy-4,5-dihydrofurfuryl alcohol but it has recently been suggested that Meinel's compound is the corresponding 2,5-derivative [4].

We have methoxylated furfuryl acetate (V) and obtained a dimethoxydihydrofurfuryl acetate (yield 66 %). It is a viscuous colourless oil boiling at about 112°/9 mm. The analysis, a methoxy, an acetoxy and a molecular weight determination demonstrate the addition of two methoxy groups to furfuryl acetate.

When the addition product is boiled with diluted sulfuric acid the characteristic greenish-yellow colour of unsaturated 1,4-dicarbonyl compounds appears. By addition of semicarbazide to the hydrolysate a white semicarbazone, which exhibits the analytical values of a monosemicarbazone of 5-acetoxypentene-(2)-one-(4)-al-(1) (VII), is precipitated in a 22 per cent yield.

Addition of hydrazine to the hydrolysate yields 3-hydroxymethylpyridazine (VIII) (yield 49 %). This condensation as well as the above evidence proves that the structure VI must be ascribed to the addition compound.



V VI VII VIII

3-Hydroxypyridazine has not previously been prepared. It is a colourless crystalline solid melting at 66°. It is only slightly soluble in benzene and ether but very soluble in alcohol and water. The structure was established by the analytical evidence, a molecular weight determination, and by the preparation and analysis of a crystalline *p*-nitrobenzoate.

Hydroxymethylpyridazine reduces Tollen's reagent immidiately at room temperature. Methylpyridazine also reduces the reagent, but not quite so rapidly.

We are well aware of the fact, that our structure determinations do not exclude the possibility that the dimethoxydihydrofurans — in spite of their narrow boling-range during distillation — are mixtures of isomers consisting mainly of the 2,5-derivatives. But as long as this has not been demonstrated, the addition products must be regarded as pure 2,5-dihydrofurans.

EXPERIMENTAL

2,5-Dimethoxy-2,5-dihydrosilvan

Preparation. 9.0 ml of silvan (0.1 mole) were methoxylated exactly as described earlier for the preparation of the corresponding furan derivative [4]. B. p. 46°/8 mm. Towards the end of the distillation the temperature was allowed to rise to 56°. Colourless liquid, yield 9.3 g = 65 %. The acetal may be distilled under atmospheric pressure without destruction; b. p. 152°. The freezing point depression of a benzene solution was measured.

$C_7H_{12}O_3$	Calc. C 58.30	H 8.40	CH_3O 43.1	Mol. wt. 144.2
	Found » 58.06	» 8.81	» 41.3	» » 141

Hydrolysis; isolation of β-acetylacrolein. 7.2 g of dimethoxy dihydrosilvan (0.05 moles) were boiled for 5 minutes with 5 ml of centinormal sulfuric acid. 5 ml of a saturated solution of calcium chloride was added and the mixture extracted with 100 ml of ether. The etheral solution was dried with 15 g of calcium chloride for 30 minutes and the ether

removed on the steam bath through a Vigreux column. The residue was distilled in vacuum and a yellow liquid boiling from 55° to 75° under 10 mm was collected; yield 3.5 g. The theoretical amount calculated as β-acetylacrolein is 4.9 g. The fraction boiling at 68° was analyzed.

$C_5H_6O_2$ (98.1)	Calc. C 61.22	H 6.14		
	Found » 59.91	» 6.58		

Hydrolysis; preparation of 3-methylpyridazine. 7.2 g of dimethoxydihydrosilvan (0.05 moles) were boiled for one minute with 40 ml of decinormal sulfuric acid. After rapid cooling with ice water and addition of 2.50 ml of hydrazine hydrate (0.05 moles) the solution was refluxed for 30 minutes. 5 g of finely powdered potassium chromate and 30 g of potassium carbonate were added, the mixture shaken at room temperature for two hours and extracted three times with in all 200 ml of ether. The etheral layer was dried with 50 g of potassium carbonate and the ether removed on the steam bath through a Vigreux column. The residue was distilled in vacuum. The methylpyridazine boiled sharply at 85°/9 mm. There was no forerun but a small amount of a higher boiling residue. Towards the end of the distillation the temperature was allowed to rise to 95°.

Colourless liquid; yield 2.6 g = 55 %.

$C_5H_6N_2$ (94.1)	Calc. C 63.81	H 6.44	N 29.77
	Found » 62.87	» 6.41	» 30.35

When boiled in the atmosphere 3-methylpyridazine rapidly turned brown. B. p. 207—211°/766 mm (Poppenberg[6]) 206°/760 mm).

2, 5 - Dimethoxy - 2, 5 - dihydrofurfuryl acetate

46.5 g of furfuryl acetate (0.33 moles) and 60 g of potassium acetate (0.6 moles) were dissolved in 400 ml of methanol. A solution of 15.0 ml of bromine (0.30 moles) in 300 ml of methanol was added at —7° during 50 minutes, and the addition product isolated in the usual manner[4]. B. p. 112—138°/20 mm.; yield 40 g = 66 %. There was a forerun of furfuryl acetate (5—6 g) and 7 g of a higher boiling residue (b. p. 140—165°/20 mm). The main fraction was a colourless liquid, which boiled sharply at 131°/20 mm by redistillation. The freezing point depression of a benzene solution was measured.

$C_9H_{14}O_5$	Calc. C 53.46	H 6.99	CH_3COO 29.2	CH_3O 30.7	Mol. wt. 202.2
	Found » 53.21	» 7.12	» 28.2	» 29.7	» » 198

Hydrolysis; isolation of a monosemicarbazone of 5-acetoxypenten-(2)-one-(4)-al-(1). 220 mg of dimethoxydihydrofurfuryl acetate were boiled with 3 ml of centinormal sulfuric acid for 30 seconds. The solution, which had a faint greenish-yellow colour, was cooled and added to 0.30 g of semicarbazide hydrochloride and 0.35 g of potassium acetate. The mixture was heated slightly for a moment and then cooled rapidly. The semicarbazone, which precipitated immediately, was filtered off, washed thoroughly with water and dried over phosphorous pentoxide in vacuum over night. Yield 52 mg = 22 %. The crude product was recrystallized from 6 ml of alcohol. Yield 41 mg = 18 %. Colourless crystals, m. p. 160—165° (Kofler stage).

$C_8H_{11}O_4N_3$ (213.2)	Calc. C 45.07	H 5.21	N 19.72
	Found » 45.14	» 5.34	» 19.56

Hydrolysis; preparation of 3-hydroxymethylpyridazine. 10.1 g of dimethoxydihydrofurfuryl acetate (0.05 moles) were boiled for one minute with 40 ml of decinormal sulfuric acid. The solution was cooled rapidly, 5.00 ml of hydrazine hydrate (0.10 moles) were added and the mixture boiled for 20 minutes. After saturating with potassium carbonate (40 g) the hydroxymethylpyridazine was extracted with 600 cc. of ether, containing 7 % of methanol, the etheral layer dried with potassium carbonate (20 + 50 g) and the ether removed on the steam bath. The residue was distilled under 1 mm. There was a colourless forerun (0.7 g) boiling at 110—120°, which crystallized readily (m. p. 40° (tube)). This substance was not further investigated. It was extremely hygroscopic.

The main fraction (b. p. 150°) consisted of the pure hydroxymethylpyridazine, which solidified to almost colourless crystals, m. p. 60°; yield 2.7 g = 49 %. M. p. after crystallisation from ether-petrolether 66° (tube, corr.). The freezing point depression of an aqueous solution was measured.

$C_5H_6ON_2$	Calc. C 54.51	H 5.50	N 25.43	Mol. wt. 110.1
	Found » 54.99	» 5.88	» 25.24	» » 105

If the synthesis was carried out with less than twice the theoretical amount of hydrazine hydrate, the reaction mixture turned dark, and the yield of hydroxymethylpyridazine was decreased considerably.

110 mg of 3-hydroxymethylpyridazine were dissolved in 0.3 ml of pyridine. 180 mg of p-nitrobenzoyl chloride were added and the mixture heated for one minute. 2 ml of water were added, the benzoate filteres off, washed thoroughly with acid sodium carbonate and water and dried over night in vacuum over phosphorous pentoxide. Yield 142 mg = 55 %. The crude product crystallized from acetone-ligroin or from methanol. M. p. 106° (tube, corr.).

$C_{12}H_9O_4N_3$ (259.2)	Calc. C 55.59	H 3.51	N 16.21 %
	Found » 55.58	» 3.63	» 16.58 »

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Zur Kenntnis der Umwandlung von Nitrito- in Nitropentamminkobalt(III)-nitrat in festem Zustande

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Der eine von uns (B. A.) hat früher [1] gefunden, dass die Geschwindigkeit der Isomerisation des Nitritopentamminkobalt (III)-ions nach der Formel

$$[Co(NH_3)_5 \cdot ONO]^{2+} \rightarrow [Co(NH_3)_5 \cdot NO_2]^{2+}$$

in wässeriger Lösung von starken Säuren, wie Salpeter-, Überchlor- und Salzsäure, mehr oder weniger erniedrigt wird. Bei gegebener Wasserstoffionenkonzentration macht sich ein deutlicher Anioneneffekt bemerkbar, und die inhibierende Wirkung nimmt in der Reihe

$$HNO_3 < HClO_4 < HCl$$

stark zu.

Es schien nun von Interesse, zu untersuchen, ob ein derartiger Anioneneffekt auch bei der Umwandlung der festen Salze zum Vorschein komme. Deshalb wurde Isoxanthonitrat $[Co(NH_3)_5 \cdot ONO](NO_3)_2$ hergestellt, und die Umwandlungsgeschwindigkeit der festen Verbindung bei 20°, 30° und 40° C extinktiometrisch bestimmt. Die Konstanten dieser Messungen konnten dann mit denjenigen einer vorhergehenden Arbeit [2] über die Isomerisation des festen Isoxanthochlorides verglichen werden.

Das zu untersuchende Isoxanthonitrat, das, wie es scheint, nicht früher in der Literatur beschrieben ist, konnte nach dem Schema

$$[Co(NH_3)_5 \cdot Cl]Cl_2 \rightarrow [Co(NH_3)_5 \cdot Cl](NO_3)_2 \rightarrow [Co(NH_3)_5 \cdot ONO](NO_3)_2$$

synthetisiert werden. Von einem nach Garrick [3] gereinigten Präparat des Chlorochlorides wurde eine bei etwa 8° C nahezu gesättigte Lösung bereitet und diese Lösung konzentrier-

ter Salpetersäure von etwa derselben Temperatur unter guter Umrührung tropfenweise zugesetzt. Das ausgefallene Chloronitrat wurde abgesaugt und dann mit verdünnter Salpetersäure, Wasser und Alkohol gewaschen. Durch Auflösen in Wasser und erneutes Fällen mit halb konzentrierter Salpetersäure konnte es gereinigt werden. Zwei Analysen gaben nun den Kobaltgehalt 19,52 % bzw. 19,39 % (ber. 19,42 %). Hauptsächlich nach dem von Jörgensen [4] für das Isoxanthochlorid angegebenen Verfahren wurde das Chloronitrat schliesslich in Isoxanthonitrat übergeführt. Hierbei war nur die Salzsäure mit Salpetersäure zu ersetzen. Der für dieses Salz gefundene Kobaltgehalt von 18,74, bzw. 18,78 % stimmte sehr gut mit dem theoretischen Wert 18,76 % überein. Die chamoisrote Farbe des Salzes war etwa die des wohlbekannten Isoxanthochlorides.

Um zur Analyse die komplexen Kobaltsalze in $CoSO_4$ überzuführen, wurden sie mit etwas mehr als der berechneten Menge verdünnter Schwefelsäure versetzt und die Lösung auf dem Wasserbade nahezu eingedampft. Das Produkt war dann in einem elektrischen Ofen bis zu etwa 550° C [5] langsam zu erwärmen und bei dieser Temperatur zu konstantem Gewicht zu erhitzen.

Wie beim Isoxanthochlorid ist beim Nitrat die Isomerisation von einer Veränderung der Farbe von Rot nach Gelb begleitet.

Da die früher [2] verwendete Methode zur optischen Bestimmung des Umwandlungsgrades des festen Isoxanthochlorides ziemlich zeitraubend ist und gewisse Extrapolationsschwierigkeiten mit sich bringt, wurde sie hier nur bei 20° C benutzt (Tabellen 1 und 2). Für 30° und 40° C haben wir ein in folgender Weise vereinfachtes und verbessertes Verfahren gewählt. Das neu hergestellte Präparat ist 20 Stunden oder mehr im Eisschrank über Phosphorpentoxyd aufzubewahren. Eine Reihe von Wägegläschen mit je 0,0785 g von dem so getrockneten Salz hängt man in einem Metallgestell auf, das seinerseits in einem Glaszylinder angebracht wird. Dieser wird dann mit einem Glasdeckel, einem Gummireifen und einer Metallklammer luftdicht verschlossen. Sein Boden ist mit einer Schicht von Phosphorpentoxyd bedeckt. Das ganze System wird gewöhnlicherweise über Nacht wieder im Eisschrank aufbewahrt und am folgenden Morgen eine bis zwei Stunden vor den Extinktionsmessungen in den Thermostat von 30° oder 40° C hineingestellt. Zu angemessenen Zeitpunkten, meistens mit Intervallen von etwa 30 Minuten, wird aus dem Inhalt eines Wägegläschens 100 ml einer Lösung von der Zusammensetzung 0,00250 *M* $[Co(NH_3)_5 \cdot ONO](NO_3)_2$ + 0,0600 *M* HCl bereitet. Die hierbei zu verwendende Salzsäurelösung wird im voraus auf 20° C temperiert. Die Extinktion solch einer Probelösung für Licht von der Wellenlänge 436 mμ wird in der früher angegebenen Weise [2] mit Multiplikatorphotozellen bestimmt. Der Zusatz von Salzsäure bringt den grossen Vorteil mit sich, dass während der Messungen die Extinktion der Versuchslösung innerhalb der Grenzen der Versuchsfehler unverändert bleibt. Bei der hier zu betrachtenden

Konzentration setzt die Salzsäure nämlich die Umwandlungsgeschwindigkeit des Isoxanthoions auf Null herab [6]. Die Extrapolationsfehler werden dadurch vermieden.

Die zu den Zeitpunkten t gefundenen Extinktionswerte E_V der salzsauren Versuchslösung in ein und derselben Küvette und bei 20°, 30° und 40° C sind in der zweiten Kolumne der Tabellen 1 bis 7 verzeichnet. In den beiden ersten Tabellen wird mit E_V der Extinktionswert bezeichnet, den man durch Extrapolation zum Auflösungsaugenblicke t des festen Komplexsalzes erhält [7]; in den übrigen Tabellen ist E_V der Mittelwert von vier oder fünf Bestimmungen und t der Zeitpunkt vollständiger Auflösung des Salzes.

Zur Berechnung der Geschwindigkeitskonstante k der Umwandlung

$$[Co(NH_3)_5 \cdot ONO](NO_3)_2 \rightarrow [Co(NH_3)_5 \cdot NO_2](NO_3)_2$$

wurde die in den früheren Arbeiten verwendete Formel

$$k = \frac{1}{t - t_0} \cdot \log \frac{E_G - E_V^\circ}{E_G - E_V} \qquad (1)$$

benutzt. E_V° und E_G sind die Extinktionswerte beim Zeitpunkte t_0 und beim Schluss der Umwandlung. t_0 ist der t-Wert der ersten Messung jeder Versuchsreihe. $(t - t_0)$ wird immer in Minuten ausgedrückt. E_G ist die Extinktion einer Lösung von der Zusammensetzung 0,00250 M $[Co(NH_3)_5 \cdot NO_2](NO_3)_2$ + 0,0600 M HCl und in der für alle Messungen benutzten Küvette. Es zeigte sich, dass alle E_V-Werte mit wachsender Zeit sich dem E_G-Wert 0,493 asymptotisch näherten. Für eine entsprechende Isoxantho c h l o r i d lösung in derselben Küvette wurde in einer vorhergehenden Arbeit [1] der E_G-Wert 0,487 gefunden. Die Übereinstimmung ist gut.

Die Gleichung (1) kann zur graphischen Bestimmung der Konstanten k zweckmässig auf folgende Weise geschrieben werden

$$\log\ (E_G - E_V) = \log\ (E_G - E_V^\circ) - k(t - t_0) \qquad (2)$$

Wir fanden, dass die experimentellen log $(E_G - E_V)$-Werte, wenn sie gegen die entsprechenden $(t - t_0)$-Werte graphisch repräsentiert wurden, in jeder Versuchsreihe um eine Gerade gut gruppierte Punkte gaben. Der Winkelkoeffizient dieser Geraden gibt den gesuchten k-Wert der monomolekularen Umwandlungsreaktion und ihre Ordinate im Nullpunkt den Wert von log $(E_G - E_V^\circ)$. Über jeder Tabelle wird die so ermittelte numerische Form der Gleichung (2)

angegeben, und die letzte Kolumne gibt die nach diesem Ausdruck berechneten E_V-Werte wieder. Sie stimmen mit den gefundenen E_V Werten zufriedenstellend überein. Die Trockenzeit des zu jeder Messungsreihe verwendeten Präparates ist auch über der Tabelle zu finden.

Tabelle 1. Umwandlung des festen Isoxanthonitrats bei 20° C.—Präparat Nr. 1; vor den Extinktionsmessungen 4 Stunden im Eisschrank und 13 Stunden im Thermostat bei 20° C über P_2O_5 getrocknet.

$$\log (0{,}4931 - E_V) = 0{,}1564 - 1 - \mathbf{0{,}284 \cdot 10^{-3}} \cdot (t - t_0)$$

$t - t_0$ Minuten	E_V gef.	E_V ber.	$t - t_0$ Minuten	E_V gef.	E_V ber.
0	0,356	0,350	420	0,387	0,384
240	0,369	0,371	575	0,396	0,395
366	0,378	0,380	636	0,399	0,399

Tabelle 2. Umwandlung des festen Isoxanthonitrats bei 20° C.—Präparat Nr. 2; vor den Extinktionsmessungen 31,5 Stunden im Eisschrank und 15 Stunden im Thermostat bei 20° C über P_2O_5 getrocknet.

$$\log (0{,}4931 - E_V) = 0{,}1346 - 1 - \mathbf{0{,}243 \cdot 10^{-3}} \cdot (t - t_0)$$

$t - t_0$ Minuten	E_V gef.	E_V ber.	$t - t_0$ Minuten	E_V gef.	E_V ber.
0	0,356	0,357	295	0,378	0,378
50	0,361	0,361	337	0,379	0,380
105	0,367	0,365	551	0,393	0,393
163	0,368	0,369	705	0,402	0,401
228	0,373	0,373			

Tabelle 3. Umwandlung des festen Isoxanthonitrats bei 30° C.—Präparat Nr. 3; vor den Extinktionsmessungen 32 Stunden im Eisschrank, 12 Stunden bei 20° C und schliesslich 1 Stunde im Thermostat bei 30° C über P_2O_5 getrocknet.

$$\log (0{,}4931 - E_V) = 0{,}0857 - 1 - \mathbf{0{,}856 \cdot 10^{-3}} \cdot (t - t_0)$$

$t - t_0$ Minuten	E_V gef.	E_V ber.	$t - t_0$ Minuten	E_V gef.	E_V ber.
0	0,365	0,371	211	0,416	0,413
51	0,387	0,383	252	0,416	0,419
97	0,394	0,393	280	0,424	0,423
141	0,400	0,401	333	0,429	0,430
173	0,408	0,407			

Tabelle 4. Umwandlung des festen Isoxanthonitrats bei 30° C.—Präparat Nr. 5; vor den Extinktionsmessungen 24 Stunden im Eisschrank und 1 ½ Stunden im Thermostat bei 30° C über P_2O_5 getrocknet.

$$\log (0{,}4931 - E_V) = 0{,}2262 - 1 - 0{,}826 \cdot 10^{-3} \cdot (t - t_0)$$

$t - t_0$ Minuten	E_V		$t - t_0$ Minuten	E_V	
	gef.	ber.		gef.	ber.
0	0,328	0,325	169	0,371	0,371
47	0,334	0,339	213	0,381	0,381
86	0,349	0,350	258	0,390	0,390
132	0,365	0,362	289	0,395	0,396

Tabelle 5. Umwandlung des festen Isoxanthonitrats bei 30° C.—Präparat Nr. 6; vor den Extinktionsmessungen 44,5 Stunden im Eisschrank und 2 Stunden im Thermostat bei 30° C über P_2O_5 getrocknet.

$$\log (0{,}4931 - E_V) = 0{,}1635 - 1 - 0{,}868 \cdot 10^{-3} \cdot (t - t_0)$$

$t - t_0$ Minuten	E_V		$t - t_0$ Minuten	E_V	
	gef.	ber.		gef.	ber.
0	0,340	0,347	197	0,397	0,395
34	0,358	0,357	222	0,403	0,400
66	0,365	0,365	247	0,402	0,404
102	0,373	0,374	281	0,408	0,410
129	0,385	0,381	309	0,412	0,415
168	0,393	0,389			

Tabelle 6. Umwandlung des festen Isoxanthonitrats bei 40° C.—Präparat Nr. 7; vor den Extinktionsmessungen 47,5 Stunden im Eisschrank und 1 Stunde im Thermostat bei 40° C über P_2O_5 getrocknet.

$$\log (0{,}4931 - E_V) = 0{,}0830 - 1 - 3{,}30 \cdot 10^{-3} \cdot (t - t_0)$$

$t - t_0$ Minuten	E_V		$t - t_0$ Minuten	E_V	
	gef.	ber.		gef.	ber.
0	0,368	0,372	127	0,445	0,447
39	0,400	0,403	160	0,456	0,457
66	0,417	0,420	188	0,464	0,464
87	0,436	0,431			

Tabelle 7. Umwandlung des festen Isoxanthonitrats bei 40° C.—Präparat Nr. 8; vor den Extinktionsmessungen 26 Stunden im Eisschrank und 1 ½ Stunden im Thermostat bei 40° C über P_2O_5 getrocknet.

$$\log (0{,}4931 - E_V) = 0{,}0447 - 1 - 3{,}12 \cdot 10^{-3} \cdot (t - t_0)$$

$t - t_0$ Minuten	E_V		$t - t_0$ Minuten	E_V	
	gef.	ber.		gef.	ber.
0	0,381	0,382	119	0,444	0,446
41	0,413	0,411	145	0,460	0,454
66	0,423	0,424	176	0,459	0,462
94	0,434	0,437	203	0,468	0,467

Tabelle 8. Die gefundenen monomolekularen Geschwindigkeitskonstanten in min^{-1} für die Isomerisation des Isoxanthonitrats und Isoxanthochlorids in festem Zustande.

Temp.	Nitrat				Chlorid
	$k \cdot 10^3$				$k \cdot 10^3$
	1. Reihe	2. Reihe	3. Reihe	Mittelw.	Mittelw.
20°	0,28 ± 0,014	0,24 ± 0,010	—	0,26	0,056
30°	0,86 ± 0,047	0,83 ± 0,031	0,87 ± 0,045	0,85	0,21
40°	3,3 ± 0,14	3,1 ± 0,20	—	3,2	0,64

Der Übersicht halber sind alle gefundenen k-Werte des Isoxantho nitrats mit ihren mittleren Fehlern und Mittelwerten, wie auch die Mittelwerte der Konstanten des Isoxantho chlorids, in der Tabelle 8 zusammengestellt. Die Parallellbestimmungen von k für das Nitrat bei einer gegebenen Temperatur zeigen, dass eine Verlängerung der Trockenzeit des Präparats über etwa 24 Stunden hinaus keine merkbare Veränderung von k mit sich bringt. Die Konstanten des Nitrats sind durchgehend 4- bis 5-mal grösser als die des Chlorids. Das kann anders so ausgedrückt werden: auch im festen Zustande wirken die Chloridionen stärker als die Nitrationen hindernd auf die Umwandlung der Isoxanthoionen ein.

Tabelle 9. Aktivierungsenergie q und Aktionskonstante α für die Isomerisation des festen Isoxanthonitrats und Isoxanthochlorids.

Salz	q cal			log α		
	30°—20°	40°—20°	Mittelw.	30°—20°	40°—20°	Mittelw.
Nitrat	20910	22860	21890	12,03	13,48	12,76
Chlorid	23380	22310	22840	13,18	12,38	12,78

In Tabelle 9 werden schliesslich die mit Hilfe der Arrheniusschen Gleichung $k = a \cdot e^{\frac{-q}{RT}}$ aus den Geschwindigkeitskonstanten berechneten Werte der Aktivierungsenergie q und der Aktionskonstanten a für die Umwandlung des festen Isoxanthonitrats zusammengestellt und mit den entsprechenden Grössen des Isoxanthochlorids [7] verglichen. Man findet, dass die Mittelwerte von log a in beiden Fällen praktisch identisch sind, dass aber q für das Nitrat etwas kleiner als für das Chlorid ist. Der Einfluss der Versuchsfehler auf q und a ist aber so gross, dass man aus diesen Daten nicht mit Sicherheit feststellen kann, ob die grösseren k-Werte des Nitrats durch eine Änderung von q oder a bewirkt werden.

ZUSAMMENFASSUNG

1. Ein neues Komplexsalz der Isoxanthoreihe, Nitritopentamminkobalt-(III)-nitrat, $[Co(NH_3)_5 \cdot ONO](NO_3)_2$ ist synthetisiert worden.
2. Die Umwandlung des festen Salzes in die entsprechende Nitroverbindung wird extinktiometrisch mit Hilfe von Multiplikatorphotozellen untersucht.
3. Die Versuchsmethodik wurde dadurch erheblich vereinfacht, dass die Probelösungen mit Salzsäure versetzt werden, wodurch die Extinktion der Probe während der Messungen unverändert bleibt.
4. Die Umwandlung ist eine Reaktion erster Ordnung mit den Geschwindigkeitskonstanten: bei 20° C $0{,}26 \cdot 10^{-3}$; bei 30° C $0{,}85 \cdot 10^{-3}$ und bei 40° C $3{,}2 \cdot 10^{-3}$ min^{-1} (dekadische Logarithmen).
5. Diese Konstanten sind 4- bis 5-mal grösser als die des früher untersuchten Chlorids derselben Reihe.
6. Dies wird so aufgefasst, dass die Chloridionen im festen Zustande wie in wässeriger Lösung stärker als die Nitrationen hindernd auf die Isomerisation der Isoxanthoionen wirken.

Zum Schluss möchten wir unsrer Assistentin, Fräulein Ingrid Lindahl, für gute Hilfe danken. Ihre Mitarbeit ist durch Mittel aus dem Chemischen Institut ermöglicht worden. Dem Institutionsvorstand, Herrn Professor Dr. S. Bodforss, der uns diese Mittel zur Verfügung gestellt hat, sprechen wir unsren herzlichsten Dank aus.

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On the Hydrolysis of the Bi^{3+} Ion

Repeated Oxygen Bridging: A new Type of Ionic Equilibrium

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It is well known that the ion Bi^{3+} easily reacts with water (is hydrolysed) in some way so that hydrogen ions are set free. On the nature of the reaction products, however, very little has been known with certainty.

PREVIOUS WORK

Smith [1] in 1923 studied

1. the conductivity of solutions of $Bi(NO_3)_3$ in excess of HNO_3,
2. the solubility of $Bi(OH)_2NO_3$ in HNO_3 of different concentrations,
3. the conductivity of solutions of $Bi(OH)_2ClO_4$ with different amounts of $HClO_4$ and
4. the emf of cells $Pt,H_2/HClO_4(NaClO_4)/Bi(ClO_4)_3,HClO_4(NaClO_4)/Bi$.

Smith concluded from 1. and 2. that bismuth is chiefly present as Bi^{3+} in solutions with excess of HNO_3. From 3. he concluded that the ion $Bi(OH)_2^+$ is rather stable but on addition of a large excess of $HClO_4$ is gradually transformed to $BiOH^{2+}$ and Bi^{3+}. He tried to evaluate 4. by assuming that all Bi is present either as BiO^+ only, or as $BiOH^{2+}$ only; no certain conclusions could however be drawn.

Swift [2] in the same year studied equilibria between Cu and Bi metals and Cu^{2+}, H^+ and bismuth (III) ions in perchlorate solutions. He assumed that Bi occurred either only as BiO^+ or only as $BiOH^+$; with the latter hypothesis the calculated molal potential of Bi came out slightly more constant.

Smith and Swift discussed only the formulae Bi^{3+}, $BiOH^{2+}$, BiO^+ (or $Bi(OH)_2^+$) and their complexes with NO_3^- or ClO_4^-. In 1936 Holmqvist [3] on one side and Prytz and Nagel [4] on the other, independently arrived at the conclusion that there must be *polynuclear* bismuth ions among the reaction products.

Prytz and Nagel concluded from measurements of the rate of diffusion of bismuth perchlorate that simple molecules prevail in solutions of high acidity. In less acid solutions the molecular weight is at first doubled and then increases further, although the latter process could not be followed for experimental reasons. On titration of Bi^{3+} with OH^- they found a number of small breaks in the curve (conductivity/added volume), which they explained in terms of the formation of $BiOH(ClO_4)_2$, $Bi(OH)_2ClO_4$, $Bi_2(OH)_2O(ClO_4)_2$, and $(BiOH)_4O_3(ClO_4)_2$. On titration of $Bi(OH)_2ClO_4$ with $HClO_4$, there was only one break in the curve (temperature/added volume) which was attributed to the formation of $BiOH(ClO_4)_2$ from $Bi(OH)_2ClO_4$. Measurements of the optical absorption of different solutions were explained in terms of the compounds mentioned. Emf titrations gave no useful results because of difficulties with the hydrogen and quinhydrone electrodes. Prytz and Nagel claim to have isolated, in addition to the previously known $Bi(OH)_2ClO_4$, the solid compounds $Bi_3(OH)_3O_2(ClO_4)_2$, $(BiO)_2OHClO_4$, and $Bi_2(OH)_3OClO_4$.

Holmqvist [3] measured the emf of different cells with Bi or quinhydrone electrodes in solutions containing bismuth perchlorate, $NaClO_4$, and $HClO_4$. From these measurements he concluded that bismuth is present almost exclusively as Bi^{3+} at $[H^+]>0.5$ C, that there is no considerable complex formation between Bi^{3+} and ClO_4^- but strong complexes between Bi^{3+} and Cl^-, and that the bismuth ions formed in less acid solutions are chiefly binuclear. By assuming that only Bi^{3+}, $Bi_2(OH)_4^{2+}$ and $Bi_2(OH)_5^+$ are formed, and that the equilibrium constants (in 0.5 C $NaClO_4$) are: $[Bi^{3+}]^2[Bi_2(OH)_4^{2+}]^{-1}[H^+]^{-4} = 0.025$, and $[Bi^{3+}]^2[Bi_2(OH)_5^+]^{-1}[H^+]^{-5} = 0.000025$, Holmqvist could explain the measured emf:s with an accuracy of 1.5 mV, which he considered to be within the limits of experimental error. He has also prepared the solid compound $Bi_2(OH)_3OClO_4$.

Holmqvist is the only investigator, whose results have had enough accuracy for permitting him to try to apply the law of mass action to the equilibrium between different bismuth ions. His measurements are, however, rather few and did not allow a very elaborate theory. Otherwise it is difficult to understand, why the two complexes $Bi_2(OH)_4^{2+}$ and $Bi_2(OH)_5^+$ should predominate over all other conceivable formulae.

It thus seemed highly desirable to make new and accurate experiments on the hydrolysis of Bi^{3+}.

CHOICE OF EXPERIMENTAL CONDITIONS

We decided to work with solutions of constant ionic strength so that the activity factors of all ions could be assumed to be approximately constant. The anions that can be assumed to have the least tendency to form complexes with Bi^{3+} are NO_3^- and ClO_4^-; of these we preferred ClO_4^- since the hydroxynitrates of bismuth are known to be less soluble than the hydroxyperchlorates.

Our solutions were prepared so as always to contain 3 C ClO_4^- and Na^+, H^+ and Bi ions. Such a high ionic strength was desirable for two reasons: the H^+ concentration had to be rather high in some of our solutions, and it was necessary to counteract as much as possible the influence on the electrostatic forces in the solution by the polyvalent Bi ions which might be formed. The total bismuth concentration was kept at 10, 25, or 50 mC. At higher concentrations the changes in ionic strength caused by the reactions of bismuth would be too large and precipitation would occur inconveniently early; at lower concentrations the changes in $[H^+]$ could not be measured accurately enough.

In these solutions it was desired to measure the concentrations $[H^+] = h$ and $[Bi^{3+}] = b$. For measuring h, a hydrogen electrode cannot be used, since bismuth metal is then formed by reduction. A glass electrode might be used, but would not be expected to give very accurate results in solutions of such high H^+ and Na^+ concentrations. Happily enough the quinhydrone electrode proved to be accurate and not affected by the presence of Bi^{3+} (see below). For the measurements of $[Bi^{3+}]$ we preferred bismuth amalgam electrodes to the bismuth metal electrodes used by Holmqvist. They were found to function very well. The solutions were prepared by titration; in this way a considerable number of measurements could be obtained with a minimum of manipulation. We found it convenient to use as reference electrode:

$$CE// = Hg,Hg_2Cl_2/4\ C\ NaCl/\ 4\ C\ NaClO_4/$$

The cells used were of the following two types (Q=quinone):

$$-\ CE//H^+,QH_2,Q/Pt\ +$$
$$E_Q = E_{0Q} + 59.16\ \log\ h + E_d = E' + 59.16\ \log\ h \qquad (1)$$

$$-\ CE//\ Bi^{3+}/Bi(\text{in Hg})\ +$$
$$E_{Bi} = E_{0Bi} + \frac{59.16}{3}\log\ [Bi^{3+}] + E_d = E' + (E_{0Bi} - E_{0Q}) + 19.72\ \log\ b \qquad (2)$$

It is not required to determine separately the constant E_{0Q} or the variable diffusion potential E_d, whereas their sum E' is an accurately determinable quantity. As H^+ ions are always present in considerable amounts on the right hand side in our cells, and as their mobility by far exceeds that of the other ions present, we can confidently assume that *the diffusion potential and thus E' is a function of the hydrogen ion concentration h only.*

Then three types of measurements were needed:

1. Quinhydrone electrode with solutions of known h (h C $HClO_4$,(3-h) C $NaClO_4$). From these experiments the values of E' for different h were calcul-

ated, and E' could be plotted and tabulated as a function of h or, more conveniently, of E_Q.

2. Quinhydrone electrode and the bismuth solution to be investigated. E' and h were obtained from the measured E_Q by means of the measurements 1/.

3. Bismuth amalgam electrode and the same bismuth solutions as in 2/. E' being known from the measurements 2/, one can calculate $E_{Bi}-E' = (E_{0Bi} - E_{0Q}) + 19.72 \log b$. If the total Bi concentration is kept constant at B, and h is increased, the hydrolysis is pressed back, and b tends to the value B. Happily enough, this seems to occur within the range of concentrations studied by us*: $E_{Bi} - E'$ tended to a limiting value, which according to (2) should be:

$$\lim_{h\to\infty} (E_{Bi} - E') = E_{0Bi} - E_{0Q} + 19.72 \log B \qquad (3)$$

When the constant $(E_{0Bi} - E_{0Q})$ had been obtained, $[Bi^{3+}] = b$ could be calculated for all solutions, by means of (2). E_{0Bi} can be expected to change with the composition of the amalgam, thus to be different for different batches of amalgam.

The following quantities were thus known for every solution:

From the analysis: B = total bismuth concentration (in mC Bi)
H = total hydrogen ion concentration (assuming all bismuth to be present as Bi^{3+})
From the emf: b = actual concentration $[Bi^{3+}]$
h = actual concentration $[H^+]$

In each series of experiments, B was kept constant. The results are conveniently given as graphs of bB^{-1} (the fraction of Bi present as Bi^{3+}) and $(h - H)B^{-1}$ (the number of H^+ set free per Bi atom) as functions of h.

EXPERIMENTAL

In all our measurements a solution T rich in H^+ was added to one S of low h and the same B. It was tried to add NaOH in order to study the equilibria at lower h, but then a precipitate was immediately formed. The apparatus used was the same as described by Sillén [5]. The bismuth amalgam electrodes consisted of a pool on the bottom of the titration vessel; the electrical connection was made by means of a Pt needle.

* In a few preliminary experiments with 1 C ClO_4^-, the limiting value could not be determined with certainty. This was one reason for our using 3 C ClO_4^- in all succeeding experiments.

Liquid bismuth amalgam containing about 1 % Bi was prepared by dissolving Bi in Hg at 90° under cover of boiled distilled water. It was stored under water. This procedure gave quite a satisfactory product and eliminated the necessity of using a N_2 atmosphere or vacuum.

The perchloric acid and sodium perchlorate were obtained and analysed as described by Sillén, the concentration of the perchloric acid being also determined by adding excess of NH_3, evaporating at 105° and weighing NH_4ClO_4 (*cf.* Holmqvist [3] p. 64). The results agreed with those of titration with NaOH within 0.2 %.

The bismuth perchlorate solutions were prepared by dissolving Bi_2O_3 (Merck pro analysi) in perchloric acid. Bismuth was precipitated with NH_3, ignited and weighed as Bi_2O_3; in the filtrate, the amount of ClO_4^- was determined by weighing as NH_4ClO_4 as above.

The E_Q and E_{Bi} measured were steady and very reproducible. Each titration was made at least twice and the difference between corresponding points seldom found to exceed 0.1 mV.

The glass electrode was a commercial one manufactured by Rudolph Grave AB. The glass electrode emf:s were measured by means of a Radiometer valve potentiometer.

COMPARISON OF GLASS AND QUINHYDRONE ELECTRODES

A number of titration experiments were made with cells

— CE//solution/glass electrode +

The emf obtained, E_{glass}, behaved in a very irregular way. In some pairs of titrations E_{glass} followed closely E_Q for the same solutions (*i. e.* $E_Q - E_{glass}$ kept almost constant) for a certain time, then suddenly E_{glass} would jump several mV, and then again follow E_Q. The quinhydrone electrode gave on the other hand very reproducible and steady potentials.

It might be feared that quinone or especially hydroquinone might form some sort of complex with Bi^{3+}; in this case E_Q could not be directly used for measuring h, and moreover all the bismuth equilibria would be displaced on addition of quinhydrone.

At high h almost all the Bi is assumed to be present as Bi^{3+} and it was found that, for a given h, almost the same E_Q was obtained whether the solution contained any Bi or not (see the discussion of δ below), and the variation of E_Q with h was identical within 0.1 mV. This seems to show that at high h no considerable error is caused by Bi-quinhydrone complexes. However, it was still desirable to make some sort of control at lower h.

Table 1. Comparison of quinhydrone and glass electrodes.

S = 2900 mC $NaClO_4$, 100 mC $HClO_4$, 100 ml
T = 2750 mC $NaClO_4$, 100 mC $HClO_4$, 50 mC $Bi(ClO_4)_3$, 0—50 ml

ml	E_Q	E_{glass}
0	413.0	61.2
5	413.5	61.8
10	414.2	62.1
15	414.8	62.8
20	415.3	63.2
25	415.9	63.7
30	416.5	64.0
35	417.0	64.6
40	417.4	65.0
45	417.9	65.4
50	418.3	65.9

In the experiments given in Table 1, the original solution S contained no Bi, whereas the added solution T contained 50 mC Bi. As seen from Table 1, E_Q and E_{glass} were parallel in the two titrations and there is no irregular behavour of E_Q on the addition of Bi. During the entire titration, E_Q is changed by 5.3 mV and E_{glass} by 4.7 mV; this is normal, as the slope of E_{glass} against log h is, in such solutions, lower than 59.16 (see Dole [6] Chapter 8).

RESULTS

From our measurements of E_Q in solutions of known h every fourth of the points is given in Table 2. In Fig. 1, E' is plotted as a function of E_Q. It was necessary to add a small correction δ in order to make E_Q values measured at different times comparable. The correction δ was the same throughout each titration and was calculated so that $E_Q + \delta$ was the same for points of the same h in all titrations. One reason for the appearance of δ is that the calomel electrodes change slowly with time. If titrations with Bi solutions were made immediately before or after such without any Bi, δ was found to be 0.4 mV lower for B = 25 mC and 0.7 mV lower for B = 50 mC than for B = O. The reason is probably the small changes in ionic strength on replacing 3 Na^+ by Bi^{3+}.

The measured E_Q and E_{Bi} in solutions containing bismuth are given in Tables 3—5. From these the quantities bB^{-1} (the part of the Bi present as

Table 2. Determination of E'. Only every fourth point is given.

A. S = 3000 mC $NaClO_4$, 100 ml; T = 2800 mC $NaClO_4$, 200 mC $HClO_4$, 0—50 ml δ = + 0.6 mV.

ml	h mC	E	E'
1	1.98	300	(460)
5	9.52	337.2	457.4
9	16.51	351.2	457.2
13	23.01	359.6	457.1
20	33.33	369.0	457.0
30	46.15	377.0	456.6
45	62.07	384.4	456.5
50	66.67	386.2	456.3

B. S = 3000 mC $NaClO_4$, 100 ml; T = 1500 mC $NaClO_4$, 1500 mC $HClO_4$, 0—50 ml δ = 0.0 mV.

ml	h mC	E	E'
1	14.9	349.5	(457.6)
5	71.4	388.2	456.0
9	123.9	400.9	454.6
16	207	412.4	452.9
25	300	420.2	451.1
35	389	425.4	449.7
45	465	428.8	448.4
50	500	430.2	448.0

C. S = 2700 mC $NaClO_4$, 300 mC $HClO_4$, 100 ml. T = 1500 mC $NaClO_4$, 1500 mC $HClO_4$, 0—50 ml. δ = 0.0 mV.

ml	h mC	E	E'
0	300	420.2	451.1
10	409	426.5	449.5
20	500	430.3	448.1
30	577	432.9	447.0
40	643	434.8	446.2
50	700	436.1	445.3

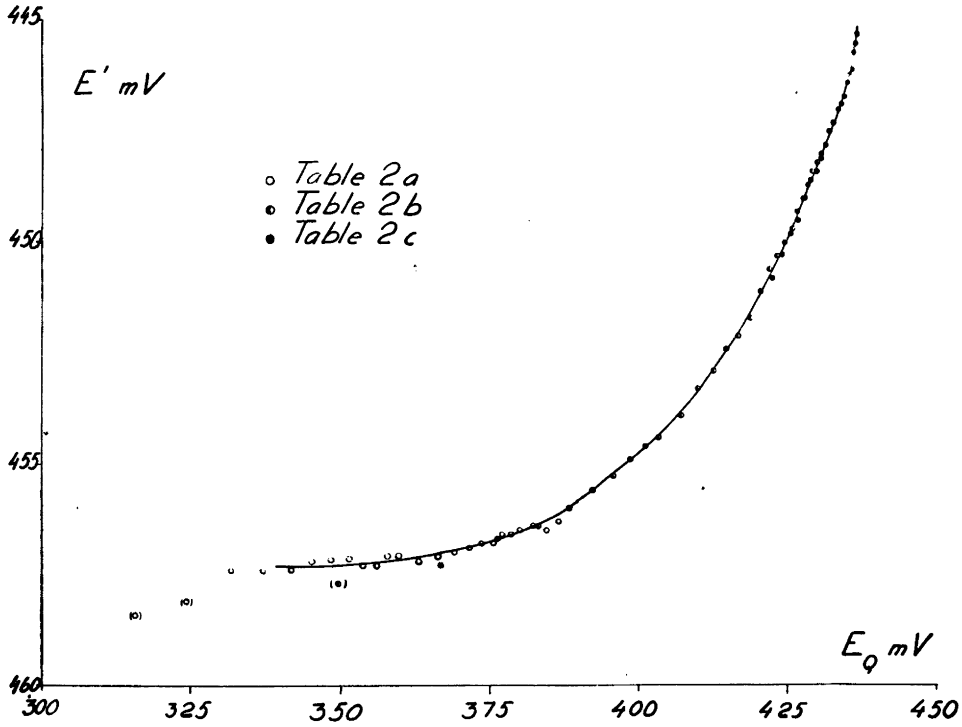


Fig. 1. E' as a function of E_Q (Tab. 1, eq. (1)).

Bi^{3+}) and $(h - H)B^{-1}$ (the number of H^+ ions set free per Bi atom) were computed by means of formulae (1), (2), and (3). They are plotted as functions of h in Figs. 2 and 3.

ON THE MECHANISM OF THE PROCESS

The simplest assumption that can be made is that, except Bi^{3+}, only mononuclear complexes $BiOH^{2+}$, $Bi(OH)_2^+$ (or BiO^+), and possibly uncharged $Bi(OH)_3$ are formed, thus

$$Bi^{3+} + H_2O \rightleftharpoons BiOH^{2+} + H^+;\ Bi^{3+} + 2H_2O \rightleftharpoons Bi(OH)_2^+ + 2H^+;\ Bi^{3+} + 3H_2O \rightleftharpoons Bi(OH)_3 + 3\ H^+$$

The equilibrium conditions would then be

$$[BiOH^{2+}] = \varkappa_1\ [Bi^{3+}]\ [H^+]^{-1} = \varkappa_1 bh^{-1};\ [Bi(OH)_2^+] = \varkappa_2 bh^{-2};\ [Bi(OH)_3] = \varkappa_3\ bh^{-3}$$

Table 3. Measurements with B = 50 mC.

S = 2850 mC $NaClO_4$, 50 mC $Bi(ClO_4)_3$
T = 1350 mC $NaClO_4$, 50 mC $Bi(ClO_4)_3$, 1500 mC $HClO_4$.
A. and B. Determination of h.
A. S = 75 ml; T = 0—65 ml; δ = — 0.7 mV.

ml	H mC	E_Q	E'	h mC	$h - H$
0	0	394.4	455.5	90.4	90.4
2	39.0	401.5	454.7	122.7	83.7
4	76.0	406.5	454.0	152.8	76.8
6	111.1	410.3	453.4	182.0	70.9
8	144.6	413.2	452.9	207.5	62.9
10	176.4	415.6	452.4	232.3	55.9
12	207	417.7	452.0	256	49
14	236	419.5	451.5	280	44
16	264	421.1	451.1	303	39
18	290	422.4	450.8	322	32
20	316	423.6	450.4	343	27
22.5	346	425.0	450.0	368	22
25	375	426.3	449.6	393	18
27.5	402	427.4	449.2	417	15
30	429	428.5	448.9	440	11
32.5	453	429.5	448.6	463	10
35	477	430.4	448.3	481	4
37.5	500	431.2	448.1	505	5
40	522	431.9	447.8	524	2
42.5	543	432.5	447.6	541	—2
45	563	433.2	447.3	562	—1
47.5	582	433.8	447.0	582	0
50	600	434.4	446.7	603	3
52.5	618	434.9	446.5	619	1
55	635	435.4	446.2	637	2
57.5	651	435.8	446.0	655	4
60	667	436.2	445.8	670	3
62.5	682	436.5	445.6	682	0
65	696	436.8	445.4	697	1

where $\varkappa_1$, $\varkappa_2$, and $\varkappa_3$ are constants. The total Bi concentration B and the amount of H^+ set free per litre would be

$$B = [Bi^{3+}] + [BiOH^{2+}] + [Bi(OH)_2^+] + [Bi(OH)_3] = b\,(1 + \varkappa_1\, h^{-1} + \varkappa_2\, h^{-2} + \varkappa_3\, h^{-3})$$
$$h - H = [BiOH^{2+}] + 2[Bi(OH)_2^+] + 3[Bi(OH)_3] = b(\varkappa_1\, h^{-1} + 2\,\varkappa_2\, h^{-2} + 3\,\varkappa_3\, h^{-3})$$

Table 3.

B. S = 100 ml, T = 0—50 ml, δ = — 0.7 mV.

ml	H mC	E_Q	E'	h mC	$h-H$
0	0	394.4	455.5	90.4	90.4
1	14.9	397.4	455.2	102.6	87.7
2	29.4	400.1	454.9	115.3	85.9
3	43.7	402.2	454.6	126.8	83.1
4	57.7	404.3	454.3	139.0	81.3
5	71.4	406.1	454.1	150.3	78.9
6	84.9	407.6	453.9	160.7	75.8
7	98.1	409.1	453.6	172.2	74.1
8	111.1	410.3	453.4	182	71
9	123.9	411.5	453.2	192	68
10	136.4	412.6	453.0	202	66
11	150.0	413.6	452.8	212	62
12	160.7	414.6	452.6	222	61
13	172.6	415.5	452.4	232	61
14	184.2	416.4	452.2	242	58
15	195.7	417.1	452.1	248	53
16	207	417.8	452.0	257	50
17	218	418.5	451.8	266	48
18	229	419.2	451.6	276	47
19	239	419.8	451.5	283	44
20	250	420.4	451.4	291	41
21	260	421.0	451.2	301	41
22.5	276	421.8	451.0	313	37
24	290	422.5	450.8	324	34
25	300	423.0	450.6	333	33
27.5	324	424.1	450.4	350	26
30	346	425.3	450.0	371	25
32.5	368	426.1	449.6	390	22
35	389	427.0	449.3	408	19
37.5	409	427.9	449.1	427	18
40	429	428.7	448.9	444	15
42.5	447	429.4	448.6	461	14
45	465	430.1	448.4	478	13
47.5	483	430.7	448.2	492	9
50	500	431.3	448.0	508	8

It is seen from the last two equations that in this case bB^{-1} and $(h-H)B^{-1}$ must be functions of h only, independent of the total bismuth concentration B. This is seen not to be true (Figs. 2 and 3). On the contrary for a given h,

Table 3.

C. Determination of b.
$S = 75$ ml $T = 0$—65 ml.
lim $(E' - E_{Bi}) = 413.7$; $E_{0Q} - E_{0Bi} = 447.2$.

ml	E_{Bi}	$E' - E_{Bi}$	b
0	16.8	438.7	2.70
1	19.4	435.7	3.84
2	21.4	433.1	5.20
3	23.0	431.3	6.41
4	24.5	429.5	7.91
5	25.6	428.1	9.31
6	26.7	426.7	10.96
7	27.6	425.5	12.62
8	28.3	424.6	14.00
9	29.0	423.6	15.7
10	29.7	422.7	17.5
11	30.3	421.9	19.2
12	30.8	421.2	20.8
13	31.3	420.5	22.6
14	31.6	420.0	24.0
15	32.0	419.4	25.7
16	32.2	419.0	26.9
17	32.5	418.5	28.6
18	32.8	418.0	30.3
20	33.3	417.1	33.7
22.5	33.7	416.3	36.9
25	33.9	415.7	39.6
27.5	34.1	415.1	42.5
30	34.1	414.8	44.5
32.5	34.0	414.6	45.0
35	33.8	414.5	45.5
37.5	33.7	414.4	46.1
40	33.5	414.3	46.7
42.5	33.2	414.4	46.1
45	33.3	414.0	48.3
47.5	33.2	413.8	49.4
50	32.9	413.8	49.4
52.5	32.7	413.8	49.4
55	32.5	413.7	50.0
57.5	32.3	413.7	50.0
60	32.1	413.7	50.0
62.5	32.0	413.6	50.6
65	31.7	413.7	50.0

Table 4. Measurements with B = 25 mC.

S = 2925 mC $NaClO_4$, 25 mC $Bi(ClO_4)_3$.
T = 1425 mC $NaClO_4$, 25 mC $Bi(ClO_4)_3$, 1500 mC $HClO_4$.
A. Determination of h.
S = 100 ml, T = 0—50 ml, δ = — 0.4 mV.

ml	H mC	E_Q	E'	h mC	$h - H$
0	0	378.4	456.7	46.8	46.8
1	14.9	384.4	456.3	60.0	45.1
2	29.4	389.0	455.9	73.0	43.6
3	43.7	392.9	455.6	85.9	42.2
4	57.7	396.0	455.3	98.0	40.3
5	71.4	398.5	455.0	109.1	37.7
6	84.9	400.8	454.7	120.8	35.9
7	98.1	402.8	454.5	131.8	33.7
8	111.1	404.6	454.3	142.2	31.1
9	123.9	406.2	454.0	153.1	29.2
10	136.4	407.7	453.8	163.7	27.3
11	150.0	409.1	453.6	174	24
12	160.7	410.3	453.4	184	23
13	172.6	411.4	453.2	193	20
14	184.2	412.5	453.0	204	20
15	195.7	413.5	452.8	213	17
16	207	414.5	452.6	223	16
17	218	415.3	452.4	232	14
18	229	416.1	452.3	240	11
19	239	416.9	452.1	250	11
20	250	417.7	451.9	260	10
21	260	418.3	451.7	269	9
22.5	276	419.4	451.5	283	7
24	290	420.3	451.3	294	4
25	300	420.9	451.1	304	4
27.5	324	422.4	450.7	327	3
30	346	423.6	450.4	347	1
32.5	368	424.8	450.0	366	—2
35	389	425.8	449.7	388	—1
37.5	409	426.7	449.4	407	—2
40	429	427.7	449.1	429	0
42.5	447	428.5	448.8	447	0
45	465	429.3	448.6	460	—5
47.5	483	430.0	448.3	483	0
50	500	430.6	448.1	498	—2

Table 4.

B. Determination of b.
$S = 75$ ml, $T = 0$—50 ml.
$\lim (E' - E_{Bi}) = 420.4$; $E_{0Q} - E_{0Bi} = 448.0$.

ml	E_{Bi}	$E' - E_{Bi}$	b
0	5.4	451.3	0.68
1	10.3	445.9	1.27
2	14.2	441.5	2.13
3	16.7	438.6	2.98
4	18.8	436.1	3.99
5	20.7	433.9	5.18
6	22.1	432.2	6.31
7	23.3	430.7	7.53
8	24.3	429.4	8.75
9	25.3	428.1	10.20
10	26.1	427.0	11.55
12	27.3	425.3	14.15
14	28.1	424.0	16.40
16	28.7	423.0	18.45
18	29.0	422.3	20.0
20	29.2	421.7	21.5
22.5	29.2	421.2	22.8
25	29.0	420.9	23.6
27.5	28.7	420.8	23.9
30	28.4	420.7	24.2
32.5	28.1	420.6	24.4
35	28.0	420.4	25.0
37.5	27.6	420.5	24.7
40	27.3	420.5	24.7
42.5	27.1	420.5	24.7
45	26.8	420.5	24.7
47.5	26.7	420.3	25.3
50	26.5	420.2	25.6

the hydrolysis goes farther (bB^{-1} is smaller and $(h—H)B^{-1}$ larger) the larger is B. Thus it is clear that there must also be *polynuclear* complexes present. At first we tried to assume that the only ionic species present in considerable amounts are Bi^{3+} and one or two ions of the type $Bi_n(OH)_m^{3n-m}$, where n and m were the unknowns sought for. Equilibrium conditions were set up assuming the combinations

$$BiOH^{2+} + Bi_n(OH)_m^{3n-m}$$

$$Bi(OH)_2^+ + Bi_n(OH)_m^{3n-m}$$

$$Bi_n(OH)_m^{3n-m} + Bi_n(OH)_{m+1}^{3n-m-1}$$

among others, and an attempt was made to determine n and m. However, we could not explain, even approximately, our experimental data by any such assumption.

Considerations of several kinds led us to propose a new type of mechanism. As is well known, the acidity of Al^{3+} and Fe^{3+} was explained by Brönsted and Volqvartz [7] by the tendency of the coordinated water molecules to split off a proton; this process is favoured by the electrostatic repulsion of the central ion (Fig. 4a). For the »aquo» ions $Fe(H_2O)_6^{3+}$ and $Al(H_2O)_6^{3+}$ K_a seems to be about 10^{-3} and 10^{-5}. (As for Fe^{3+} see also Brosset [8] and Lindstrand [9].) For Bi^{3+} with its larger radius and thus lower Coulomb forces, the aquo acidity might be expected to be less; actually, however, the main part of the H^+

Table 5. Measurements with B = 10 mC.

S = 2970 mC $NaClO_4$, 10 mC $Bi(ClO_4)_3$, 100 ml.
T = 1470 mC $NaClO_4$, 10 mC $Bi(ClO_4)_3$, 1500 mC $HClO_4$, 0—50 ml.
A. Determination of h.
$\delta = -1.6$ mV.

ml	H mC	E_Q	E'	h mC	$h - H$
0	0	358.4	457.3	20.0	20.0
1	14.9	371.5	457.1	33.6	18.7
2	29.4	379.9	456.7	47.3	17.9
3	43.7	385.8	456.3	60.5	16.8
4	57.7	390.4	456.0	73.1	15.4
5	71.4	394.2	455.6	86.1	14.7
6	84.9	397.3	455.3	98.4	13.5
8	111.1	402.2	454.7	121.9	10.8
10	136.4	406.0	454.2	143.9	7.5
12	160.7	409.1	453.8	165.2	4.5
14	184.2	411.9	453.3	187.5	3.3
16	207	414.3	452.9	209	2
18	229	416.4	452.5	231	2
20	250	418.3	452.1	252	2
25	300	421.9	451.2	301	1
30	346	424.7	450.4	343	—3
35	389	427.1	449.6	392	3
40	429	428.9	449.1	429	0
45	465	430.5	448.6	465	0
50	500	431.9	448.1	500	0

B. Determination of b
lim $(E' - E_{Bi}) = 428.1$ mV; $E_{0Q} - E_{0Bi} = 447.8$ mV

ml	E_{Bi}	$E' - E_{Bi}$	b
0	—8.9	466.2	0.117
1	0.0	457.1	0.339
2	5.6	451.1	0.682
3	9.4	446.9	1.11
4	12.2	443.8	1.60
5	14.5	441.1	2.19
6	16.3	439.0	2.80
8	19.2	435.5	4.22
10	21.2	433.0	5.65
12	22.6	431.2	7.06
14	23.3	430.0	7.93
16	23.9	429.0	9.00
18	23.8	428.7	9.33
20	23.6	428.5	9.55
25	23.0	428.2	10.12
30	22.3	428.2	10.12
35	21.5	428.1	10.00
40	21.0	428.1	10.00
45	20.4	428.2	10.12
50	20.1	428.0	9.89

seems to be split off at about $h = 10^{-2}$ C. Thus it seems unlikely that the acidity of Bi^{3+} is mainly an aquo acidity.

If, as the experiments seems to indicate, there is a process which can couple Bi ions together to polynuclear complexes $Bi_n(OH)_m$, it would be remarkable if complexes of some n were present and not of others; it seems more likely that all n: 1,2,3, . . . are possible. One would thus be inclined to search for a *general* process by which Bi atoms can be added one by one to the complex.

A closer study of the measurements revealed that at points of the same bB^{-1} but different B (10, 25, 50, mC), the quantity bh^{-2} had almost the same value (Fig. 2) and thus bB^{-1} seemed to be a function of the variable

$$y = bh^{-2} \tag{4}$$

The same is true for $(h\text{-}H)B^{-1}$. Moreover, y seemed to approach to a limiting value at low h. All this indicates that in the equation of the general reaction there is one Bi^{3+} on one side and two H^+ on the other. The following equation immediately suggests itself

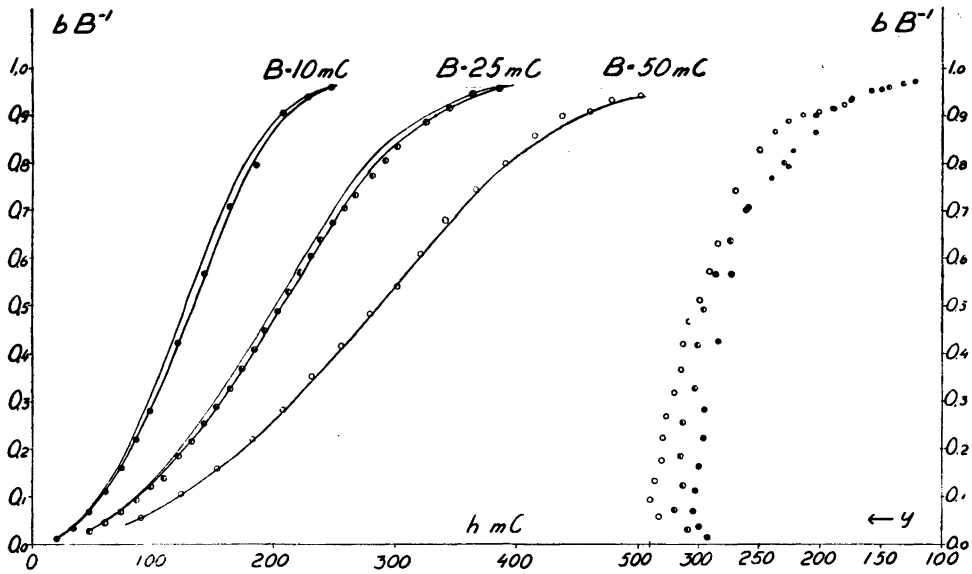


Fig. 2. $bB^{-1} = [Bi^{3+}]_{free}/[Bi]_{total}$ *as a function of* $h = [H^+]$ *and* $y = bh^{-3}$. *Circles = experimental values. Thick lines are calculated with eq. (11) and (4) and the constants: for B = 50 mC* $k_1 = 0{,}065$ *C* $k = 2.80$ *C; for B = 25 mC* $k_1 = 0{,}070$ *C* $k = 2.95$ *C; and for B = 10 mC* $k_1 = 0{,}060$ *C* $k = 3.15$ *C. Thin lines for 25 and 10 mC are calculated with the constants for B = 50 mC. The scale gives 1000 y.*

$$Bi^{3+} + H_2O + Complex^{q+} \rightarrow BiOComplex^{(q+1)} + 2H^+$$

The simplest reaction of this type would be

$$Bi^{3+} + H_2O + Bi^{3+} \rightarrow Bi_2O^{4+} + 2H^+$$

Thus a water molecule forms an *oxygen bridge* between two bismuth atoms, and at the same time two H^+ are set free (Fig. 4b). We shall see that a repetition of this process and a few simple assumptions gives a resonable explanation of the experimental data.

PROPOSED MECHANISM

We assume that the hydrolysis of Bi^{3+} takes part in the following steps:

	Equilibrium const.	
$Bi^{3+} + Bi^{3+} + H_2O \rightarrow Bi_2O^{4+} + 2H^+$	k_1	(5_1)
$Bi_2O^{4+} + Bi^{3+} + H_2O \rightarrow Bi_3O_2^{5+} + 2H^+$	k_2	
$Bi_3O_2^{5+} + Bi^{3+} + H_2O \rightarrow Bi_4O_3^{6+} + 2H^+$	k_3	

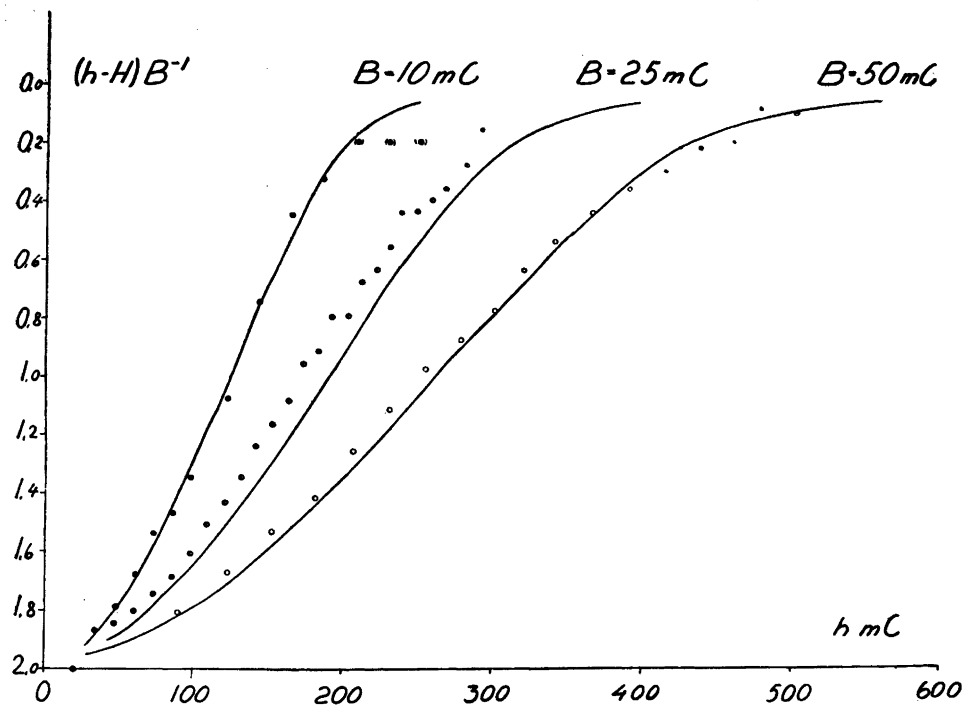


Fig. 3. (h-H)B^{-1} (average number of H$^+$ set free per Bi atom) as a function of h. Circles = experimental values. The curves are calculated with eq. (11) and (12) and the constants: for B = 50 mC k_1 = 0,065 C k = 2.80 C; for B = 25 mC k_1 = 0,070 C k = 2.95 C and for B = 10 mC k_1 = 0,060 C k = 3.15 C.

or generally

$$Bi_nO_{n-1}^{(n+2)+} + Bi^{3+} + H_2O \rightarrow Bi_{n+1}O_n^{(n+3)+} + 2H^+ \qquad k_n \qquad (5_n)$$

We shall moreover make the simplifying assumption *that except for k_1, all constants k_2, k_3 . . . . . k_n are assumed to be equal* and that there is no limit to n:

$$k_2 = k_3 = k_4 \ldots\ldots = k_n = \ldots\ldots = k \qquad (6)$$

With the notations

$$bh^{-2} = y \qquad (4)$$
$$ky = kbh^{-2} = z \qquad (7)$$

we find

$$c_1 = [Bi^{3+}] = b \qquad (8_1)$$

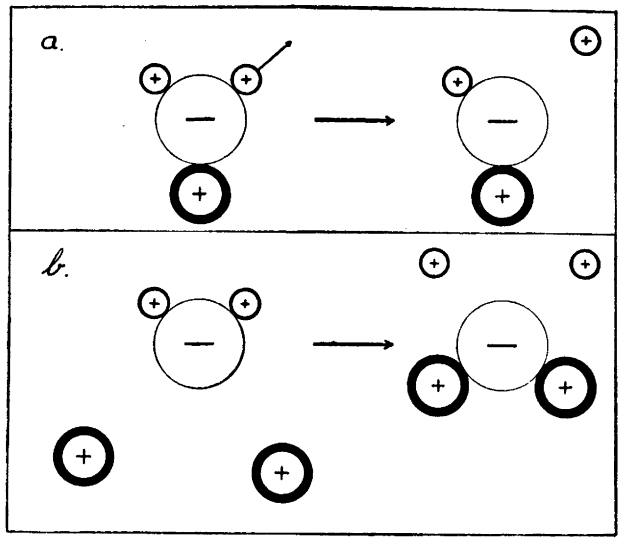


Fig. 4. Schematic picture of a) aquo acidity; a proton of a coordinated water molecule is repelled by the electrostatic forces from the central cation. b) oxygen bridging; a water molecule is split up so that an oxygen bridge is formed between two cations, and two protons are set free. — In the figure the other water molecules coordinated to the cation or to the protons have been left out.

$$c_2 = [Bi_2O^{4+}] = k_1 b^2 h^{-2} = k_1 by$$
$$c_3 = [Bi_3O_2^{5+}] = k[Bi_2O^{4+}]bh^{-2} = k_1 byz \qquad (8_2)$$
$$c_4 = [Bi_4O_3^{6+}] = k[Bi_3O_2^{5+}]bh^{-2} = k_1 byz^2$$
$$c_n = [Bi_nO_{n-1}^{(n+2)+}] = k_1 byz^{n-2} \qquad (n > 1) \qquad (8_n)$$

For the total bismuth concentration B and the amount of H^+ set free, h—H, we find

$$B = [Bi^{3+}] + 2[Bi_2O^{4+}] + 3[Bi_3O_2^{5+}] + \ldots = \Sigma\, nc_n = \\ = b + k_1 by(2 + 3z + 4z^2 + \ldots) \qquad (9)$$
$$h - H = 2[Bi_2O^{4+}] + 4[Bi_3O_2^{5+}] + 6[Bi_4O_3^{6+}] + \ldots. = \\ = 2\,\Sigma\,(n-1)c_n = 2k_1 by\,(1 + 2z + 3z^2 + \ldots) \qquad (10)$$

If these expressions are summed to infinity we find

$$B = b + k_1 by(2 - z)\,(1 - z)^{-2} \qquad (11)$$
$$h - H = 2k_1 by\,(1 - z)^{-2} \qquad (12)$$

In these equations, B,b,H,h and $y = bh^{-2}$ are directly measured variables, whereas k and k_1 are unknown constants and $z = ky$. The unknown constants can be found by a graphical method.
From (11) and (7) we find:

$$\log [(B - b)\ (by)^{-1}] = \log k_1 + \log [(2 - z)\ (1 - z)^{-2}] \quad (13)$$
$$\log y = \log z - \log k \quad (14)$$

On one graph one can construct the curve giving $\log [(2-z)\ (1-z)^{-2}]$ as a function of $\log z$ and on another plot to the same scale the experimental values of $\log [(B-b)(by)^{-1}]$ against $\log y$.

If our assumptions are permissible, it is possible, by horisontal and vertical translations, to make the experimental points coincide with the curve. Moreover, from the positions of the curves it is possible to obtain $\log k$ and $\log k_1$.

Finally, if k and k_1 are known, the measured quantities b, h and $h-H$ are conveniently calculated as follows:

$$b = B\ [1 + k_1 y\ (2 - ky)\ (1 - ky)^{-2}]^{-1} \quad (11)$$
$$h = \sqrt{by^{-1}} \quad (4)$$
$$h - H = 2k_1 by\ (1 - ky)^{-2} \quad (12)$$

COMPARISON WITH THE MEASUREMENTS

In Fig. 5 the experimental values of $\log [(B-b)\ (by)^{-1}]$ are plotted against $\log y$. (In those points where only one of b and h had been measured, the other was found by graphical interpolation.) Within the limits of experimental error the points are seen to fit the theoretical curves. When the points for each value of B were regarded separately, the following constants were found:

$B = 50$ mC	$k_1 = 0.065 \pm 0.005$ C	$k = 2.80 \pm 0.05$ C
25 mC	$k_1 = 0.070 \pm 0.010$ C	$k = 2.95 \pm 0.10$ C
10 mC	$k_1 = 0.060 \pm 0.010$ C	$k = 3.15 \pm 0.20$ C

By means of these constants, b and $h-H$ were calculated as functions of h in the way indicated in the preceding section (cf. Tab. 6). In Fig. 2, the thin curves for $B = 25$ and 10 mC have been calculated by means of the constants 0.065 and 2.80, whereas the thick curves are in Figs. 2 and 3 are calculated by means of the »best values» given above.

The agreement for bB^{-1} is seen to be surprisingly good in view of the crude assumptions involved. For $(h-H)B^{-1}$ the deviations are somewhat larger, but it must be remembered that this quantity is the difference between two

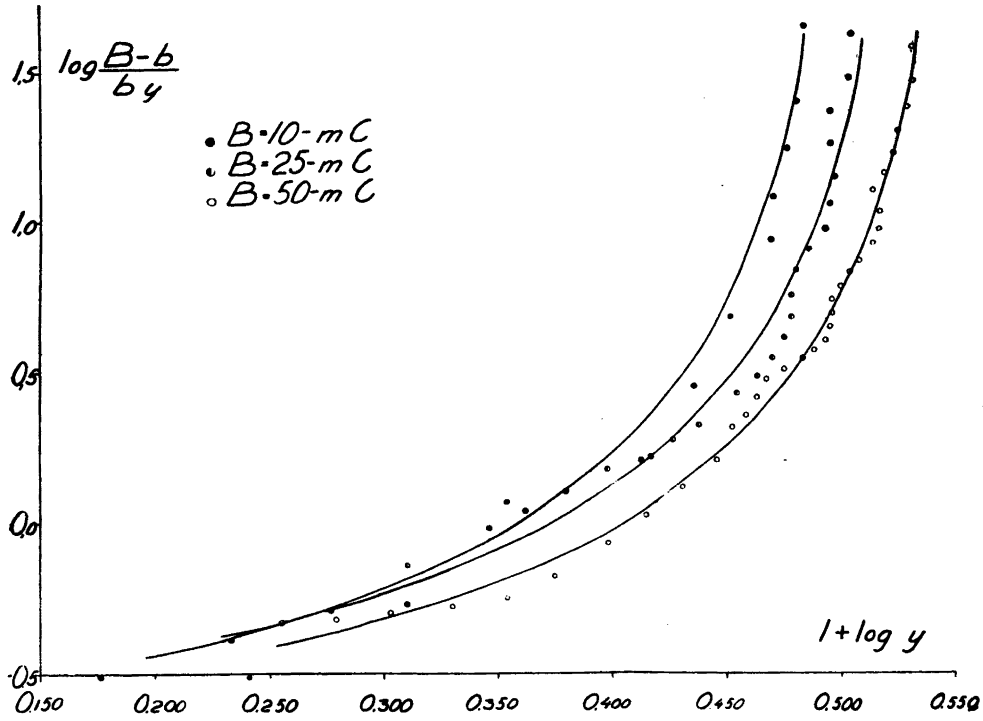


Fig. 5. Circles: experimental values of log (B—b) $(by)^{-1}$ as a function of log y. Curves: theoretical curves assuming, from right to left, $k_1 = 0{,}065$ C $k = 2.80$ C; $k_1 = 0{,}070$ C $k = 2.95$ C; $k_1 = 0{,}060$ C $k = 3.15$ C.

larger quantities and can therefore not be determined very accurately. In view of this fact the agreement must be said to be satisfactory.

The measurements give no indication of any systematic variation of k_1 with B; it may very well be constant at 0.065 in all the solutions studied. However, k seems to decrease somewhat with increasing B. This is not improbable since the ionic environment would be changed at higher B by the considerable concentration of polyions $Bi_nO_{n-1}^{(n+2)+}$ of large charge.

Our rather crude assumptions, involving only two equilibrium constants k_1 and k, give a good explanation of the experimental data. Actually, if it is desired to make the agreement still better by introducing a third constant, *e. g.* by assuming $k_2 \neq k$, or by assuming some different type of reaction (OH_2 acidity, formation of $(BiO)_n^{n+}$), it is hard to decide in which way to make the correction.

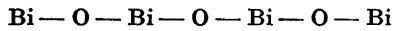
Table 6. Calculated values of b, h and h — H for round y values at B = 50 mC.

$k_1 = 0.065 \pm 0.01$ $k = 2.80 \pm 0.05$

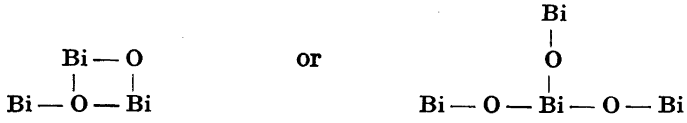
y	b	h	$h - H$
0.345	2.38	83	92
0.340	4.50	115	86.5
0.335	7.10	146	80.5
0.330	10.0	174	74.2
0.320	16.0	224	61.7
0.310	21.6	264	50.0
0.300	26.6	298	40.5
0.290	30.6	325	33.7
0.280	33.9	348	27.4
0.270	36.6	368	22.4
0.250	40.5	402	14.5
0.225	43.6	440	9.3
0.200	45.6	477	6.1
0.175	46.9	518	4.1
0.150	47.8	565	2.8

DISCUSSION

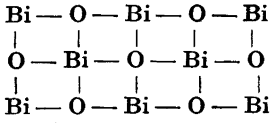
The general formula $(Bi_nO_{n-1})^{(n+2)+}$ may apply equally weli to a straight or puckered chain.



as to a branched one such as



The first one can be formed for example by bending in of a straight chain Bi — O — Bi — O — Bi, a process which would be favoured by electrostatic attraction. By repetition of this process one can imagine complexes to be formed such as



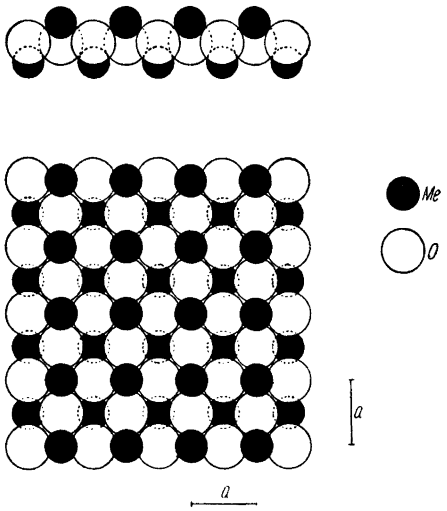
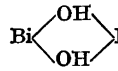


Fig. 6. Tetragonal metal-oxygen sheet in bismuth oxyhalides, viewed along a and c axes.

which can be considered as nuclei of the tetragonal $(MeO)_n$ sheets found in the crystal structures of many bismuth oxyhalides (Bannister and Hey [10]; Sillén [11, 12]) as well as in bismuth oxycarbonate (Lagercrantz and Sillén [13]) (Fig. 6). This is one reason why we prefer the formulation with oxygen bridges Bi — O — Bi to one with double hydroxyl bridges  . The latter would otherwise be equally consistent with the experimental data, which of course, give no means of distinguishing between two formulae differing by one or by several molecules of H_2O. (Our experiments, for similar reasons, give us no means of deciding whether or not there are ClO_4^- ions bound to the bismuth complexes.)

Our calculations suggest that the first oxygen bridging is much less easily brought about than the following steps since k_1 comes out at about 40 times smaller than k. Maybe the forces withstanding an attack by $(Bi^{3+} + H_2O)$ are much weaker in Bi_2O^{4+} than in Bi^{3+}, even if the formation of a straight chain $(Bi\text{-}O\text{-}Bi\text{-}O\text{-}Bi)^{5+}$ is the result; at any rate the »bending in» of the complexes must greatly stabilize the larger complexes from $Bi_3O_2^{5+}$ on.

In Fig. 7 is given the distribution of bismuth (in weight fractions nc_nB^{-1}) over complexes of different n with four different h for $B = 50$ ml. The average n by number has been computed from the formula

$$\bar{n}_n = \frac{\Sigma n c_n}{\Sigma c_n} = \frac{B}{b\,(1 + k_1\,y\,(1-z)^{-1})} = 1 + k_1\,y\,(1-ky)^{-1}\,(1-k+k_1y)^{-1} \quad (15)$$

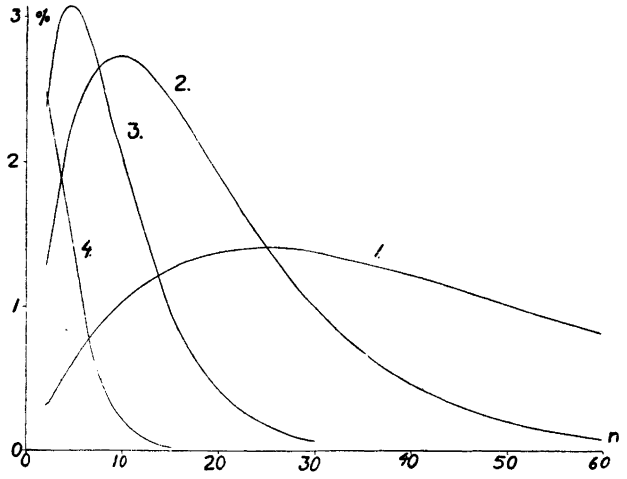


Fig. 7. Distribution of bismuth over complexes $(Bi_nO_{n-1})^{n+2}$ *of varying n in four differen solutions with B = 50 mC, calculated from eq. (15) assuming* $k_1 = 0{,}065$ *C,* $k = 2.80$ *C*

No.	z	y C^{-1}	b mC	h mC	H mC	$\bar{n}_w$	$\bar{n}_n$
1	.960	.3429	3.23	97.0	7.1	46.0	9.95
2	.900	.3214	15.16	207.4	144.1	13.7	2.73
3	.800	.2857	32.11	287.7	305.5	3.98	1.42
4	.600	.2143	44.57	457.0	449.2	1.39	1.08

The »average n by weight» was calculated from

$$\bar{n}_w = \frac{\Sigma n^2 c_n}{\Sigma n c_n} = \frac{b\,[1 + k_1\,y\,(z^2 - 3\,z + 4)\,(1-z)^{-3}] =}{B}$$

$$= 1 + \frac{2\,k_1\,y\,(1-z)^{-1}}{(1-z)^2 + k_1\,y\,(2-z)} \qquad (16)$$

For the special case of ky close to 1, we have

$$\bar{n}_n \approx (1 - ky)^{-1};\ \bar{n}_w \approx 2\,(1 - ky)^{-1}$$

A possible type of complex not included in our formulae is that containing rings of formula $(BiO)_n^{n+}$ such as

At lower h, which we have not been able to investigate because of precipitations, the aquo acidity of Bi — OH_2 may begin to appear. When this happens, one Bi may set free more than $2H^+$, the highest value obtained by the mechanism of oxygen-bridging proposed here.

It would be interesting to study the spatial arrangement of the Bi (and O) atoms by means of the X-ray diffraction of Bi solutions. If the three atoms Bi — O — Bi form a straight line, the Bi — Bi distance would be about 4.6 Å. On the other hand, in the crystal structures referred to, every O is surrounded by an almost regular tetrahedron of Bi atoms, as is seen *e. g.* from the Bi — Bi distances tabulated by Sillén [11] (p. 73). If, as we find probable, the angles Bi — O — Bi are »tetrahedral» in solution also, the smallest distance Bi — Bi would be about 3.9 Å. It should not be very difficult to decide between these alternatives.

SUMMARY

The hydrolysis of the Bi^{3+} ion has been studied by measuring the concentrations $[H^+] = h$ and $[Bi^{3+}] = b$ in solutions with $[ClO_4^-] = 3$ C, and Na^+ as the only foreign cation. The total bismuth concentrations used were $B = 50$, 25 and 10 mC.

The results cannot be explained by assuming that only mononuclear complexes $BiOH^{2+}$ and BiO^+ are formed, nor by assuming that one or two polynuclear complexes $Bi_x(OH)_y$ predominate.

However, a surprisingly good agreement with the experimental data is obtained by assuming the main process to be a chain of reactions:

$$Bi^{3+} + Bi^{3+} + H_2O \rightleftharpoons Bi_2O^{4+} + 2H^+$$
$$Bi_2O^{4+} + Bi^{3+} + H_2O \rightleftharpoons Bi_3O_2^{5+} + 2H^+$$
$$Bi_3O_2^{5+} + Bi^{3+} + H_2O \rightleftharpoons Bi_4O_3^{6+} + 2H^+$$
$$Bi_nO_{n-1}^{(n+2)+} + Bi^{3+} + H_2O \rightleftharpoons Bi_{n+1}O_n^{(n+3)+} + 2H^+$$

and the equilibrium constant of all reactions except the first to have the same value k.

At 25° C and in the ionic medium used the first constant is found to be $k_1 = 0.065 \pm 0.005$ C. The constant k seems to decrease slowly with increasing B from 3.15 ± 0.20 C at $B = 10$ mC and 2.95 ± 0.10 at $B = 25$ mC to 2.80 ± 0.05 at $B = 50$ mC.

The more facile formation of the higher complexes (from $Bi_3O_2^{4+}$ on) can be explained by a »bending in» of these complexes; by this process sheets can be formed which may be nuclei of the $(BiO)_n$ sheets found in the crystal structures of many bismuth oxyhalides.

Our thanks are due to fil. mag. Börje Andersson, who prepared our Bi stock solutions and made a series of preliminary measurements which aided us greatly in choosing suitable experimental conditions.

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Lunarine, an Alkaloid from *Lunaria biennis*

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From the cruciferae only one alkaloid has so far been isolated in a pure state, *viz.*, the lunarine found by Hairs[1] and Reeb[2] in seeds of *Lunaria biennis.*

Recently Steinegger and Reichstein[3] have isolated the alkaloid in fairly large quantities, and their product seems to be identical with that of Hairs. The Swiss chemists propose the empirical formula $C_{19}H_{26}N_2O_4$ for lunarine with one basic nitrogen atom.

The present author also has isolated lunarine following the directions of Hairs. The substance was purified chromatographically on aluminum oxide. Contrary to the findings of Steinegger and Reichstein (*l. c.*) the analysis, a molecular weight determination and a microhydrogenation were found to be in agreement with the formula $C_{25}H_{33}N_3O_5$. The analysis of a chloroaurate showed the presence of two basic nitrogen atoms.

Lunarine is soluble in ethanol' chloroform, pyridine, aqueous hydrochloric acid and aqueous sodium hydroxide, but insoluble in water, ether, benzene and the week bases. The solution in sodium hydroxide is yellow, perhaps due to enolization. Lunarine is precipitated by the usual alkaloid reagents (picric acid etc.) the precipitates being amorphous. *p*-Diazobenzenesulphonic acid produces a red colour when dropped into a solution of lunarine in sodium hydroxide. Lunarine gives a positive colour reaction for enols[4] but doubtful reactions for reactive metylene groups with sodium 1,2-naphthaquinone-4-sulphonate[5] and for phenols with Millon's reagent[6]. The reaction with ferri chloride as well as reactions for primary and secondary amino groups with flourescein chloride[7] and for the indole ring with the reagent of Hopkins-Cole-Winkler[8] were negative. Methoxyl-, methylenedioxy-[9] and N-methyl groups could not be found.

EXPERIMENTAL

Isolation of lunarine

900 g of finely ground seeds of *Lunaria biennis* were mixed with 1 l of gasoline and left standing for 24 h. The mixture was filtered and the seeds extracted once more in the same way. Now the well dried seeds were extracted several times with boiling ethanol and the combined ethanolic extracts evaporated in vacuum to sirupy consistence. The residue was shaken with a solution of 15 g of tartaric acid in 500 ml of water, the mixture filtered and the filtrate extracted with chloroform. The aqueous layer was made alkaline with soda and extracted twice with 500 ml of chloroform. After drying with sodium sulphate the chloroform solution was evaporated to dryness and the residue (10 g) dissolved in 40 ml of boiling ethanol. The solution was partly decolourized with a little charcoal and filtered. On cooling, 3 g of yellowish needles separated. The crude material was repeatedly recrystallized from ethanol. Micro m. p. 224°—226°.

Purification by chromatography

One g of the crude product was dissolved in 25 ml of chloroform and poured through a column of aluminum oxide, 1 cm in diameter. A yellow band, 2 cm high, was formed, and the column was eluated with a mixture of chloroform and ethanol (20 : 1) and four fractions of about 25 ml were collected. Fraction II contained the yellow band and yielded the alkaloid in a pure crystalline state by evaporation of the solvent.

$C_{25}H_{33}N_3O_5$ (455.5)	Calc. C 65.91	H 7.30	N 9.23	
	Found » 66.09	» 7.52	» 9.14	(Kjeldahl)
			9.23	(Dumas)

The molecular weight was determined osmometrically (Barger's method). Azobenzene was used as comparison substance.

Found: 460, 470 (in chloroform) 410 (in pyridine).

The hydrogen absorption was measured in the apparatus of Clauson-Kaas and Limborg[9]. Ethanol and acetic acid were used as solvents.

In ethanol: equiv. wt. found 152.5
calc. for 2 ⊢: 151.8
In acetic acid: equiv. wt. found 92.5
calc. for 5 ⊢: 91.1

Chloroaurate

A saturated solution of hydrogen auri chloride was added dropwise to a solution of 20 mg of lunarine in 2 ml of 4 *N* hydrochloric acid. The amorphous precipitate was filtered and dried in vacuum at 20°. Micro m. p. 170° (dec.).

$C_{25}H_{33}N_3O_5$, 2 $HAuCl_4$ (1135.6)	Calc. Au 34.73	Cl 24.98	
	Found » 34.55	» 24.40	

Colour reaction with diazobenzenesulphonic acid

A few mg of lunarine were dissolved in 1 ml of 4 *N* hydrochloric acid and the solution made alkaline with 5 ml of 2 *N* sodium hydroxide. Hereby a yellow solution was obtained, to which diazotized sulfanilic acid was added dropwise. A red colour immediately occurred but after a few seconds the colour became orange. Acidification with hydrochloric acid produced a yellow colour.

SUMMARY

The alkaloid lunarine from *Lunaria biennis* has been isolated and the empirical formula $C_{25}H_{33}N_3O_5$ is proposed.

The author wishes to thank the director of the Chemical Laboratory of the University of Copenhagen, Professor Dr. A. Langseth for permission to carry out this work in his laboratory, and Dr. K. A. Jensen for his encouragement during the investigation.

I am indebted to Mr. N. Clauson-Kaas and Mr. F. Limborg for their assistance in performing the microanalyses.

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Über die Geschwindigkeit der Umwandlung von 1,6-Dinitrito- in 1,6-Dinitrodipyridindiamminkobalt(III)-nitrat in wässeriger Lösung

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In früheren Arbeiten [1, 2, 3, 4] dieser Reihe ist der zeitliche Verlauf der Isomerisation des Nitritopentamminions nach der Formel

$$[Co\ (NH_3)_5\ ONO]^{2+} \rightarrow [Co\ (NH_3)_5\ NO_2]^{2+} \qquad (1)$$

unter verschiedenen Versuchsbedingungen extinktiometrisch untersucht worden. Es war der Zweck vorliegender Arbeit, der Umwandlung eines komplexen Kobaltions zu folgen, das z w e i Nitritogruppen enthält. Nach den halbqualitativen Angaben der Literatur schien das 1,6-Dinitritodipyridindiamminkobalt (III)-nitrat für diesen Zweck geeignet. Es ist von Werner [5] zum ersten Mal hergestellt und beschrieben worden.

Zu dessen Synthese wurde hier folgender Arbeitsgang verwendet:

$$NH_4[Co(NH_3)_2\ (NO_2)_4] \rightarrow [Co(NH_3)_2\ (H_2O)_2\ Cl_2]Cl \rightarrow$$
$$[Co(NH_3)_2\ py_2\ (H_2O)\ (OH)]Cl_2 \rightarrow [Co(NH_3)_2\ py_2\ (ONO)_2]NO_3\ ,\ 1\ H_2O$$

Erstens wurde Ammoniumtetranitrodiamminkobaltiat nach Jörgensen [6] hergestellt und umkrystallisiert. Durch Einwirkung von bei 0° C gesättigter HCl liess es sich in grünes Dichlorodiaquodiamminkobalt (III)-chlorid überführen [7], das luftgetrocknet und dann mit Pyridin und Kaliumchlorid in Hydroxoaquodipyridindiamminkobalt (III)-chlorid umgewandelt wurde [8]. Aus diesem Salz konnte schliesslich das gesuchte 1,6-Dinitritodipyridindiamminkobalt(III)-nitrat, hauptsächlich nach der von Werner [9] für das entsprechende Bromid angegebenen Methode, synthetisiert werden.

Die kupferfarbige Verbindung war meistens mit einer citronengelben, ziemlich schwerlöslichen Verunreinigung gemischt. Diese konnte dadurch entfernt werden, dass das Hauptprodukt in der eben hinreichenden Menge Wasser gelöst und dann aus der filtrierten Lösung mit festem Natriumnitrat wieder ausgefällt wurde. Es zeigte nun fast nahezu

den theoretischen Kobaltgehalt ($CoC_{10}N_7H_{16}O_7 + 1\ H_2O$, ber. 13,93 %, gef. 13,94 % Co). Die Analyse wurde wie in der vorigen Arbeit [4] ausgeführt.

Die rotbraune Farbe einer wässerigen Lösung des zu betrachtenden Salzes geht etwa von einem Tage zu dem anderen in gelborange über. Das wird von Werner durch die Umwandlung

$$\left[\begin{matrix}(1)\ ONO \\ (6)\ ONO\end{matrix}\ Co\ \begin{matrix}py_2 \\ (NH_3)_2\end{matrix}\right]^+ \rightarrow \left[\begin{matrix}(1)\ O_2N \\ (6)\ O_2N\end{matrix}\ Co\ \begin{matrix}py_2 \\ (NH_3)_2\end{matrix}\right]^+ \qquad (2)$$

Auch das feste kupferrote Dinitritosalz ist nach ungefähr 6 Wochen in die entsprechende orange Dinitroverbindung übergegangen [5]. Vorläufig wurde aber nur die Isomerisation in wässeriger Lösung folgender Zusammensetzung untersucht:

$$aM\ [Co(NH_3)_2py_2(ONO)_2]NO_3 + bM\ HOCOCH_3 + bM\ NaOCOCH_3;$$

a ist etwa 0,004 und b 0,01.

Die Extinktion E_V der Versuchslösung in einer gegebenen Küvette (von etwa 3 cm Dicke) und für Licht der Wellenlänge $\lambda = 546$ mμ wurde mit Hilfe von zwei Multiplikatorphotozellen wie früher [2, 10] bestimmt. Zur Monochromatisierung diente ein Schottsches Didymglasfilter für die fragliche Wellenlänge. Zwischen den einzelnen Messungen war die Versuchslösung vor Licht aus der Quecksilberlampe geschützt. Die Versuchstemperatur war 20,0° C.

Tabelle 1. Die Isomerisation bei 20,0° C in wässeriger Lösung der Zusammensetzung: 0,00400 M *Dinitritosalz + 0,00973* M *$HOCOCH_3$ + 0,00973* M *$NaOCOCH_3$. — Das feste Salzpräparat (Nr. 4) vor Bereitung der Versuchslösung etwa 45 Stunden bei Zimmertemperatur luftgetrocknet.*

$$\log\ (E_V - 0{,}1977) = 0{,}3562 - 1 - 1{,}51 \cdot 10^{-3} \cdot (t - t_0)$$

$t - t_0$ Minuten	E_V		$t - t_0$ Minuten	E_V	
	gef.	ber.		gef.	ber.
0	0,424	0,425	325,3	0,271	0,271
28,3	0,403	0,404	381,6	0,256	0,258
55,7	0,383	0,385	592,5	0,220	0,227
92,8	0,365	0,362	639,1	0,210	0,222
124,7	0,346	0,345	1994	0,182	—
157,0	0,328	0,329	2058	0,189	—
182,9	0,318	0,318	3538	0,198	—
208,8	0,305	0,308	3557	0,199	—
240,9	0,296	0,296	4949	0,196	—
267,3	0,288	0,287	7075	0,198	—
300,5	0,281	0,278			

Tabelle 2. Die Isomerisation bei 20,0° C in wässeriger Lösung der Zusammensetzung: 0,00400 M *Dinitritosalz + 0,00994* M $HOCOCH_3$ *+ 0,00994* M $NaOCOCH_3$. — *Das feste Salzpräparat (Nr. 6) vor Bereitung der Versuchslösung etwa 23 Stunden bei Zimmertemperatur luftgetrocknet.*

$$\log (E_V - 0{,}1982) = 0{,}3649 - 1 - 1{,}52 \cdot 10^{-3} \cdot (t - t_0)$$

$t - t_0$ Minuten	E_V		$t - t_0$ Minuten	E_V	
	gef.	ber.		gef.	ber.
0	0,429	0,430	238,5	0,298	0,299
42,5	0,394	0,398	272,3	0,284	0,288
73,7	0,374	0,377	306,8	0,278	0,277
117,2	0,357	0,352	335,1	0,270	0,270
139,1	0,344	0,341	361,6	0,264	0,264
167,8	0,329	0,327	3494	0,197	—
198,5	0,316	0,314	4217	0,199	—

Tabelle 3. Die Isomerisation bei 20,0° C in wässeriger Lösung der Zusammensetzung: 0,00400 M *Dinitritosalz + 0,00994* M $HOCOCH_3$ *+ 0,00994* M $NaOCOCH_3$. — *Das feste Salzpräparat (Nr. 7) vor Bereitung der Versuchslösung etwa 17 Stunden bei Zimmertemperatur luftgetrocknet.*

$$\log (E_V - 0{,}1982) = 0{,}4581 - 1 - 1{,}54 \cdot 10^{-3} \cdot (t - t_0)$$

$t - t_0$ Minuten	E_V		$t - t_0$ Minuten	E_V	
	gef.	ber.		gef.	ber.
0	0,483	0,485	177,5	0,354	0,351
40,8	0,446	0,447	205,9	0,336	0,337
77,2	0,414	0,417	237,6	0,328	0,322
107,6	0,396	0,394	276,9	0,302	0,306
143,1	0,372	0,371	313,1	0,291	0,293

In den Tabellen 1—5 sind die zu verschiedenen Zeitdifferenzen $t - t_0$ gehörigen E_V-Werte zusammengestellt. Jeder E_V-Wert ist der Mittelwert von 4—5 Bestimmungen; t ist der entsprechende t-Mittelwert. Das erste (E_V, t)-Paar jeder Messungsreihe wird mit (E_V°, t_0) bezeichnet. Im Haupte der Tabellen wird die Zusammensetzung der Versuchslösung und die Trockenzeit des benutzten Salzpräparates angegeben. Die in der Tabelle 4 gewählten Messungszeiten sind zum Studium der Extinktionsveränderungen beim letzteren Teil der Umwandlung des Dinitritosalzes geeignet. Man findet, dass die Extink-

Tabelle 4. Die Isomerisation bei 20,0° C in wässeriger Lösung der Zusammensetzung: 0,00378 M *Dinitritosalz + 0,00994* M $HOCOCH_3$ *+ 0,00994* M $NaOCOCH_3$. — *Das feste Salzpräparat (Nr. 8) vor Bereitung der Versuchslösung etwa 11 Stunden bei Zimmertemperatur luftgetrocknet.*

$$\log (E_V - 0{,}1868) = 0{,}5050 - 1 - 1{,}53 \cdot 10^{-3} \cdot (t - t_0)$$

$t - t_0$ Minuten	E_V		$t - t_0$ Minuten	E_V	
	gef.	ber.		gef.	ber.
0	0,506	0,507	930,0	0,193	0,199
583,0	0,228	0,228	980	0,186	—
615,0	0,224	0,223	1022	0,190	—
644,3	0,220	0,220	1062	0,186	—
677,3	0,215	0,216	1091	0,186	—
721,0	0,209	0,212	1272	0,185	—
790,8	0,201	0,207	1312	0,189	—
822,4	0,196	0,204	1343	0,187	—
854,0	0,194	0,203	2140	0,186	—
887,2	0,194	0,201	5037	0,187	—

Tabelle 5. Die Isomerisation bei 20,0° C in wässeriger Lösung der Zusammensetzung: 0,0037 M *Dinitritosalz + 0,00994* M $HOCOCH_3$ *+ 0,00994* M $NaOCOCH_3$. — *Das feste Salzpräparat (Nr. 9) nach Waschen mit Alkohol und Äther und Durchsaugen von Luft unmittelbar zur Bereitung der Versuchslösung benutzt.*

$$\log (E_V - 0{,}1809) = 0{,}4646 - 1 - 1{,}55 \cdot 10^{-3} \cdot (t - t_0)$$

$t - t_0$. Minuten	E_V		$t - t_0$ Minuten	E_V	
	gef.	ber.		gef.	ber.
0	0,474	0,472	208,7	0,316	0,319
34,6	0,446	0,439	252,2	0,300	0,299
69,8	0,420	0,408	287,3	0,282	0,285
114,9	0,375	0,374	1247	0,178	—
162,7	0,341	0,344	1268	0,184	—

tion E_V mit wachsender Zeit kontinuierlich abnimmt und sich einem Grenzwert nähert. Sie bleibt dann tagelang mit unregelmässigen, kleinen Fluktuationen praktisch konstant. Das geht besonders gut aus den Tabellen 1, 2 und 4 hervor. Dieser Grenzwert E_G kommt dem Extinktionswert einer Versuchslösung sehr nahe, den man durch Ersatz der Dinitrito- mit der Dinitro-ver-

Tabelle 6. Zusammenfassung der gefundenen k-Werte in den Interpolationsformeln

$$\log (E_V - E_G) = \log (E_V^\circ - E_G) - k\,(t - t_0)$$

Tabelle Nr.	Trockenzeit des Präparates in h	$k \cdot 10^3$
1	45	1,51 ± 0,02
2	23	1,52 ± 0,02
3	17	1,54 ± 0,04
4	11	1,53 ± 0,02
5	0	1,55 ± 0,04
	Mittelwert	1,53

bindung erhält. So lässt sich aus den Tabellen 1 und 2 für 0,00400 *M* $[Co(NH_3)_2py_2(ONO)_2]NO_3$ in 0,00973 bzw. 0,00994 *M* Acetatpufferlösung der mittlere Grenzwert $E_G = 0{,}198$ berechnen, während die Lösung 0,00400 *M* $[Co(NH_3)_2py_2(NO_2)_2]NO_3$ + 0,00999 *M* $HOCOCH_3$ + 0,00999 *M* $NaOCOCH_3$ in derselben Küvette den Extinktionswert 0,190 zeigte. Das im letzteren Falle verwendete Salz war dadurch bereitet, dass die feste Dinitritoverbindung bei 30° C drei Tage und 20° C 42 Tage lang im Dunkel trocken aufbewahrt wurde. Es scheint also berechtigt, zu schliessen, dass die Isomerisation (2) vollständig von links nach rechts verläuft.

Mit Ausgangspunkt von den Ergebnissen früherer Arbeiten dieser Reihe lag es nahe, die gefundenen log $(E_V - E_G)$-Werte graphisch gegen die $(t - t_0)$-Werte einzuzeichnen. Es zeigte sich, dass für jede Versuchsreihe die resultierenden Punkte mit guter Annäherung auf einer Geraden lagen. Ihr Winkelkoeffizient k und ihre Ordinate im Nullpunkt log $(E_V^\circ - E_G)$ konnten graphisch bestimmt werden. Mit Hilfe der so erhaltenen Daten wurden die Interpolationsformeln ermittelt, die in den Tabellen angegeben sind. Die nach diesen Formeln berechneten E_V-Werte sind neben den gefundenen verzeichnet.

Tabelle 6 gibt einen Überblick über die k-Werte der verschiedenen Versuchsreihen. Sie stimmen mit einander sehr gut überein. Man findet weiter, dass eine Steigerung der Trockenzeit des verwendeten festen Salzpräparates bis zu etwa 45 Stunden keine merkliche Änderung von k mit sich bringt. Es gilt also folgender Zusammenhang

$$E_V - E_G = (E_V^\circ - E_G) \cdot 10^{-0{,}00153\ (t - t_0)} \tag{3}$$

oder

$$E_V - E_G = (E_V^\circ - E_G) \cdot e^{-0{,}00352\ (t - t_0)} \tag{4}$$

Will man die hier experimentell untersuchte Isomerisation von dem 1,6-Dinitritodipyridindiamminkobalt(III)-ion $\left[\begin{matrix}(1)\ ONO \\ (6)\ ONO\end{matrix}\ Co\ \begin{matrix}py_2 \\ (NH_3)_2\end{matrix}\right]^+$ theoretisch behandeln, so macht die Erfahrung von anderen Gebieten der physikalischen Chemie, z. B. von dem der Dissoziation der mehrbasischen Säuren und der Verseifung ihrer Ester, die Annahme wahrscheinlich, dass diese Isomerisation stufenweise, d. h. über ein Nitronitritodipyridindiamminion $\left[\begin{matrix}(1)\ O_2N \\ (6)\ ONO\end{matrix}\ Co\ \begin{matrix}py_2 \\ (NH_3)_2\end{matrix}\right]^+$ als Zwischenglied vor sich geht.

Bei Bereitung einer M-molaren Lösung des Salzes seien im Auflösungsaugenblicke wegen der Isomerisation im festen Zustande von den M Molen in einem Liter der Lösung nur a Mole unverändertes Dinitritosalz, b Mole in Nitronitritosalz und c Mole in Dinitrosalz umgewandelt. Nach t Minuten seien durch die Isomerisation in der Lösung x Mole Dinitrito- in Nitronitrito- und y Mole Nitronitrito- in Dinitroverbindung übergeführt. Man habe also, falls man die Zeit t von dem Auflösungsaugenblicke rechnet und alle Salze vollständig ionisiert sind, folgende Komplexionenkonzentrationen in der Lösung:

Tabelle 7. Komplexionenkonxentrationen.

Zeit in Minuten	Komplexionenkonzentrationen in Grammionen/Lit.		
	Dinitrito-ion	Nitronitrito-ion	Dinitro-ion
0	a	b	c
t	$a - x$	$b + x - y$	$c + y$

Da die Umwandlung des Nitritopentamminkobalt (III)-ions, $[Co(NH_3)_5 ONO]^{2+}$, in das entsprechende Nitroion nach den Resultaten der vorigen Untersuchungen in Lösung wie in fester Phase monomolekular verläuft, scheint es berechtigt, anzunehmen, dass auch die beiden Isomerisationen

$$\left[\begin{matrix}(1)\ ONO \\ (6)\ ONO\end{matrix}\ Co\ \begin{matrix}py_2 \\ (NH_3)_2\end{matrix}\right]^+ \rightarrow \left[\begin{matrix}(1)\ O_2N \\ (6)\ ONO\end{matrix}\ Co\ \begin{matrix}py_2 \\ (NH_3)_2\end{matrix}\right]^+ \qquad (5)$$

und

$$\left[\begin{matrix}(1)\ O_2N \\ (6)\ ONO\end{matrix}\ Co\ \begin{matrix}py_2 \\ (NH_3)_2\end{matrix}\right]^+ \rightarrow \left[\begin{matrix}(1)\ O_2N \\ (6)\ O_2N\end{matrix}\ Co\ \begin{matrix}py_2 \\ (NH_3)_2\end{matrix}\right]^+ \qquad (6)$$

dem monomolekularen Schema folgen. Die Geschwindigkeitskonstanten für (5) und (6) seien mit k_1 und k_2 bezeichnet. Dann müssen folgende Gleichungen gelten:

$$a + b + c = M \tag{7}$$

$$\frac{dx}{dt} = k_1 \, (a - x) \tag{8}$$

$$\frac{dy}{dt} = k_2 \, (b + x - y) \tag{9}$$

Schon a priori ist es wahrscheinlich, dass k_1 und k_2 zwar von derselben Grössenordnung, aber nicht gleich sind. Rein statistisch wäre zu erwarten, dass k_1 doppelt so gross wie k_2 sei, denn das Dinitritoion kann auf zwei Wegen, nämlich durch die Umlagerung jeder der beiden ONO-Gruppen für sich in das Nitronitritoion übergehen, während letztgenanntes Ion nur eine umwandelbare ONO-Gruppe besitzt. Deshalb wird in der folgenden Auseinandersetzung der Fall $k_1 = k_2$ ausgeschlossen.

Aus der mittleren der drei obigen Gleichungen folgt unmittelbar

$$x = a - a \cdot e^{-k_1 t} \tag{10}$$

Durch Einsetzung in (9) kommt man weiter zu der Differentialgleichung

$$\frac{dy}{dt} + k_2 \cdot y = k_2 \, (a + b) - a k_2 \cdot e^{-k_1 t} \tag{11}$$

die folgende allgemeine Lösung hat:

$$y = a + b + a \cdot \frac{k_2}{k_1 - k_2} \cdot e^{-k_1 t} - \left[\frac{a k_1}{k_1 - k_2} + b \right] \cdot e^{-k_2 t} \tag{12}$$

Aus den obigen Ausdrücken (10) und (12) für x und y lassen sich die Ionenkonzentrationen beim Zeitpunkte t berechnen (vgl. Tabelle 7). Die Dinitritoionenkonzentration wird:

$$a - x = a \cdot e^{-k_1} \tag{13}$$

die Nitronitritoionenkonzentration

$$b + x - y = - \frac{a k_1}{k_1 - k_2} \cdot e^{-k_1 t} + \left[\frac{a k_1}{k_1 - k_2} + b \right] \cdot e^{-k_2 t} \tag{14}$$

und die Dinitroionenkonzentration

$$c + y = a + b + c + \frac{ak_2}{k_1 - k_2} \cdot e^{-k_1 t} - \left[\frac{ak_1}{k_1 - k_2} + b\right] \cdot e^{-k_2 t} \tag{15}$$

Die lichtabsorbierende Schichtdicke der Versuchslösung sei d cm und die molekularen Extinktionskoeffizienten der Dinitrito-, der Nitronitrito- und der Dinitroverbindung seien a_1, a_2 und a_3. Under Voraussetzung, dass Beers Gesetz für alle drei Salze gilt, findet man für die Extinktion E_V der Versuchslösung beim Zeitpunkte t:

$$E_V = da_1 a e^{-k_1} - \frac{da_2 ak_1}{k_1 - k_2} \cdot e^{-k_1 t} + da_2 \left[\frac{ak_1}{k_1 - k_2} + b\right] \cdot e^{-k_2 t} +$$
$$+ da_3 a + da_3 b + da_3 c + \frac{da_3 ak_2}{k_1 - k_2} \cdot e^{-k_1 t} - da_3 \left[\frac{ak_1}{k_1 - k_2} + b\right] \cdot e^{-k_2} \tag{16}$$

oder vereinfacht

$$\frac{E_V}{d} = a_3 (a + b + c) + a \left(a_1 - a_2 \cdot \frac{k_1}{k_1 - k_2} + a_3 \cdot \frac{k_2}{k_1 - k_2}\right) e^{-k_2 t} +$$
$$+ (a_2 - a_3) \left(\frac{ak_1}{k_1 - k_2} + b\right) e^{-k_2 t} \tag{17}$$

E_G ist die Extinktion der Versuchslösung, wenn die Isomerisation zu Ende gegangen ist, d. h. wenn alles vorhandene Komplexsalz in die Dinitroform umgewandelt ist. E_G kann als der Grenzwert betrachtet werden, dem sich E_V für unendlich grosse t-Werte nähert. Aus (17) folgt deshalb

$$\frac{E_G}{d} = a_3 (a + b + c) \tag{18}$$

und aus (17) und (18)

$$E_V - E_G = ad \left(a_1 - a_2 \cdot \frac{k_1}{k_1 - k_2} + a_3 \cdot \frac{k_2}{k_1 - k_2}\right) e^{-k_1 t} +$$
$$+ d (a_2 - a_3) \left(\frac{ak_1}{k_1 - k_2} + b\right) \cdot e^{-k_2 t} \tag{19}$$

In ein und derselben Versuchsreihe sind die Koeffizienten von $e^{-k_1 t}$ und $e^{-k_2 t}$ beide Konstanten, die mit A und B bezeichnet seien. Das gibt

$$E_V - E_G = A \cdot e^{-k_1 t} + B \cdot e^{-k_2 t} \tag{20}$$

wenn

$$A = ad\,(a_1 - a_2 \cdot \frac{k_1}{k_1 - k_2} + a_3 \cdot \frac{k_2}{k_1 - k_2}) \tag{21}$$

bzw.

$$B = d\,(a_2 - a_3)\,(\frac{ak_1}{k_1 - k_2} + b) \tag{22}$$

Andererseits konnten für eine gegebene Versuchsreihe die Resultate der Extinktionsmessungen nach (4) in einer Formel

$$E_V - E_G = (E_V^\circ - E_G)\, e^{k_n t_0} \cdot e^{-k_n t} \tag{23}$$

zusammengefasst werden. k_n ist gleich 0,00352. t ist die Zeit von einem willkürlichen Nullpunkt gerechnet und t_0 der Zeitpunkt der ersten Messung. Als Nullpunkt sei hier wie in der obigen theoretischen Diskussion der Auflösungsaugenblick des festen Komplexsalzes gewählt. Dann kann (23) folgenderweise vereinfacht werden:

$$E_V - E_G = C \cdot e^{-k_n t} \tag{24}$$

wo C eine für jede Versuchsreihe charakteristische Konstante ist, die durch die Formel

$$C = (E_V^\circ - E_G)\, e^{k_n t_0} \tag{25}$$

berechnet werden kann.

Es entsteht nun die Frage, unter welchen Bedingungen die empirische Gleichung (24) mit der theoretischen (20) in Einklang zu bringen ist, d. h. für welche Werte der Konstanten k_1, k_2, A und B man berechtigt ist,

$$C \cdot e^{-k_n t} = A \cdot e^{-k_1 t} + B \cdot e^{-k_2 t} \tag{26}$$

zu setzen. Dabei muss man sich erstens erinnern, dass k_1 wahrscheinlich zwar grösser als k_2, aber etwa von derselben Grössenordnung wie k_2 ist. Weiter nimmt E_V mit wachsender Zeit ab, und E_G ist kleiner als E_V; a_3 ist also auch kleiner als a_1. Es ist ebenfalls zu erwarten, dass a_2 zwischen a_1 und a_3 liegt, d. h. dass

$$a_1 > a_2 > a_3 \tag{27}$$

Man könnte sich nun denken, dass die Umwandlung des festen Salzes vor der Auflösung in Wasser so weit fortgeschritten sei, dass im Auflösungsaugen-

blicke keine Dinitritoionen mehr vorhanden wären. Dann müsste in allen Formeln a gleich Null gesetzt werden und nach (21) A verschwinden. Nach (22) und (26) würde weiter k_n gleich k_2 und C gleich $d\ (\alpha_2 - \alpha_3) \cdot b$ sein. Dieser Erklärungsgrund ist aber sehr unwahrscheinlich, denn die Isomerisation in fester Phase geht sehr langsam vor sich (siehe S. 660 oben) und kann besonders im letzten Versuche (Tabelle 5), wo das Salz schon etwa 50 Minuten nach seiner Herstellung aufgelöst war, keine vollständige Umwandlung der Dinitritoform verursachen.

A kann aber auch dadurch verschwinden, dass in (21) die Parenthese

$$\alpha_1 - \alpha_2 \cdot \frac{k_1}{k_1 - k_2} + \alpha_3 \cdot \frac{k_2}{k_1 - k_2} \tag{28}$$

gleich Null wird. Daraus würde folgen, dass $k_n = k_2$ und $C = B$ seien. k_1 liesse sich nicht berechnen. Diese Annahme wird jedenfalls von den Versuchsdaten nicht widersprochen. Wenn man von dem statistischen Verhältnis $k_1/k_2 = 2$ ausgeht, wird der fragliche Ausdruck zu $\alpha_1 - 2\alpha_2 + \alpha_3$ vereinfacht, und falls α_2 der Mittelwert von α_1 und α_3 ist, nimmt dies Trinomium den Wert Null an. Es soll aber stark betont werden, dass man auch, wenn die Beziehungen $k_1/k_2 = 2$ und $\alpha_2 = \frac{1}{2} \cdot (\alpha_1 + \alpha_3)$ theoretisch möglich sind, aus dem vorliegenden Versuchsmaterial auf ihre Richtigkeit natürlich nicht schliessen darf. Der exakte oder annähernde Nullwert des ursprünglichen Ausdruckes (28) kann ja durch mehrere andere Zusammenhänge zwischen den eingehenden Konstanten verursacht werden.

Gleichung (26) könnte auch in dem Falle für alle t-Werte gelten, dass $B = 0$, $C = A$ und $k_1 = k_n$ wären. Da α_2 mit grösster Wahrscheinlichkeit von α_3 verschieden ist, a und $b \gtrless 0$, k_1 und $k_1 - k_2 > 0$ sind, kann B aber nur für $a = b = 0$, d. h. wenn die Isomerisation ganz zu Ende ist, den Wert Null annehmen.

Es ist schliesslich folgendes zu beachten: Wenn die experimentell bestimmbare Grösse $E_V - E_G$ nach (20) eine Summe oder Differenz von z w e i e-Funktionen wäre, dann würde sich, falls man log $(E_V - E_G)$ gegen t graphisch repräsentierte, eine gekrümmte Kurve ergeben, die eventuell in einem begrenzten t-Gebiet annähernd als eine Gerade betrachtet werden könnte. In Wirklichkeit resultierten Punkte, die über ein sehr grosses Zeitintervall ($0 \leqq (t - t_0) <$ etwa 700 Minuten) nahe an einer Gerade lagen. Für grössere $(t - t_0)$-Werte fielen die Punkte etwas unter die Linie (vgl. Tabelle 4). Hier befindet sich das System aber so nahe an seinem Endzustand, dass $E_V - E_G$ sehr klein ist und von den Versuchsfehlern äusserst stark beeinflusst wird. Die genannte Abweichung von der Geraden entspricht übrigens einer g r ö s-

seren Abfallsgeschwindigkeit der Extinktion E_V als in dem früheren $(t - t_0)$-Gebiete, während man, falls $(E_V - E_G)$ eine t-Funktion von der Form (20) wäre, eine Verminderung der Isomerisationsgeschwindigkeit zu erwarten hätte, denn für grosse t-Werte müsste die langsamer abfallende Funktion $e^{-k_2 t}$ vorherrschen $(k_2 < k_1)$.

Nach der obigen Erörterung scheint es also möglich, die Versuchsresultate theoretisch nur so zu deuten, dass in den Gleichungen (20) und (21) der Ausdruck $a_1 - a_2 \cdot \frac{k_1}{k_1 - k_2} + a_3 \cdot \frac{k_2}{k_1 - k_2}$ und damit A exakt oder annähernd Null wird und k_2 gleich der experimentell bestimmten Konstante k_m ist. k_1 kann aber aus den Versuchsdaten nicht berechnet werden.

Untersuchungen über die Isomerisation anderer komplexen Kobaltverbindungen mit zwei Nitritogruppen in der inneren Sphäre sind im Gange.

ZUSAMMENFASSUNG

1. Die Umwandlung von 1,6-Dinitritodipyridindiamminkobalt (III)-nitrat in die entsprechende Dinitro-Verbindung in acetatpufferhaltiger Lösung von der Zusammensetzung

$$aM \text{ Komplexsalz} + bM \text{ } HOCOCH_3 + bM \text{ } NaOCOCH_3$$

wird bei 20,0° C extinktiometrisch untersucht; a ist etwa 0,004 und b 0,01.

2. Die Isomerisation ist mit einer Farbenänderung der Lösung von rotbraun in gelborange begleitet.

3. Die Extinktion der Versuchslösung E_V in ein und derselben Küvette von etwa 3 cm Dicke und für Licht von der Wellenlänge $\lambda = 546$ mμ wird mit Hilfe von zwei Multiplikatorphotozellen nach einer früher beschriebenen Methode bestimmt.

4. Es zeigt sich, dass die Extinktion E_V mit wachsender Zeit abnimmt und sich einem Grenzwert E_G kontinuierlich nähert. Sie bleibt dann während mehrerer Tage innerhalb der Versuchsfehlergrenzen konstant. Dieser Grenzwert kommt dem Extinktionswert der Lösung

$$aM \text{ Dinitroverbindung} + bM \text{ } HOCOCH_3 + bM \text{ } NaOCOCH_3$$

sehr nahe. Das ursprüngliche Salz scheint also vollständig in die Dinitroverbindung überzugehen.

5. Es wird gefunden, dass die Gleichung

$$^{10}\log\ (E_V - E_G) = {}^{10}\log\ (E_V^{\circ} - E_G) - k\ (t - t_0)$$

oder die mit dieser gleichwertigen

$$E_V - E_G = (E_V^{\circ} - E_G) \cdot e^{-k_n \cdot (t - t_0)}$$

gilt. E_V° und t_0 sind die zur ersten Messung jeder Versuchsreihe gehörigen Werte der Extinktion und Zeit; t und t_0 werden in Minuten angegeben, k ist gleich $1{,}53 \cdot 10^{-3}$ und k_n gleich $3{,}52 \cdot 10^{-3}$. Die Trockenzeit und damit auch das Alter des benutzten festen Salzpräparates übt praktisch keinen Einfluss auf den k-Wert aus.

6. Die Resultate werden unter der Annahme theoretisch erörtert, dass die Isomerisation stufenweise nach dem Schema

$$\left[\begin{matrix}(1)\ \mathrm{ONO} \\ (6)\ \mathrm{ONO}\end{matrix}\ \mathrm{Co}\ \begin{matrix}\mathrm{py_2} \\ (\mathrm{NH_3})_2\end{matrix}\right]^+ \rightarrow \left[\begin{matrix}(1)\ \mathrm{O_2N} \\ (6)\ \mathrm{ONO}\end{matrix}\ \mathrm{Co}\ \begin{matrix}\mathrm{py_2} \\ (\mathrm{NH_3})_2\end{matrix}\right]^+ \rightarrow \left[\begin{matrix}(1)\ \mathrm{O_2N} \\ (6)\ \mathrm{O_2N}\end{matrix}\ \mathrm{Co}\ \begin{matrix}\mathrm{py_2} \\ (\mathrm{NH_3})_2\end{matrix}\right]^+$$

vor sich geht.

7. Dann ergibt sich der Zusammenhang

$$E_V - E_G = A \cdot e^{-k_1 t} + B \cdot e^{-k_2 t} \quad \text{mit}$$

$$A = ad\ (a_1 - a_2 \cdot \frac{k_1}{k_1 - k_2} + a_3 \cdot \frac{k_2}{k_1 - k_2}) \text{ und}$$

$$B = d\ (a_2 - a_3)\ (\frac{ak_1}{k_1 - k_2} + b)$$

a_1, a_2 und a_3 sind die molekularen Extinktionskoeffizienten der Dinitrito-, der Nitronitrito- und der Dinitro-ionen, a und b die Konzentrationen der beiden ersten Ionenarten im Auflösungsaugenblicke, d die lichtabsorbierende Schichtdicke sowie k_1 und k_2 die monomolekularen Geschwindigkeitskonstanten für die Umwandlung der Dinitrito- in die Nitronitritoform, bzw. für die der letzteren in die Dinitroform.

8. Der obige theoretische Ausdruck für $E_V - E_G$ kann, wie es scheint, mit der empirischen nur so in Einklang gebracht werden, dass die Parenthese $a_1 - a_2 \cdot \frac{k_1}{k_1 - k_2} + a_3 \cdot \frac{k_2}{k_1 - k_2}$ und damit A exakt oder annähernd gleich Null wird. Dann muss die experimentell gefundene Konstante k_n $(= 3{,}52 \cdot 10^{-3})$ gleich der monomolekularen Geschwindigkeitskonstanten für die Isomerisation

der Nitronitritoionen sein. k_1 lässt sich nicht aus dem Versuchsmaterial berechnen.

9. Rein statistisch ist es zu erwarten, dass $k_1 = 2\ k_2$ wäre.

Die grundlegenden Synthesen dieser Arbeit sind im Sommer 1945 im Chemischen Institut der Universität zu Uppsala ausgeführt worden. Ich danke dessen Vorstand, Herrn Professor Dr. G. Hägg, herzlichst für seine Gastfreiheit und Entgegenkommen.

Die Arbeit ist dieses Jahr im Chemischen Institut der Universität zu Lund wieder aufgenommen und abgeschlossen worden. Dem Institutionsvorstand, Herrn Professor Dr. S. Bodforss, bin ich zu grossem Dank verplichtet. Er hat mir Geldmittel zur Anstellung einer Assistentin zur Verfügung gestellt. Dieser Assistentin, Fräulein Rut Landqvist, habe ich für gute Mithilfe zu danken.

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On the Structure of the Protein Molecule *

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In 1920 the author initiated an investigation of the proteins according to new methods, proceeding from the working hypothesis that the protein molecule essentially consists of heterocyclic ring systems which are easily split by acids, alkalies and enzymes, thereby producing the amino acids [1].

For the past 50 years the most important method of investigating the structure of the proteins has involved boiling with strong mineral acids — a method which, to say the least, seems rather one-sided in its treatment of these substances that are so important to life.

One thing that the previous theories have been unable to account for is the great similarity of elementary composition displayed by the proteins. Calculated on the basis of 10 carbon atoms, all proteins have approximately the same formula $C_{10}H_{16}N_{2.6}O_{3.2}S_{0.1-0.03}$ (12) [2], and this alone must justify an investigation on whether the protein should not have a structure that is more regular than indicated by the polypeptide theory.

The present investigation, in which is employed a reductive cleavage of the protein molecule, has shown the justification of this idea.

There have been isolated and identified four piperazines, piperidine, α-methyl pyrrolidin, and analyses have been made of a long series of high molecular weight cleavage products, obtained by stepwise degradation of widely different proteins. The results show that about 70 % of the nitrogen of the proteins is present in systems having the formulae $C_{10}H_{16}N_2O_{3.4}$ and $C_{11}H_{18}N_2O_{3.4}$ and that the former is by far the more predominant one.

These sub-units offer an explanation of why all proteins have the uniform

* With regard to preparation, fractionation, analyses, identification of isolated bases and preparations, reference is made to various papers in scientific publications and handbooks and to the author's monograph: *On the Structure of the Protein Molecule* (Einar Munksgaard, Copenhagen, and Humphrey Millford, Oxford University Press, London). In the copies of the monograph sent out after May 1947 pp. 55 and 56 have been replaced by other pages with a somewhat amended text. Reference to the monograph is by the page number in paranthesis ( ).

elementary composition, a fact that cannot be realized when the protein is boiled with concentrated hydrochloric acid.

If the proteins simply were composed of long aliphatic chains the cleavage method employed should produce large amounts of aliphatic amines, amides and amino alcohols — but of such components it has been possible to demonstrate only isoamyl-amine, and that has perhaps its origin in a reaction with the amyl-alcohol employed.

It is the author's hypothesis that the peculiar properties of the individual proteins are to some degree accountable for by the varying position of the oxygen atom in macro- and micro-sub-units.

A brief description is here given of the cleavage method and fractionation employed, followed by a report of analytical results. A more detailed description is to be found in the author's monograph (see also Troensegaard [3]).

Anhydrous solvents have been used for the proteins, especially anhydrous methanol-KOH and glacial acetic acid. Proteins like gelatin, casein and globin are transferred to dry amyl-alcohol and here subjected to a hydrogenation with metallic sodium at a temperature of 130—145° (113) [3]. Ether and water in the form of ice are added to the reaction mixture, and the ether-amyl-alcoholic phase separated from the aqueous phase. The reaction products in the two phases are fractionated according to basicity and solubility.

From the ether-amyl-alcoholic phase one obtains the fractions B_1, B_2, C_1 and C_2; from the aqueous phase D_1, D_2 and D_3.

The D_2- (the proteol) and C-fractions contain 60 % of the protein nitrogen. 12 % are found in the D_3-fraction which chiefly contains intact protein. So it must be assumed that about 70 % of the entirely hydrogenated protein are found in the D_2- and C-fractions. The remainder of the nitrogen is present in the B-fractions as well as in the form of ammonia split off in the hydrogenation (A-fraction).

The *B-fractions* consist of weakly basic substances which are not bound by primary sodium phosphate. They turn very dark upon oxidation and contain, *inter alia*, pyrrole- and indigo-like compounds. For details, see the monograph (21—22).

The C_1- and C_2-fractions are strong bases which can be extracted by shaking with primary sodium phosphate. The C_1-bases, nearly all of them oxygen-free, have their origin in the C_2-bases since they can be prepared from the latter by hydrogenation. They can be extracted from the aqueous solution with ether after saturation with K_2CO_3. The C_2-bases are obtained by extraction of the same solution by shaking with alcohol. After neutralization of the C_2-fraction with hydrochloric acid it can be fractionated into 2 groups which are soluble and insoluble in acetone.

The *D_1-fraction* is only a small fraction which is obtained by extraction with ether. It gives the pyrrole reactions, but is decomposed in the course of a day or so.

The *D_2-fraction* is obtained from the aqueous phase by salting out in alcohol with K_2CO_3. It is the largest fraction and must represent the »nucleus» of the protein. It has been given the name »proteol».

The *D_3-fraction* contains the methanol-soluble part of the aqueous phase and consists, as mentioned, presumably of protein which is but slightly affected by the hydrogenation.

The *ammonia* formed must, *e. g.*, originate from the guanidine group of the arginine and from the free amino group of the lysine.

Many proteins which are soluble in methanol-KOH cannot be transferred to amyl-alcohol before being acetylated. In the acetylation 2—5 % of the peripheral layer of the molecule is split off. The split-off, ether-soluble acetyl compounds are especially rich in nitrogen [4].

In Chapters 6 and 7 of the author's monograph it is shown that this acetylation has no influence on the nature of the hydrogenation products formed, inasmuch as there have been identified the same bases from *acetylated* as from *non-acetylated* protein. Several reviewers of the author's monograph have apparently *failed to read these chapters and therefore given a wrong picture of the investigation.*

It must be assumed that the hydrogenation involves not only *an elimination* of the oxygen but also *a rupture* of carbon chains by the heating in the strong sodium amylate. It must be assumed that the proteol and the part of the C_2-fraction which forms acetone-soluble hydrochlorides represent such rupture products. They have the same oxygen content as the original proteins and it must be supposed that they have not been hydrogenated.

After this *introduction* and *review* the investigation will now be reported in more detail.

The *proteol* (D_2) contains about 30 % of the nitrogen of the protein. The hydrochlorides of this fraction from serum globulin, casein and gliadin have very nearly the same elementary composition $C_{10}H_{16}N_2O_{3.2}(HCl)_{0.5}$ calculated on the basis of 2 nitrogen atoms (54).

It is very interesting that the acid combining capacity is 1 mole HCl per 4 nitrogen atoms. This indicates that the proteol must consist of systems having the elementary composition $C_{20}H_{32}N_4O_6$ or perhaps $C_{20}H_{32}N_4O_7$ which rather must be regarded as »macro-sub-units».

The empirical composition $C_{10}H_{16}N_{2.6}O_{3.2}S_{0.1—0.03}$ of the proteins is very close to that of the proteol (54). The nitrogen which is in excess in the protein

Table 1. The elementary composition of the D_2-fraction from the different proteins investigated.

Analysis no.	D_2-fraction	Empirical composition
1	Pt-salt of D_2-gliadin, methylated with methyl iodide	$C_{10.3}H_{16}N_2(CH_3)_{0.85}(OCH_3)_{0.65}O_{2.6}[PtCl_6]_{0.5}$
2	Pt-salt of D_2-gliadin, methylated with dimethyl sulphate	$C_{11}H_{17}N_2(CH_3)_{1.8}(OCH_3)_{0.8}O_{3.3}[PtCl_6]_{0.5}$
3	From gliadin	$C_{10}H_{16}N_2O_{3.4}$
3a	Hydrochloride from acetylated gliadin	$C_{9.6}H_{16}N_2O_{2.9}(HCl)_{0.5}$
4	From acetylated casein, methylated with methyl iodide	$C_{10.4}H_{16}N_2(CH_3)_1O_{3.8}$
5	Pt-salt from acetylated casein, methylated with methyl iodide	$C_{10}H_{18}N_2(CH_3)_{1.3}(OCH_3)_{0.4}O_2[PtCl_6]_{0.5}$
6	Hydrochloride from non-acetylated casein	$C_{9.8}H_{15}N_2O_{3.2}(HCl)_{0.52}$
7	Hydrochloride from acetylated serum globulin	$C_{10.3}H_{16}N_2O_{3.5}(HCl)_{0.5}$
8	Hydrochloride from globin	$C_{8.4}H_{14}N_2O_{2.4}(HCl)_{0.5}$
9	Pt-salt from methylated, non-acetylated gelatin	$C_{10.6}H_{20}N_2(CH_3)_{2.2}(OCH_3)_{0.6}O_{2.2}[PtCl_6]_{0.82}$

(Analyses nos. 1, 2 and 3 (gliadin): N. Troensegaard and E. Fischer, *Z. physiol. Chem.* **142** (1925) 54, 57.)

must be the nitrogen which is split off as ammonia in the hydrogenation, or which is found in the systems rich in nitrogen that are split off during the acetylation.

The proteol has, however, other chemical properties than those of the protein itself. As distinct from polypeptides it gives no biuret reaction, and it can be salted out of the aqueous solution in alcohol with K_2CO_3.

If the proteol is methylated by means of methyl iodide, both methoxyl and methylimide are formed. Thus it is possible to introduce from 30 up to 50 methoxyl groups and up to 50 methylimide groups for every 100 nitrogen

atoms. The methoxyl is easily split off by alkalies. The formation of methoxyl signifies that the more central part of the protein molecule contains very labile atom systems which do not occur in the aliphatic polypeptides. The proteins react only to a slight degree with methyl iodide. The hydrochloride of proteol is *insoluble* in acetone.

That the proteol must be the central part of the molecule is proved by the fact that this fraction from gliadin on acid hydrolysis yields 32 % proline [6]. The proteol of serum globulin gave $3^1/_4$ % amino acids which were soluble in absolute alcohol. Gliadin yields on acid hydrolysis 13 % proline, and serum globulin yields 2.5 % proline. These findings are in agreement with the assumption, based on enzymatic investigation, that the proline belongs to the central part of the protein molecule.

The proteol from the globular proteins has a more »compact» structure than the peripheral layers. It is difficult to split by renewed hydrogenation; a great deal of resinifying occurs, and very small amounts of ether-soluble bases are formed. This does not apply to the fibroid protein gelatin (66).

THE C_2-FRACTIONS

The C_2-fraction whose hydrochlorides are soluble in acetone

The hydrochloride of gliadin in this fraction has the composition $C_{11}H_{18}N_2O_{3.4}(HCl)_{0.9}$ (calculated on the basis of 2 nitrogen atoms). The fraction presumably consists of fragments of the median layer that encloses the proteol. That it is a question of fragments is evident from the fact that a methylation product gave a platinum salt having the elementary composition $C_{12}H_{22}N_2(CH_3)_3(2HCl,PtCl_4)$, but that only one-half of the nitrogen of the preparation was precipitated as platinum salt. On methylation this fraction gave no methoxyl, but the methylimide showed the presence of both secondary and tertiary nitrogen. On renewed hydrogenation it was in all essentials changed into the oxygen-poorer C_2-bases, the hydrochlorides of which are insoluble in acetone, and into the C_1-bases. Hence the fraction must be regarded as intermediary hydrogenation products.

The C_2-fraction whose hydrochlorides are insoluble in acetone (4 2)

This fraction can after methylation be divided into 4 subfractions. No methoxyl is formed. The methylation products from the different proteins can be divided into 2 fractions, one soluble and the other insoluble in acetone.

Table 2. Composition of the platinum salts of methylated C_2-bases.

From	Fraction	Analysis no.	Composition
Non acetylated gelatin	a	4	$C_{9.7}H_{17}O_{2.7}N_2(CH_3)_{2.7}[PtCl_6]_{0.87}$
	b	5	$C_{9.2}H_{19}O_{3.5}N_2(CH_3)_{2.1}[PtCl_6]_{0.72}$
	c	6	$C_8H_{17}O_{2.7}N_2(CH_3)_{2.2}[PtCl_6]_{0.76}$
	d	7	$C_9H_{18}O_{1.4}N_2(CH_3)_{1.9}[PtCl_6]_{0.81}$
Non acetylated casein	a	1	$C_{9.1}H_{16}O_2N_2(CH_3)_{2.4}[PtCl_6]_{0.63}$
	b	2	$C_{8.9}H_{17}O_{2.8}N_2(CH_3)_2[PtCl_6]_{0.59}$
	c	3	$C_{9.5}H_{18}O_{2.2}N_2(CH_3)_{2.2}[PtCl_6]_{0.67}$
	d		gave no Cu salt
Acetylated casein, not methylated	1	18	$C_9H_{16.5}O_{2.8}N_2$
	2	19	$C_{10.1}H_{19}O_{2.3}N_2$
Non-acetylated globin	a	8	$C_{8.3}H_{15}N_2(CH_3)_{2.1}[PtCl_6]_{0.85}$
	b	9	$C_{7.8}H_{12}O_{2.7}N_2(CH_3)_{1.8}[PtCl_6]_{0.45}$
	c	10	$C_{8.2}H_{13}O_{1.2}N_2(CH_3)_{0.6}[PtCl_6]_{0.52}$
	d	11	$C_{8.2}H_{12}O_{3.2}N_2(CH_3)_{1.1}[PtCl_6]_{0.24}$
Acetylated globin	a	16	$C_{11.6}H_{21}O_{2.5}N_2(CH_3)_{2.1}[PtCl_6]_{0.65}$
	c	17	$C_{10}H_{18}O_{1.8}N_2(CH_3)_{2.1}[PtCl_6]_{0.59}$
Acetylated gliadin	a		$C_{11.2}H_{22}O_{2.2}N_2(CH_3)_{2.4}[PtCl_6]_{0.8}$
	b		$C_{10}H_{18}O_{2.2}N_2(CH_3)_2[PtCl_6]_{0.8}$
	c		$C_{11.4}H_{23}O_2N_2(CH_3)_2[PtCl_6]_{0.8}$
	d		$C_{10}H_{19}O_{1.8}N_2(CH_3)_2[PtCl_6]_{0.8}$
Oxygen free bases from the hydrolysed C_2-bases of gliadin			$C_{8.6}H_{17.1}N_2(H_2PtCl_6)_{0.8}$
			$C_{10.6}H_{18.6}N_2(H_2PtCl_6)_{0.8}$
			$C_{9.2}H_{16}N_2(H_2PtCl_6)_{0.8}$
Acetylated serum globulin	a	12	$C_{10.6}H_{21}O_{2.3}N_2(CH_3)_2[PtCl_6]_{0.82}$
	b	13	$C_{9.7}H_{19}O_{1.6}N_2(CH_3)_{2.3}[PtCl_6]_{0.71}$
	c	14	$C_{9.5}H_{17}O_{1.6}N_2(CH_3)_{2.1}[PtCl_6]_{0.66}$
	d	15	$C_{10.2}H_{19}O_{2.1}N_2(CH_3)_2[PtCl_6]_{0.67}$
Non-distillable C_1-bases from gelatin (methylated)	Analyses nos. 11 and 12 in the chapter on gelatin		$C_{9.8}H_{20}O_{2.8}N_2(CH_3)_{2.1}[PtCl_6]_{0.7}$
			$C_{10.8}H_{20}O_{1.6}N_2(CH_3)_2[PtCl_6]_{0.7}$
Non-distillable C_1-bases from non-acetylated casein	Analysis in the chapter on casein		$C_{11.4}H_{16.7}O_2N_2(CH_3)_2[PtCl_6]_{0.74}$

(Analyses of gliadin: N. Troensegaard and E. Fischer, *Z. physiol. Chem.* **142** (1925) 45, 47.)

These 2 fractions can in turn be divided into 2 sub-fractions by precipitation with alcoholic copper acetate which only precipitates one-half of the preparation (43). The fractions have been investigated as platinum salts and the elementary composition of 30 such platinum salts is recorded in Table 2 (44) where the analyses have been calculated on the basis of 2 nitrogen atoms.

The analyses show that the bases from the non-acetylated proteins have a little higher nitrogen content than those from the acetylated proteins. As mentioned above, this is because the systems particularly rich in nitrogen partly have been removed by the acetylation — as especially evident from the analyses of the C_2-bases of globin.

A large part of the analyses show a platinum content of $(PtCl_6)_{0.8}$ per 2 atoms of nitrogen. In some of the fractions too little platinum chloride has been used, but since it is a question of high molecular weight substances the bases are nevertheless precipitated.

This circumstance points also to a considerable similarity of the structure of these cleavage products in the case of the different proteins, like gelatin, gliadin and serum globulin, included in the present investigation. In the case of gliadin exactly the same is found to apply to the *oxygen-free* base fractions of high molecular weight which are obtained by the acid hydrolysis. *One-fifth of the nitrogen is not basic.*

Since the methylated C_1-bases contain rather substantial amounts of tertiarily bound nitrogen and these bases have their origin in the C_2-fraction, it follows that tertiary nitrogen must be predominant in these cleavage products.

The C_2-bases from the different proteins contain from 15 to 20 % of amino nitrogen (van Slyke), and since the methylimide content of the exhaustively methylated bases is rather uniformly $(CH_3)_{1.1}$ for each nitrogen atom, this circumstance is in reasonably good agreement with the fact that the tertiary nitrogen is predominant, when taking into account that one-fifth of the nitrogen is non-basic and that the amino nitrogen binds more than one methyl group.

The molecules are larger than indicated by the formulae, the molecular weight being from 800 upward.

By *cautious acid hydrolysis* of these bases from gliadin, Wrede has isolated methyl piperazine, isopropyl piperazine, and pyrrolidyl carbinol (43, 30). These products show the ketopiperazine structure.

The analyses, which are recorded in the tabulation of the acetylated proteins gliadin, globin, serum globulin and casein, can with close approximation be interpreted as representing hydrogenated cleavage products from systems having an elementary composition $C_{10}H_{16}N_2O_{3.2}$ and $C_{11}H_{18}N_2O_{3.2}$, converting the hydrogen content according to the oxygen content of the C_2-bases whose hydrochlorides are soluble in acetone and from which these bases originate (42). *Most of the bases belong to the systems* $C_{10}H_{16}N_2O_{3.2}$. The amount of oxygen displaced in the reduction varies from 0 to 3 atoms per 2 atoms of nitrogen.

The *C_1-fraction* contains the bases which, as mentioned, are produced by complete hydrogenation of the C_2-bases. The marked uniformity of elementary composition which distinguishes the other fractions is not nearly so prominent here. The bases are soluble in ether.

Of *hydrogenation products* from this fraction there are, in the case of gliadin, gelatin and casein, isolated piperidine, methyl piperazine, trans-2,5-dimethyl piperazine, isopropyl piperazine, α-methyl pyrrolidine and isoamyl amine. These products too show the ketopiperazine structure (23, 33, 58, 74).

The most volatile bases from the 3 proteins just mentioned consist only of these compounds. If the protein molecule were composed of aliphatic peptide chains, one should here expect the occurrence of other aliphatic amines than isoamyl amine, but such are not found.

From the hydrogenation products of casein and gliadin several bases are prepared: $C_{11}H_{22}N_2$, $C_9H_{20}N_2$ and $C_{14}H_{22}N_2$. The first one is identified as isobutyl pyrrolidino piperazine, while the others have not yet been identified.

In the case of gliadin 10 different fractions have been analysed (33, 77, 82) with boiling points 100°/16 mm Hg—170°/0.1 mm Hg, but of approximately the same composition as the identified base $C_{11}H_{22}N_2$, isobutyl pyrrolidino piperazine. The bases contain secondarily and tertiarily but no primarily bound nitrogen. They have been analysed as gold and platinum salts. They all smell like piperazines. They must be assumed to have their origin in tricyclic sub-units of composition $C_{11}H_{16}N_2O_3$ or $C_{11}H_{14}N_2O_4$ with a central diketopiperazine ring (27). The piperazines demonstrated must be fragments of three-dimensional lattice structures. The three-dimensional structure is plausible because the piperazine of which the best yield is obtained is trans-2,5-dimethyl piperazine.

Of the C_1-fraction from casein 5 bases of high boiling point are investigated (35—39). Their composition shows that they can be regarded as *homologues of the base* $C_{10}H_{22}N_2$, thus $C_{10}H_{22}N_2(CH_2)_x$, where x is a small number. If the hydrogen is converted corresponding to the oxygen content of the preparation before the hydrogenation, one obtains the composition $C_{10}H_{16}N_2O_3(CH_2)_x$. It will be seen that it is the same sub-units which constantly reappear, but here they are attached to shorter carbon chains.

It is only to some degree possible to compare the C_1-bases from the serum proteins with those mentioned above — *though the proteol and C_2-fraction of serum globulin are in close agreement,* both as regards composition and chemical properties, with those of the other proteins.

Piperidine and isoamyl amine are found both in the case of serum globulin and in that of serum albumin, but piperazines cannot be demonstrated.

As in the case of the C_1-fraction from casein there are in that from serum globulin found a series of homologues of the type $C_{10}H_{16}N_2O_3(CH_2)_x$ or $C_{11}H_{18}N_2O_3(CH_2)_x$, where x is a small number. These homologues do not smell like piperazines but have a closer resemblance to the piperidines (98—101). Several of them are precipitated by $H_4Fe(Cy)_6$ which indicates that the nitrogen is tertiarily bound.

In the case of both serum proteins the C_1-fraction consists of many different bases of which some are very unstable. At the distillation of one of the fractions from serum albumin an oil of the composition $C_9H_{14}O$ was split off. It is presumably a *diterpene* (102—104).

In the case of both serum proteins the fractions with the highest boiling point (150—200° / 0.1 mm Hg) have yielded bases having the composition $C_{27}H_{45}N_3O_2$, $C_{24}H_{42}N_3O$ and $C_{27}H_{45}N_3$. Even though the distillation temperature was high, but little discoloration occurred. (Chapters 10 and 11.)

The content of carbon and hydrogen in two of these bases is in good agreement with that of the cholesterol, the third has 3 carbon atoms less. Although it is probable that the bases have their origin in systems containing more oxygen, it must be assumed that they represent a transitional stage of the synthesis of the sterols and that the completion of this synthesis involves a deamination in the liver. Similar bases could not be demonstrated in the hydrogenation products from the other proteins investigated.

So little is known regarding the function of the serum proteins in the organism — this is especially true of the serum albumin [7].

So far it has been assumed that the sterols are synthezised in the organism from nitrogen-free compounds such as acetaldehyde, methyl-glyoxal and glycerose [8]. Bloch, Borek and Rittenberg [9] have demonstrated the final synthesis of cholesterol in the liver. Liver slices from rats were suspended in Krebs' phosphate buffer, *acetic acid* labeled with C^{13} or D_2O was added, and the isotopes were demonstrated in the cholesterol isolated.

Thus the investigation has shown that the globular proteins gliadin, casein and serum globulin can be regarded as built up of a central nucleus (the proteol) of rather compact structure *surrounded* by systems of a uniform elementary composition. From these systems from gliadin and casein there are formed the C_2-bases which must have diketopiperazine structure. No piperazines are produced by the hydrogenation of the C_2-bases from the serum proteins.

Outside of these systems are layers particularly rich in nitrogen, which contain the hexone bases.

There must be some reason for the fact that the C_1-fraction from the serum proteins deviates so much from the C_1-fraction of the other proteins.

SUMMARY

This paper gives the results of an analytical investigation of the fractionation products from hydrogenated proteins and presents a new view on the structure of the protein molecule. The proteins are dissolved in anhydrous solvents and subjected to a hydrogenation with metallic sodium in dry amyl alcohol. By the various cleavages that follow and by fractionation it is shown that in this way one obtains a series of intermediary degradation products, which cannot be obtained in acid hydrolysis.

A large analytical material shows that 70 % of the nitrogen content can be referred to various systems having the elementary composition $C_{10}H_{16}N_2O_{3.2}$ and $C_{11}H_{18}N_2O_{3.2}$, chiefly the former. (The D_2- and C-fractions). This is very nearly the case of all proteins. The analyses are calculated for 2 nitrogen atoms in the molecule.

The remainder of the nitrogen is present in the peripheral systems especially rich in nitrogen, and in the B-fractions.

These degradation products explain, why all proteins, calculated on the basis of 10 carbon atoms, have an elementary composition very close to $C_{10}H_{16}N_{2.6}O_{3.2}S_{0.1-0.03}$ (12). As there is 0.6 nitrogen atom per 10 carbon atoms more in the protein molecule than in the above mentioned two systems, the other part of the protein molecule must be especially rich in nitrogen. Further it involves that the D_2- and C-fractions constitute about 85 % of the weight of the protein.

The actual building stones of the protein molecule must be systems of uniform composition, »sub-units» with the indicated formula. It has been possible (Wrede) to identify one of these sub-units in hydrogenated form; it proved to be isobutyl pyrrolidino piperazine, $C_{11}H_{22}N_2$.

It is found that the globular proteins investigated consist of a »compact» nucleus, »proteol», of uniform elementary composition and uniform acid combining capacity for the proteins investigated — $C_{10}H_{16}N_2O_{3.2}(HCl)_{0.5}$. The proteol is surrounded by systems of uniform elementary composition $C_{10}H_{16}N_2O_{3.2}$ and $C_{11}H_{18}N_2O_{3.2}$. In the case of gliadin and casein these systems contain diketopiperazine compounds. In addition there is a third peripheral system rich in nitrogen, which contains the hexone bases.

The most-hydrogenated decomposition products originate mainly from the C_2-fraction. There have been identified: piperidine, methyl piperazine, *trans*-2,5-dimethyl piperazine, isopropyl piperazine, isobutyl pyrrolidino piperazine, isoamyl amine and α-methyl pyrrolidine. Other aliphatic amines cannot be demonstrated. It is possible that the piperidine has its origin in the most peripheral layer.

From the serum proteins there are isolated three bases with the composition $C_{27}H_{45}N_3O_2$, $C_{24}H_{42}N_3O$ and $C_{27}H_{45}N_3$ as well as a »diterpene» $C_9H_{14}O$. It must be assumed that these compounds represent intermediary products in the synthesis of cholesterol and that the final synthesis takes place in the liver as shown by Bloch, Borek and Rittenberg.

APPENDIX

The author takes this occasion to mention an incorrect theory which frequently is called »Troensegaard's Pyrrole Theory». It has its origin in the author's first paper in *Z. physiol. Chem.* **112** (1920) 89 which gives a model formula composed of 3 pyrrole nuclei. This has been misinterpreted by someone presumably having no further knowledge of the author's work and its purpose. The fact is that on page 90 of the paper mentioned it is definitely stated that it is *not* the author's opinion that the proteins are composed of such systems — but this has escaped the attention of several other authors, thus for example, the co-workers of Carl L. A. Schmidt's book: *The Chemistry of the Amino Acids and Proteins* (1945).

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As it will be seen the above paper is a review of the experimental material from earlier papers by the same author, and especially from the book mentioned in the note p. 672. The reason why it has been decided nevertheless to publish it in this journal is that the material from the book may not be easily accessible to the reader. The author informs us that as long as copies of the 2nd edition of his book are still in stock, he will be glad to send on request a copy free of charge to any reader, who may be interested in the subject.

The Editors.

Preliminary Communications

A New Isomer of Hexachloro-Cyclohexane with Zero Dipole Moment

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The determination of the molecular structure of the different benzene hexachlorides $(CHCl)_6$ would be of great interest, but demands a considerable amount of precise work. The only isomer the configuration of which is well established is the symmetrical β compound [1] in which the Cl atoms have the positions $\varkappa,\varkappa,\varkappa,\varkappa,\varkappa,\varkappa$. Besides this substance a second isomer with zero dipole moment should exist in which the chlorine atoms 1 and 4 are in ε positions $(\varepsilon,\varkappa,\varkappa,\varepsilon,\varkappa,\varkappa)$. It is known that the α, γ and δ isomers have considerable dipole moments, the moment of the ε isomer [2] has not been determined.

Cyclohexane and monochloro-cyclohexane were treated with gasous chlorine under cooling but using strong artificial light until no further absorbtion of chlorine could be observed. From the different fractions obtained by distillation *in vacuo* of the reaction product some new substances were prepared. One of these is a chloride $C_6H_6Cl_6$ of m. p. 145° C obtained from the most volatile part of the product.

$C_6H_6Cl_6$		
Calc.	Mol. wt. 290.9	Cl 73.1
Found	» » 285, 286	» 72.0, 72.5

(Cryoscopic molecular weight determination in benzene.)

Monoclinic unit cell contains 4 molecules ($a = 11.1$, $b = 6.78$, $c = 14.0$; $\beta = 98°$).

The result of dielectric constant measurements of very dilute solutions in benzene and carbon tetrachloride may be expressed by the ratio $\frac{\Delta\varepsilon}{x}$ (x being the mole fraction of hexachloride):

	C_6H_6	CCl_4	
New substance:	0.552	0.536	(x ranging from 0,001 —0,007)
β isomer:	0.847	—	(x = 0,002)

There can be little doubt that the dipole moment is in fact zero. If we exclude the possibility (which seems very improbable) that the substance contains two CCl_2-groups and two CH_2-groups, the conclusion must be drawn that the new substance is in fact a sixth isomer in the series of benzene hexachlorides having the configuration of Cl atoms given above ($\varepsilon,\varkappa.\varkappa.\varepsilon,\varkappa,\varkappa$). (Small quantities of the converted, energetically less stable configuration $\varkappa,\varepsilon,\varepsilon,\varkappa,\varepsilon,\varepsilon$ may occur in solution or in the vapour). We therefore propose the designation ζ benzene hexachloride for the new substance.

Preliminary experiments on insects indicate that the isomer is almost inactive as a contact poison.

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Note on the Clevage of Insulin by Chymotrypsin

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Crystalline insulin has been digested with crystalline chymotrypsin until the amino nitrogen was constant. The reaction

was followed with chromatograms on paper strips [1] using ninhydrin as a developer. The formation of six distinct components was observed with different R_F-values in pyridine-amyl alcohol: 0.11; 0.20; 0.25; 0.29; 0.38; 0.55. These components all appeared early in the digestion and apparently increased in amount with time but no formation of additional split products was observed. During the digestion, however, a considerable amount of a gelatinous precipitate was formed and this has not been investigated.

Attempts were also made to separate the split products. Partial separation was achieved through the use of chromatography on starch[2] in pyridine-amyl alcohol. One of the component, $R_F = 0.55$, was obtained in the form of needle-shaped crystals. Other fractions were not homogeneous as judged by the paper strip method. These experiments are now continued with the aim of obtaining a more complete separation and larger amounts of the components.

Concerning the nature of the observed split products nothing definite can now be said although it seems likely that at least some of them are peptides. It is hoped that a closer investigation of these products will throw some light on the structure of insulin.

The fact that paper strip chromatograms on samples from different stages of the digestion do not differ qualitatively is taken as an indication that the enzyme splits one substrate molecule to completion before it attacks the next one. This gives additional support to the hypothesis of protease action advanced by Tiselius and Eriksson-Quensel [3].

A detailed paper will be published later.

1. Consden, R., Gordon, A. H., and Martin, A. J. P. *Biochem. J.* **38** (1944) 224.
2. Elsden, S. R., and Synge, R. L. M. *Biochem. J.* **38** (1944) ix.
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Preliminary Communications

A New Isomer of Hexachloro-Cyclohexane with Zero Dipole Moment

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The determination of the molecular structure of the different benzene hexachlorides $(CHCl)_6$ would be of great interest, but demands a considerable amount of precise work. The only isomer the configuration of which is well established is the symmetrical β compound [1] in which the Cl atoms have the positions $\varkappa,\varkappa,\varkappa,\varkappa,\varkappa,\varkappa$. Besides this substance a second isomer with zero dipole moment should exist in which the chlorine atoms 1 and 4 are in ε positions $(\varepsilon,\varkappa,\varkappa,\varepsilon,\varkappa,\varkappa)$. It is known that the α, γ and δ isomers have considerable dipole moments, the moment of the ε isomer [2] has not been determined.

Cyclohexane and monochloro-cyclohexane were treated with gasous chlorine under cooling but using strong artificial light until no further absorbtion of chlorine could be observed. From the different fractions obtained by distillation *in vacuo* of the reaction product some new substances were prepared. One of these is a chloride $C_6H_6Cl_6$ of m. p. 145° C obtained from the most volatile part of the product.

$C_6H_6Cl_6$		
Calc.	Mol. wt. 290.9	Cl 73.1
Found	» » 285, 286	» 72.0, 72.5

(Cryoscopic molecular weight determination in benzene.)

Monoclinic unit cell contains 4 molecules ($a = 11.1$, $b = 6.78$, $c = 14.0$; $\beta = 98°$).

The result of dielectric constant measurements of very dilute solutions in benzene and carbon tetrachloride may be expressed by the ratio $\frac{\Delta\varepsilon}{x}$ (x being the mole fraction of hexachloride):

	C_6H_6	CCl_4	
New substance:	0.552	0.536	(x ranging from 0,001 —0,007)
β isomer:	0.847	—	(x = 0,002)

There can be little doubt that the dipole moment is in fact zero. If we exclude the possibility (which seems very improbable) that the substance contains two CCl_2-groups and two CH_2-groups, the conclusion must be drawn that the new substance is in fact a sixth isomer in the series of benzene hexachlorides having the configuration of Cl atoms given above ($\varepsilon,\varkappa.\varkappa.\varepsilon,\varkappa,\varkappa$). (Small quantities of the converted, energetically less stable configuration $\varkappa,\varepsilon,\varepsilon,\varkappa,\varepsilon,\varepsilon$ may occur in solution or in the vapour). We therefore propose the designation ζ benzene hexachloride for the new substance.

Preliminary experiments on insects indicate that the isomer is almost inactive as a contact poison.

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Note on the Clevage of Insulin by Chymotrypsin

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Crystalline insulin has been digested with crystalline chymotrypsin until the amino nitrogen was constant. The reaction

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Catalase Activity

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The destruction of hydrogen peroxide by catalase is probably one of the most intensively studied enzymatic reactions as its abundant literature attests. Those studies, whether based upon titrimetric or manometric procedures have been consistently affected by the fragility of the enzyme; either the reaction-velocity constant decreases throughout the reaction or is constant at only a small fraction of its initial value. Using stronger catalase solutions and modified techniques it has been found that the reaction-velocity constant is large and nearly constant and that some effects previously attributed to the nature of the catalatic reaction were probably due to inactivation of the enzyme. The velocity constant for the reaction of enzyme and substrate has been evaluated in the proper units and the effects of substrate concentration, temperature, pH, and type of catalase upon this velocity constant have been determined. Some of the data obtained previously are re-evaluated and the conclusions are re-interpreted.

The value for the reaction velocity of hydrogen peroxide and catalase during the conversion of hydrogen peroxide into water and oxygen having been re-evaluated, it is compared with the reaction velocity of the formation of the intermediate compound recently found in the catalase hydrogen peroxide reaction [1].

THE MEANING OF KAT. F.

Nearly all of the previous data have been expressed in terms of Kat. F.[2] for this unit is very useful for representing the purity of catalase preparations. Since the data of this paper are in the usual units (seconds and moles per liter) a simple conversion formula has been given below. A similar calculation has

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been made by Haldane [3] and Moelwyn-Hughes [4] but on an iron basis. The factor given by Sizer [5] differs from the one given here.

The kinetics of the disappearance of hydrogen peroxide closely follow the first-order equation, especially when corrections are made for the inactivation of the enzyme [6]. In some manometric experiments an exponential curve is obtained [7, 8], while in others a linear function is found [5, 9, 10]. There are many data which indicate that the zero-order reaction is not due to saturation of catalase with substrate. Also the values of Kat. F. obtained manometrically appear to lie between 0.5 and less than 0.1 of those obtained titrimetrically [11]. In the recent manometric data of George [12], the activity during the initial rapid phase is later calculated in this paper from the values of $\Delta O_2/E$ and from Fig. 2 to be about 2 % of that found here at the same substrate concentrations ($\approx 0.01\ M$).*

Since the highest activity is determined in a first-order reaction, the following equation (1) is probably valid for intact catalase. Further data to support this point will be presented later.

$$\text{Kat. F.} = \frac{\log_{10} \frac{x_0}{x}}{tW/50\text{ ml}} = \frac{k_1}{W/50\text{ ml}} \qquad (1)$$

where x_0 is the substrate concentration at $t = 0$,
x is the substrate concentration at t (minutes),
W is the grams of catalase used in the 50 ml reaction mixture **.

The molecular weight (M) of a number of catalases has been found to be about 225,000 and the proper units, seconds, moles per liter, and natural logarithms can now be used. W grams of catalase per 50 ml is $eM/20$ moles per liter where e is the enzyme concentration. The complete expression for the first-order reaction is:

$$\log_e \frac{x_0}{x} = k_1 e t' \qquad (2)$$

where t' is in seconds. This is only a restatement of Eq. 1 but in proper units. From Eqns. 1 and 2,

$$k' = \frac{\log_{10} \frac{x_0}{x}}{t} = \frac{60\ \log_e \frac{x_0}{x}}{2.3\ t'} = \frac{60\ k_1 e}{2.3} \qquad (3)$$

---

* See footnote p. 696.

** The volume is often omitted in this definition; Kat. F. is inversely proportional to the volume employed.

$$\text{Kat. F.} = \frac{520\ k_1}{M} = \frac{k_1}{431} \qquad (4)$$

Eq. 2 is used throughout this paper for evaluating k_1.

Particular forms of Eqns. 3 and 4 have been used by Haldane [3], Moelwyn-Hughes [4], and Sizer [5], to calculate the rate of combination of enzyme and substrate so that this rate might be compared with the theoretical reaction velocity.

Three of the more obvious factors which may cause k_1 to be less than the correct value are:

1. Hematin iron impurities in e.
2. Inactivation of e by x.
3. Partial saturation of e.

At the present time pure crystalline preparations of catalase may be obtained by several methods [13] and accurate spectrophotometric and molecular weight data are available [14, 15]. Factor 1 does not contribute to the error of the present determinations. There is, however, some question as to the number of active hematins in the liver catalases, but in most of these experiments erythrocyte catalase was used. Factors 2 and 3, however, appear to be inadequately studied at present and will be discussed in detail.

ENZYME INACTIVATION DURING THE CATALATIC REACTION

The inactivation of catalase during the decomposition of hydrogen peroxide has been discussed by many workers and many methods have been devised to correct for it by various equations; for a summary see Elliott [16]. It has not been our purpose here to study the inactivation, we have attempted to find the experimental conditions by which inactivation could be minimized. Under such conditions a larger and more accurate value of k_1 may be determined.

Two fairly obvious factors in the inactivation of catalase would seem to be:

1. The spreading of catalase over the various interfaces in monolayers as shown by Harkins, Fourt, and Fourt [17] or by simply increasing the surface area of the reaction vessel by the addition of glass beads (Agner—personal communication).

2. The chemical inactivation of catalase by its substrate by reversible or irreversible reactions.

Both these factors would be more serious in dilute catalase solutions. Thus the amounts of catalase used should be large compared with the amount that will be inactivated at the surfaces of the reaction vessel and its concentra-

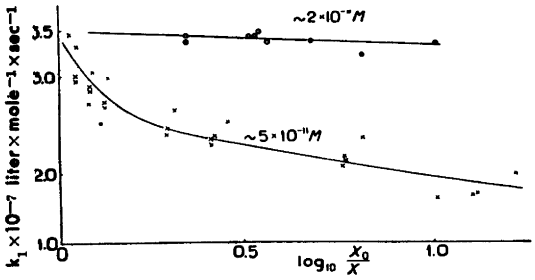

Fig. 1. The effect of enzyme concentration upon the kinetics of the decomposition of hydrogen peroxide by horse blood catalase. The lower curve was obtained using conditions recommended for the determination of Kat. F. (13). 5 mM H_2O_2, 23° C, k_1 calculated by Eq. 2. The values of t may be found from this equation.

tion should be large enough so that each catalase molecule collides with its substrate a comparatively small number of times during the reaction.

Since the half-time required for the decomposition of hydrogen peroxide is inversely proportional to the enzyme concentration, the methods for measuring the activity of stronger catalase solutions must respond rapidly. Only a few of the possible methods are listed below:

1. More rapid manipulation of the titration technique.
2. The use of the flow method
   a. with the platinum microelectrode
   b. with the two-mixer technique.

According to Sumner [13], the enzyme concentration in the determination of Kat. F. is adjusted so that $0.04 > k > 0.025$ where k is in min^{-1} and $\log_{10}$. Using Eqns. 2 and 4 and a value of Kat. F. of 60,000 the enzyme concentration is found to be on the order of $10^{-11}M$ and the half-time of the reaction is about 8 minutes. Typical results for such titrations at 22° C are shown in the lower curve of Fig. 1. At 0° C the inactivation proceeds more slowly [18].

With more rapid manipulation of the titration technique the enzyme concentration can be increased about 100-fold and the half-time decreased to about thirty seconds. The results of the titrations are calculated according to Eq. 2 and are plotted not against time but against the extent of reaction in order that the two curves might be easily compared.

METHOD OF RAPID TITRATION

The same values of k_1 have been obtained over a wide range of conditions and some typical experimental procedures are given here. 2 ml of hydrogen peroxide solution (0.7 ml »Perhydrol» in 50 ml water) are added to 50 ml 0.01 M phosphate buffer (pH = 7) in an Erlenmeyer flask. A 2 ml sample is withdrawn and is blown out into a 2 % sulfuric acid solution and titrated with 0.01 N permanganate to give the initial peroxide concentration.

The catalase solution is diluted in 0.01 M phosphate buffer (pH = 7) to about 1 μM and its density is measured at the peak of the Soret band in the Beckman spectrophoto-

meter. About 0.03 ml of this solution is pipetted into a watch-glass. Simultaneously the stop-watch is started, the watchglass is dropped into the flask containing the substrate, and the liquid in the flask is rapidly swirled.

At 13 seconds a 2 ml sample is withdrawn and is rapidly blown into a swirling 2 % sulfuric acid solution. The time at which the pipette is blown out is noted. When clean pipettes are used the differential error in delivery from the pipettes is less than 11 %. A second sample is withdrawn at 28 s and a third at 43 s. The values of k_1 are calculated by Eq. 2 and may or may not be extrapolated to $t = 0$.

A satisfactory alternative procedure is to withdraw the first sample a few seconds after adding the enzyme and then to take a second sample fifteen seconds later. k_1 is calculated using the ratio of the two values of hydrogen peroxide concentration and the time interval between the measurements. Although k_1 is assumed to be a first-order constant over this interval, this method has the advantage that the slowness of mixing enzyme and substrate causes no error and the error in timing is only the differential error of the two measurements.

In other cases the reaction has been stopped by blowing a few ml of sulfuric acid into the reaction vessel at the desired time. No appreciable difference in the values of k_1 was found with these variations of the method.

Fig. 1 shows a striking difference between the course of the two reactions; with dilute enzyme the initial portions of the curve are sharply inflected and catalase inactivation occurs which is soon complete. The residual activity is relatively constant and is the phase of the catalatic reaction upon which some of the catalase activity data has previously been based. It seems, however, that this residual activity might not have the same characteristics as intact catalase and therefore the curve found with the stronger enzyme is preferable. In this case there appears no abrupt decrease in activity up to the earliest time measured. The slight inactivation that occurs during the course of the reaction is at a constant rate and no difficulty is encountered in extrapolating to an initial rate. In fact, there is so little difference between the rate obtained in the early portion of the reaction (between 15 and 30 s) and that obtained by extrapolation (3 %) that the unextrapolated value may be used in nearly all cases.

It appears that the curve with dilute enzyme could possibly be extrapolated to the same initial activity as obtained with the stronger enzyme. The spread of the experimental data attest the inaccuracy of such a procedure. On the other hand, extrapolation from points obtained at 3, 6, and 9 minutes as recommended by Sumner [13] gives a definitely smaller activity (about three quarters).

The data of Fig. 1 were not taken with sufficient time resolution to prove that the stronger catalase solution did not have a similar initial decrease of activity in the first few seconds of the reaction. The activity of strong catalase solutions was therefore measured by a quite different technique. A modified

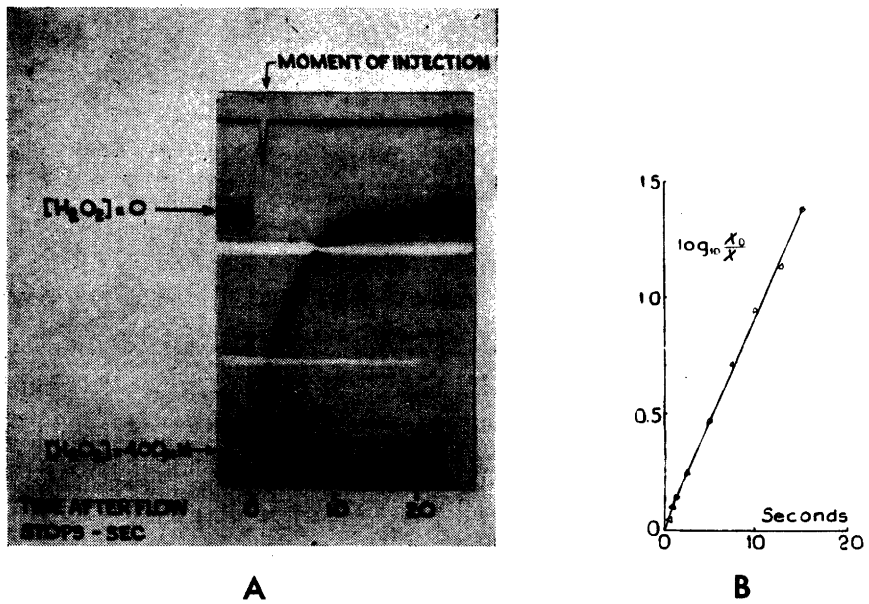

Fig. 2. The kinetics of the catalase reaction measured by the platinum microelectrode with anodic polarization. In A is shown the original data recorded by a mirror oscillograph and in B the graph of the data according to the first-order equation. 5.7 × 10⁻⁹ M horse liver catalase, 400 μM hydrogen peroxide, 0.01M phosphate buffer, pH = 6.5, T = 25° C, electrode polarized at +1V, $k_1 = 4 \times 10^7$ liter × mole^{-1} × second^{-1}. Expt. 90 C.

circuit for the platinum microelectrode [19] gives a linear response to the hydrogen peroxide concentration within a fraction of a second. A very small electrode has been inserted into the capillary cuvette of the rapid flow apparatus so that advantage may be taken of the rapid mixing. A typical record and a logarithmic plot of the data are shown in Fig. 2A and B. The moment of injection of the mixed enzyme and substrate solutions into the capillary is shown on the top trace of Fig. 2A. The electrode current suddenly increases due to the initial hydrogen peroxide concentration as shown by the sharp drop in the lower trace. The flow stops in about one second and the catalatic reaction proceeds along an exponential curve. As the logarithmic plot of Fig. 2B shows, there is no abrupt discontinuity which would indicate that catalase has a higher activity in the first few seconds of the reaction. Thus the data of Fig. 2B fully justify the straight-line extrapolation used in the upper curve of Fig. 1. Also the value of k_1 for Fig. 2B is 4×10^7 liter × mole^{-1} × second^{-1} in fairly good agreement with the data of Fig. 1. The results with

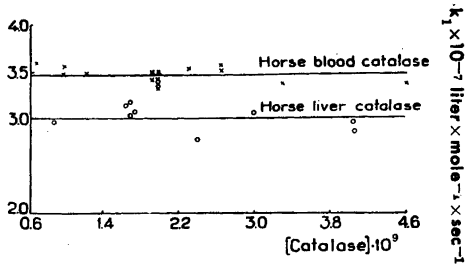

Fig. 3. The effect of enzyme concentration upon k_1 for liver and blood catalases. 5 mM H_2O_2 20° C. t = 15 sec.

the platinum electrode are somewhat less accurate than those obtained by titration and a typical series of data is shown later in Table 7. No significance is yet attached to the small numerical differences between the values of k_1 determined by the two methods.

It is of importance to learn just how much enzyme is required to give negligible inactivation in the first fifteen seconds of the reaction. This is illustrated by Fig. 3 which shows a substantially constant value of k_1 for the range of enzyme concentration tested. With more dilute enzyme the value of k_1 decreases as might be expected from Fig. 1. The value of k_1 was constant from 10^{-9} to 10^{-8} M catalase when using the platinum electrode technique in the capillary of the flow apparatus.

It is clear that only a portion of catalase is intact when its activity is measured by the older methods and that many of the factors previously found to affect the activity of catalase may only have affected the rapidity of its inactivation. One would first expect this factor to be clearly demonstrated in experiments in which the substrate concentration is varied for there the number of times that each catalase molecule must react will vary greatly.

THE EFFECT OF SUBSTRATE CONCENTRATION UPON THE ACTIVITY OF CATALASE

The substrate-activity characteristic of an enzyme is of great fundamental importance since it gives much information on the enzyme mechanism. If the reaction velocity is governed by the rate of breakdown of an enzyme-substrate compound in accordance with the Michaelis theory, then the Michaelis constant for such a complex must be determined in order to calculate the absolute reaction velocity (see Factor 3). From this point on the distinction between a hypothetical Michaelis compound governing the rate of destruction of hydrogen peroxide and the recently reported catalase hydrogen peroxide complex [1] must be kept clearly in mind. The latter compound is present at its saturation value during the destruction of hydrogen peroxide but the rate

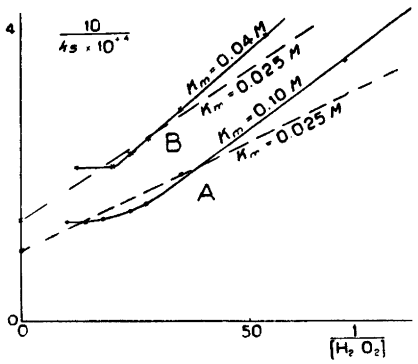

Fig. 4. The data of Euler's (20) Table 5 (circles) and Table 7 (crosses) plotted in accordance with Lineweaver and Burke's (22) equation, $\frac{1}{V} = \frac{1}{x_0} \cdot \frac{K_m}{V_{max}} + \frac{1}{V_{max}}$ *where* $\frac{10}{k \cdot s \cdot 10^4} = \frac{1}{V}$. *The dashed lines are drawn in accordance with the values of* $\frac{1}{V_{max}}$ *and* K_m *shown on Figures 2 and 3 of (20).*

of its monomolecular breakdown does not govern the catalatic activity. To avoid confusion this catalase-hydrogen peroxide complex will be consistently termed »intermediate compound» while the hypothetical Michaelis compound will be termed »Michaelis compound».

Although the mere existence of a logarithmic relation between substrate concentration and time rules out the possibility of significant amounts of a Michaelis compound in catalase action, many investigators have neglected this fact and have attached much significance to the maximum found in the activity-substrate relation; it has been concluded that the activity of catalase involves a Michaelis compound of $K_m = 0.025$ M[20] and 0.033 M[21]. It appears to be desirable to review the data on which those conclusions were based.

Following the experience of Lineweaver and Burke [22] that Eq. 5 gives the better placement of experimental data at low substrate concentrations,

$$\frac{1}{V} = \frac{1}{x_0} \cdot \frac{K_m}{V_{max}} + \frac{1}{V_{max}} \qquad (5)$$

the data of v. Euler [20], Stern [21], and Williams [6] have been replotted in Figs. 4, 5, and 6. Lineweaver and Burke [22] have previously made a plot of Stern's data

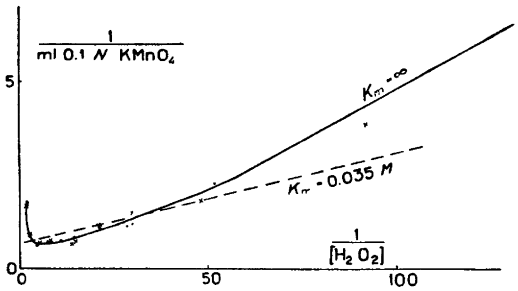

Fig. 5. The data of Stern's (21) Figures 1 and 2 (points) and 3 and 4 (crosses) plotted as in Fig. 4 except $\frac{1}{ml\ 0.1\ N\ KMnO_4} = \frac{1}{V}$ *and the values of S are converted into moles per liter. The dashed line corresponds with the value of* K_m *given by Stern (0.033 M). The data of Figures 2 and 4 are adjusted to the scale of Figures 1 and 3 respectively by means of the values of* V_{max} *shown on Stern's figures.*

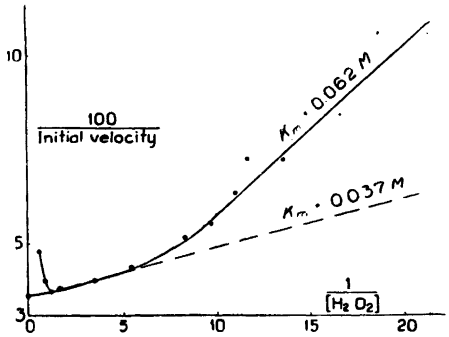

Fig. 6. The data of William's (6) Figures 3 and 3a plotted as in Figure 4 except $\frac{100}{M/L\ H_2O_2/min} = \frac{1}{V}$. *The dashed line gives the value of* K_m *that might have been determined by Williams had he reasoned as had Euler and Stern.*

using a form less suited to low substrate concentrations (Fig. 3 B II′ [22]) and, by neglecting those data at lower substrate concentrations, have obtained values of K_m and V_{max} which are later used to show that an active and an inactive intermediate compound may satisfy Stern's catalase data. We prefer to emphasize the data at low substrate concentrations and to evaluate the slopes of Figs. 4, 5, and 6 for large values of $\frac{1}{[H_2O_2]}$ which give values of K_m of 0.07, ∞, and 0.62 *M*.

These values indicate that the Michaelis constant for catalase activity, if it exists, is considerably greater than previous authors had assumed.

Fig. 7 affords an explanation of these effects. Here the reaction velocity obtained in the 15 s titration of strong enzyme (A) is compared with that obtained in the 3, 6, and 9 minute titration of dilute enzyme (B) at various values of substrate concentration. Curve A clearly corresponds to $K_m > 1\ M$ in agreement with the large values obtained above. The severe inactivation of the enzyme is clearly indicated by the difference between curves A and B. While the bulk of Stern's data was consistent with Lineweaver and Burke's mechanism, their proposal is quite inadequate to explain the effect of varying enzyme concentration demonstrated by curves A and B. There appears as yet to be little evidence supporting the existence of significant concentrations

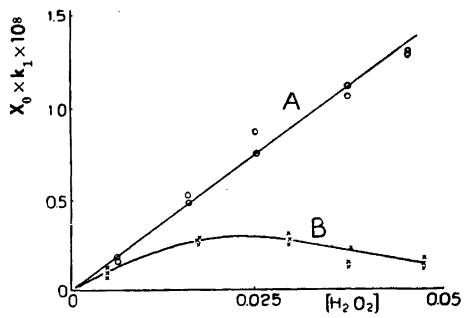

Fig. 7. The variation of reaction velocity with substrate concentration. The circles are obtained with rapid titration (15 sec) in strong enzyme while the crosses were obtained according to the previous methods with dilute enzyme and measurements at 3, 6, and 9 minutes (from top to bottom). Horse blood catalase, 3° C, pH 6.8, 0.01 M phosphate.

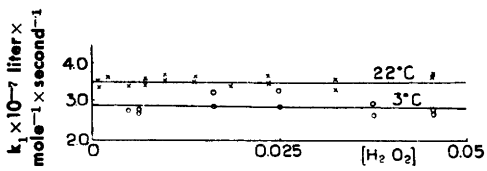

Fig. 8. The effect of a variation of initial substrate concentration upon k_1, at two temperatures as determined by 15 sec. titrations. Horse blood catalase pH = 6.8, 0.01 M phosphate.

of active or inactive compounds of the type postulated by Michaelis or by Lineweaver and Burke. On the other hand the peroxidatic catalase — hydrogen peroxide intermediate compound is readily demonstrable [1], but is saturated with substrate over the entire range that the activity has been studied and therefore does not cause the reaction velocity to be affected by substrate concentration.

Since the constancy of k_1 and not its variation with substrate concentration is to be evaluated, the data of Fig. 7 are replotted and repeated at room temperature as shown in Fig. 8. The constancy of k_1 is apparent. It is of considerable interest that the temperature coefficient of k_1 is independent of substrate concentration since this affords additional proof of the absence of an appreciable concentration of a Michaelis compound.

Similar results are readily obtained with the platinum microelectrode and are shown in Fig. 9. Not only is k_1 independent of substrate concentration but its numerical value is approximately the same as in Fig. 8. At lower substrate concentrations there is an apparent increase of k_1 which is believed to be due to errors in the electrode.

A number of years ago the activity of strong catalase was studied by a two-mixer technique similar to that used by Hartridge and Roughton [23] in hemoglobin studies*. Since the results can now be readily interpreted, they are included here. Buffered enzyme and substrate were mixed and caused to flow through a tube at constant velocity [24]. After a distance of flow equivalent to 0.2 s the catalatic reaction was stopped in a second mixing chamber

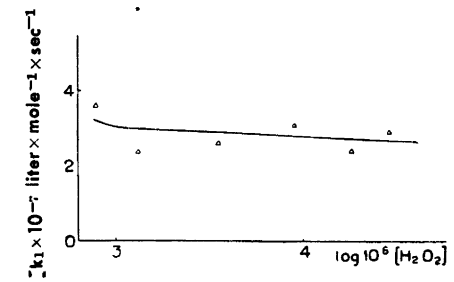

Fig. 9. The effect of a variation of initial substrate concentration upon k_1 as determined by the platinum microelectrode 5.9 × 10^{-9} M/L horse liver catalase, Expt. 87a, pH = 6.5, 0.01 M phosphate, 25° C.

* These experiments were mainly the work of the late G. A. Millikan who was assisted by P. McLaughlin and were carried out at the Johnson Foundation for Medical Physics, University of Pennsylvania.

by the addition of sulphuric acid. A sample of the reaction product was then titrated with permanganate.

After some difficulty due to the decomposition of hydrogen-peroxide in the storage syringe was eliminated by coating the parts with glyptal, the data of Table 1 were obtained.

Table 1. Activity determinations by the flow method.

x_0, moles per liter	0.05	0.3	1.0
x_0/x	1.25	1.17	1.22
t—s	0.2	0.2	0.2
k—s^{-1} ($\log_e$)	1.2	0.78	1.1
Catalase dilution	100	100	100
$k \times$ dilution	120	78	110

From the values of k the catalase concentration was about 3×10^{-8} M. The value of $k \times$ dilution obtained by the usual 5-minute titration was only 65. These tests therefore confirm these later data and show that the reaction velocity constant of very strong catalase is nearly independent of the substrate concentration up to 1 M and is in excess of that obtained by the old titration method.

These three independent methods show no variation of k_1 in excess of the experimental error over the entire range of substrate concentration tested. The properties of catalase found by these methods differ considerably from those obtained with more dilute enzyme and this difference may be due to enzyme inactivation or to the properties of partially-inactivated catalase. In any case these data give no support for the existence of an appreciable concentration of a Michaelis compound (see bottom of p. 691).

The recent note of George [12] on manometric studies of catalase activity adds support to these data in the region where a comparison is possible. Apparently the rate of initial oxygen evolution R_r, which precedes the enzyme inhibition, is linearly related to the initial substrate concentration up to 0.3 M hydrogen peroxide as shown by the data of Table 2. Georges' data, which clearly do not fit a hyperbolic relationship required by the Michaelis theory, do agree with the experiments of this paper.

Table 2. The variation of the initial rate of oxygen production in George's [12] manometric experiments (Fig. 2).

Initial hydrogen peroxide (x_0—M)	0.05	0.1	0.15	0.2	0.3
R_r as measured from Fig. 2 (μl/min)	290	640	1100	1400	2100
$R_r/x_0 \times 10^{-3}$ (μl $\times$ min^{-1} $\times$ liter $\times$ $mole^{-1}$)	5.8	6.4	7.3	7.0	7.0

Above this concentration an inhibition is observed that was not found in the titrimetric experiments of Table I and resembles the older titration data with dilute enzyme.

It may be that the conditions of the manometric experiment, rapid shaking over a large surface, are more severe than in these experiments and that some inhibition of catalase activity occurs even during the initial phase of oxygen evolution. A calculation of k_1 is desirable.

Although the enzyme concentration used in George's experiments is not given, it may be estimated from the value of $\Delta O_2/E \approx 1 \times 10^6$ moles oxygen per mole catalase and the value of $\Delta O_2 = 460$ μl in 0.5 M hydrogen peroxide (see Fig. 1A). $E \approx 2 \times 10^{-5}$ μ moles*.

The quantity R_r/x_0 of Table 2 is converted into these units: $\frac{7.0 \times 10^3}{22.4 \times 60} =$ 5.2 μmoles $\times$ second^{-1} $\times$ liter $\times$ mole^{-1}. The absolute reaction-velocity constant, k_1, is equal to $\frac{dx}{dt} \cdot \frac{1}{x_0\, e}$ in the initial phases of the reaction. Since $\frac{dx}{dt} =$ R_r and x and e are both in μmoles, $k_1 \approx 2 \times 10^5$ liter $\times$ mole^{-1} $\times$ second^{-1} which is roughly 1 % of the value found in these tests (3×10^7 liter $\times$ mole^{-1} $\times$ second^{-1}.

The value of k_1 for the steady rate at 0.05 M hydrogen peroxide is considerably smaller, $\approx 5 \times 10^4$ liter $\times$ mole^{-1} $\times$ second,$^{-1}$ corresponding to 0.2 % of the full activity of catalase.

In accepting the data of Table 2 in support of these experiments it must be assumed that the small fraction of the initial catalase concentration active in George's experiments had the same characteristics as the intact catalase of these experiments.

THE EFFECT OF TEMPERATURE UPON THE VELOCITY OF THE CATALATIC REACTION

Another important characteristic of the catalase reaction on which misleading data may have been obtained is the temperature coefficient.

The importance of the order of the overall enzymatic reaction upon the interpretation of thermal data has been emphasized by Sizer [25] since the tem-

* A letter from Dr. George indicates that the actual enzyme concentration was 7.4×10^{-6} μ moles in a volume of 4 ml. Thus $\frac{\Delta O_2}{E}$ was near its minimum value (0.3×10^6) in his Fig. 1 A and k_1 is 7×10^5 liter $\times$ mole^{-1} $\times$ second^{-1} about 2.5 % of this value. Dr. George prefers to explain this discrepancy by stating that his enzyme preparation had a smaller activity than that of these crystalline preparations.

perature coefficient of a zero-order overall reaction must represent the temperature coefficient of the break-down of an intermediate compound while the temperature coefficient of a first-order overall reaction usually represents the temperature coefficient of the combination of enzyme and substrate. In Sizer's recent measurements of the temperature coefficient of the catalase reaction [5] the manometric data closely approximate a zero order reaction. In other manometric experiments the values of Kat. F. have been found to be low and variable [11, 12].

Using the titration technique discussed previously a series of values for the energy of activation (μ) have been obtained as shown by Table 3. It would be desirable to prove the constancy of μ over this temperature range but the small change of velocity taxes the accuracy of the titration method.

Table 3. Titration data on the energy of activation of the catalase-hydrogen peroxide reaction.

Type of Catalase	T_2 (°C)	T_1 (°C)	$(k_1)_2 \times 10^{-7}$	$(k_1)_1 \times 10^{-7}$	μ cal
Horse blood	25.5	1.5	3.50	2.66	1880
Horse blood	23.5	7.2	3.50	3.11	1490
Horse blood	22.0	2.0	3.54	2.84	1800
Horse blood	23.5	3.0	3.50	2.86	1760*
Horse liver	22	2.0	3.00	2.55	1320

The values are considerably smaller than those obtained by Sizer (4200 cal)[5]. The values obtained by Williams [6] are closer to the present values than those of other workers. Apparently the energy of activation for the reaction of intact catalase with its substrate is less than that for the reaction of partially inactivated catalase studied by previous workers.

It is of interest to recalculate the theoretical value of reaction velocity of catalase and hydrogen peroxide on the basis of the value of μ for horse blood catalase. This calculation closely follows the pattern of Sizer [5] with two important exceptions; $\mu = 1700 \pm 100$ calories, $k_1 = 3.5 \times 10^7$ liter $\times$ mole^{-1} $\times$ second^{-1}.

The number of activated collisions of enzyme and substrate per second (or the number of substrate molecules destroyed per second) is

$$k = \frac{3}{13} \bar{\mu}\, n_e n_x \pi \sigma^2 \Sigma^{-\mu/RT} \qquad (5)$$

when n_e and n_x are the numbers of enzyme and substrate molecules per ml. Since k is calculated from the activity data as follows,

* See Fig. 8.

$$k = n_x \frac{\log_e \frac{x_0}{x}}{t} = n_x k_1' e = n_x k_1' \frac{10^3 n_e}{6.06 \times 10^{23}} \qquad (6)$$

Eq. 6 may be simplified by eliminating k and solving for k_1',

$$k_1' = 1.4 \times 10^{20} \bar{u}\, \pi\sigma^2 \Sigma^{-\mu/RT} \qquad (7)$$

Thus the teoretical reaction velocity constant, k_1' (liter $\times$ mole^{-1} $\times$ second^{-1}), for the rate of reaction of enzyme and substrate is seen to depend only upon the velocity of the substrate molecule $\bar{u}$ (assuming a stationary catalase molecule), the area of the catalase molecule (here taken to be the entire area $= \frac{u\sigma^2}{4}$) and the fraction of collisions that are effective ($\Sigma^{-\mu/RT}$). Using the values of $\bar{u}$ and σ calculated by Sizer [5],

$$k_1' = 1.24 \times 10^{13} \Sigma^{-\mu/RT} \qquad (8)$$

For $\mu = 1700$ and $T = 300°$ K, $\Sigma^{-\mu/RT}$ is 1/17 and

$$k_1' = 7.3 \times 10^{11} \text{ liter} \times \text{mole}^{-1} \times \text{second}^{-1} \qquad (9)$$

If this value is decreased by 5.2×10^{-3} on the basis that the active center has the area of four iron atoms as suggested by Sizer [5], a value of 4×10^9 is obtained which is 100 times larger than the observed rate. The accuracy of the theory underlying the theoretical calculation is probably not high, especially since Moelwyn-Hughes [26] mentions in a calculation of the molecular statistics of saccharase action that the theory of Hinchelwood would possibly increase the collision number by several powers of ten.

Reasonable conclusions of this comparison are: — 1. the experimental value for the reaction of catalase and hydrogen peroxide (3.5×10^7 liter $\times$ mole^{-1} $\times$ second^{-1}) is considerably less than the smallest theoretical value that can be calculated on the basis of reasonable assumptions — and 2. the probability of an effective collision of enzyme and substrate is extremely large ($\Sigma^{-\mu/RT} = 6$ %).

It appears that an even larger fraction of the collisions of oxygen and hemoglobin are effective for in this case Hartridge and Roughton [23] found the reaction-velocity change to be within their experimental error over the 10° interval. The latter was calculated to be 2.4 % from the nine data of their Table 2 (2900 ± 70, probable error of arithmetic mean). The value of μ must therefore be less than 350 cal and more than 50 % of the collisions are effective.

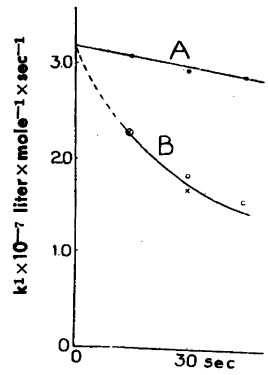

Fig. 10. The effect of pH upon the reaction velocity constant (k_1). Curve A, pH = 7.0; Curve B, pH = 3.5 0.001 M phosphate (acidified with phosphoric acid).

A similar situation exists in the reaction of peroxidase and hydrogen peroxide when the following preliminary data were obtained [27], but were previously unpublished since the error was relatively large.

Table 4. Preliminary values of the effect of temperature upon the formation of the peroxidase-hydrogen peroxide complex [27].

T°C	3	25	42
$k_1 \times 10^{-6}$ liter × mole^{-1} × second^{-1}	8	9	9

There is no doubt that an appreciable percentage of the collisions is effective.

These data therefore indicate that a large fraction of the collisions of enzyme and substrate are effective.

THE EFFECT OF pH

When a neutral catalase solution is diluted in a neutral phosphate buffer the turnover of substrate proceeds with very little decrease of k_1 as upper Curve A of Fig. 10 shows. If an acid phosphate solution is used, k_1 falls from its initial value at pH = 7 to much lower values as Curve B of Fig. 10 shows. Apparently there is no immediate inhibition of catalase by the acid solution; but there is a progressive decrease of the enzyme activity during the course of the reaction as if the inactivation of catalase by hydrogen peroxide were proceeding more rapidly in acid solutions.

The data of Table 5 show that a portion of the activity is lost by diluted catalase in the absence of substrate but that a much more extensive loss occurs when substrate is present. The inactivation of catalase in the presence of substrate can also be greatly increased by acetate in neutral solution as the

data of Table 5 show. Phosphate and formate do not cause much loss of activity in neutral solutions. As a check of these data the equilibrium constant for formate inhibition is calculated to be 3.7×10^{-3} *M* (pH = 7, 7° C) and is compared with 4.2×10^{-3} *M* obtained by extrapolating and interpolating the data of Agner and Theorell [28] for pH and temperature respectively.

Table 5. The effects of dilution at pH = 7 and 3.5 upon the activity of catalase. Horse blood catalase, $k_1 = 2.85 \times 10^7$ liter $\times$ mole$^{-1} \times s^{-1}$ at 7° C. 5 mM hydrogen peroxide, 0.001 M phosphate buffer, acidified with phosphoric acid to give pH = 3.5.

Treatment of catalase	pH	$k_1 \times 10^7$ (by 30 s titration)	% Activity remaining
Diluted to 10^{-9} for 9 min and then hydrogen peroxide was added for the activity test.	7.0	2.32	79
Ditto	3.5	0.94	32
Diluted to 10^{-9} in 5 *mM* hydrogen peroxide for 9 min and then fresh hydrogen peroxide was added for the activity test.	7.0	1.96	66
Ditto	3.5	0.40	14

Table 6. The effect of various anions upon the decrease of k_1 during the decomposition of 5 mM hydrogen peroxide at pH = 7.0. In both cases the activity was tested after 9 min. by the addition of fresh substrate (30 sec. data).

Conditions of experiment	0.01 *M* phosphate	0.16 *M* formate	0.37 *M* acetate
Percent activity remaining after 9 min. dilution to 10^{-9} *M* in solution containing anion alone.	79	88	75
Percent activity remaining after 9 min. dilution to 10^{-9} *M* in solution containing both anion and 5 *mM* initial hydrogen peroxide.	66	81	12

THE RELATION BETWEEN THE NUMBER OF INTACT HEMATINS AND ACTIVITY

The fourth important characteristic of catalase activity is the number of hematins that are required for maximal activity. This question has been studied by Lemberg [29]. Although their values of Kat. F. were carefully cor

rected for enzyme inactivation, they are considerably less than values obtained by these methods and therefore some of the inactivation may not have been accounted for.

Since pure horse blood and liver catalases are now available and have been proved to have identical protein components [15], the comparison has been made on the basis of these two preparations instead of upon liver catalases of varying bile pigment content [24]. The titration data of Fig. 3 show that the ratio of the activities of horse-liver to horse-blood catalase is 0.86. The platinum electrode data of Table 7 give an average ratio of 0.89. These enzyme concentrations were determined on a dry weight and molecular weight basis which was in agreement with the spectrophotometric determination of concentration at the peak of the Soret band.

These activity ratios are much larger than those computed by Lemberg [29] and suggest that these liver catalases contain considerably less bile pigment. The spectra of the carbon monoxide hemochromogens obtained from these catalases have been compared with that of Lemberg's [29] Fig. 2. The ratio of bile pigment to total heme for this horse-liver catalase is calculated by Lemberg's formula [29] $[(\Sigma_{630}/\Sigma_{570}) - 0.047]/[(0.55\Sigma_{630}/\Sigma_{570}) + 0.96]$ where the second term of the numerator has been increased to equal $\Sigma_{630}/\Sigma_{570}$ for horse blood catalase carbon-monoxide hemochromogen. A value of 9 % is found which is equivalent to 91 % non-bile-pigment hemoprotein.

The hemochromogen spectra of these catalases also differ markedly from that found by Lemberg, the value of $\Sigma_{650}/\Sigma_{557}$ was 0.25 in his experiments while this horse-liver catalase preparation gives a value of only 0.11. By correcting both ratios for that of horse-blood catalase (0.035) and by assuming that Lemberg used a catalase of the same bile pigment content for this test as for Fig 2 [29] a ratio of bile pigment to total heme of 14 % is calculated for this horse-liver catalase or 86 % non-bile-pigment hemoprotein. These two values appear to be in good agreement with the ratio of the hematin iron content of horse-liver and blood catalases as determined by pyridine hemochromogen (83 %, Bonnichsen [15]).

Although these ratios are in agreement with Agner's early determinations of the protohematin content of liver catalase [30], his later values [14] and Theorell and Agner's [11] magnetic titration data gave values of about 75 %. Agner and Theorell [28] found the ratio of the extinctions of liver and blood catalase fluorides to be 81 % (calculated from their Fig. 4) until m/40 fluoride concentration was reached when a value of about 75 % was obtained. A similar spectrophotometric test with cyanide gave 75 %.

DISCUSSION

A fifty-fold increase in the amount of catalase used in activity determinations appears to have made inactivation and other effects unimportant. The values of activity can be determined without introducing a personal element into the determination by extrapolating a sharply inflected curve. Since the values of activity determined by this method are quite independent of variations of initial substrate concentration, are only slightly influenced by temperature changes, and give a constant activity for a wide range of enzyme concentrations, it is recommended that future determinations of catalase activity be made in this way. No set procedure has been formulated simply because the range of conditions over which constant activity may be obtained is relatively large. The value of k_1 is constant in the titrimetric method from 0.6 to 5 $\times$ 10^{-9} M catalase. In the platinum electrode tests which were carried out in the 1 mm capillary of the flow-apparatus k_1 was constant from 10^{-9} to 10^{-8} M catalase*.

Since the catalase concentration is now readily calculated on a molar basis either from the known molecular weight or from spectrophotometric data, it is suggested that the results obtained by this new method be given in terms of k_1 (see Eq. 2), the velocity constant for the disappearence of hydrogen peroxide, instead of Kat. F. There are two reasons for this:

1. Values of Kat. F. determined in this manner are consistently higher unless the inflected curves obtained by the old method are optimistically extrapolated. In order to avoid unnecessary qualifications as to how the activity was determined, a change of units is desirable.

2. The quantity k_1 is expressed in accepted units and has a definite physical-chemical meaning; the activity of catalases expressed in this manner are immediately comparable with data on other enzymes.

The value of k_1 is found to be 3.5 $\times$ 10^7 liter $\times$ mole^{-1} $\times$ second^{-1} at 22° C for horse blood catalase and corresponds to a Kat. F. of 61,000 at 0° C. From Fig. 1 k_1 found by extrapolation of the 3, 6, and 9 minute data is only 2.75 $\times$ 10^7 liter $\times$ mole^{-1} $\times$ second^{-1} or a Kat. F. of 48,000 at 0° C. However, the Kat. F. actually found at 0° C for this catalase by the 3, 6, and 9 minute extrapolation [15] was 65,000. This discrepancy may be caused by an extrapolation error or by a change in the shape of the catalase kinetics from the sharply inflected curve of Fig. 1 to a more slowly changing curve at 0° C (see, for example, Sumner [18]). Extrapolation errors and the change of the rate of inactivation of catalase with temperature, initial substrate concentration, pH, etc.,

* It is surprising that this is so for the ratio of area to volume in the capillary is probably an order of magnitude greater in the capillary than in the flasks used for the titration experiments. Air-water interfaces are, however, absent.

make the older method of activity determination very dangerous to use for a comparison of catalase activities or for kinetic studies. It is therefore recommended that the 3, 6, and 9 minute extrapolation be abandoned in favor of this titration method and that the activity values be given in terms of k_1 at 20° C for enzymes of known molecular weight (horse liver [31] and beef liver [32], horse erythrocyte [15]). Since the correct values of k_1 are given in this paper for horse liver and blood catalases, the enzyme concentration (e) can be readily determined by an activity test and is calculated from Eq. 2. The enzyme concentration of a large number of purified preparations have been determined in this manner and have been found to be equal to that determined in the Beckman spectrophotometer at the peak of the Soret band using $\Sigma = 380$ $cm^{-1} \times mM^{-1}$ for horse-erythrocyte catalase and $\Sigma = 340$ $cm^{-1} \times mM^{-1}$ for horse-liver catalase prepared according to Bonnichsen [15]. The purity of the preparation is then expressed as the ratio of e found by activity test or spectrophotometrically to the enzyme concentration found from the dryweight and molecular weight. The spectrophotometric method of determining the enzyme concentration is more rapid than the activity test and is, therefore, often used as a test of the purity of the preparation. The final stage of purification is, however, best judged by the extinction coefficient at 280 mμ and has been found to be $\Sigma = 280$ $cm^{-1} \times mM^{-1}$ for the horse blood and liver preparations [15].

For catalases of unknown molecular weight Kat. F. is still useful but this titration procedure should be used.

This new method for obtaining catalase activity now gives data on the enzyme mechanism which do not appear to be obscured by inactivation effects. Therefore four of the fundamental factors in the mechanism of the enzyme have been re-examined and new conclusions have been reached in three cases. It appears that several other conclusions on the mechanism of catalase action will have to be re-examined in the near future.

In the discussion which follows the distinction between the hypothetical Michaelis compound and the recently reported catalase-hydrogen peroxide intermediate compound must be remembered (see bottom of p. 691).

Between 1 and 100 mM substrate concentration the value of k_1 is constant; and no saturation effect is observed as is required by the Michaelis theory. Although this conclusion is at variance with the results obtained by previous workers, a re-examination of their data clearly shows that the maxima in the activity-substrate relationships that they obtained fitted the Michaelis theory over only a very narrow range of substrate concentration; the data are affected by variable inactivation of the enzyme by its substrate.

A most convincing proof of the independence of k_1 and substrate concentration is given by the fact that this relation obtains at both 2 and 22° C.

The absence of any saturation affect in the activity of strong catalase had been found in 1940 in experiments conceived and largely carried out by the late G. A. Millikan. Due to the lack of independent confirmation and the imminence of the war, the data remained unpublished until now.

Independent experiments [1] show that the catalatic activity is appreciable when the substrate concentration is very small-roughly equal to that of the peroxidatic intermediate compound. The value of k_1 under these conditions is calculated to be about 1.2×10^7 liter $\times$ mole^{-1} $\times$ second^{-1}. This indicates the tremendous range of substrate concentration over which k_1 is nearly constant — from about 1×10^{-6} to 0.1 M.

Table 7. Platinum electrode experiment $[H_2O_2] = 0.3$ *mM* ($T = 25°C$).

Type of catalase	L	B	L	B	L	B	L	B	L	B	L	B
Concentration-$M \times 10^9$	2.24	1.69	5.6	4.23	11.2	8.45	4.48	2.38	2.24	1.69	1.32	0.994
Average t ½-sec	8.6	10	4.6	5.6	3.0	3.6	6.8	8.7	14.3	13.2	22	27.8
$k\frac{0.693}{(t\ ½)}$ sec^{-1}	0.081	0.070	0.15	0.12	0.23	0.19	0.10	0.08	0.049	0.053	0.030	0.025
$(k_1 = k/e) \times 10^7$-liter $\times$ mole^{-1} $\times$ second^{-1}	3.6	4.1	2.7	2.9	2.1	2.3	2.4	2.4	2.2	3.1	2.4	2.5
Ratio of activity $\frac{\text{Liver}}{\text{Blood}}$	0.87		0.93		0.90		1.0		0.70		0.96	
Average of all determinations	0.89											

These experiments do not rule out the existence of a Michaelis compound governing the catalatic activity, they merely require its lifetime to be very short. Since the error of the determinations of k_1 are less than 3 % such reactions must be at least 30 times more rapid than the observed rate of reaction of enzyme and substrate at the highest substrate concentration used (≈ 0.1 M). If only one intermediate is formed its break-down constant must exceed $30 \times 0.1 \times 3 \times 10^7$ s^{-1} or about 1×10^8 s^{-1}. Furthermore if the proposed mechanism involves a reaction with a second molecule (such as oxygen) this must

proceed with a second-order rate constant in excess of about 1×10^8 liter $\times$ mole^{-1} $\times$ second^{-1}. While the collision theory indicates that such a rate of reaction is possible, it is much more rapid than the reaction of oxygen and myoglobin. The rate of such a reaction with oxygen would have to be several orders of magnitude more rapid if catalase activity is to have no appreciable induction period when the ratio of substrate to oxygen concentration is large.

The recent data of Brdicka and Wiesner [33] on the rate of reaction of ferrohemin and hydrogen peroxide at the surface of the dropping mercury electrode give a very large value ($\approx 10^{11}$ liter $\times$ mole^{-1} $\times$ second^{-1}). Although neither the oxidation nor complex formation of ferrohematin with hydrogen peroxide has been proposed as a part of the catalase mechanism, it is interesting that their value of the reaction velocity constant for ferrohemin and hydrogen peroxide is large enough to fulfil the requirements above.

The energy of activation for the catalatic activity obtained from older methods has been found to be several fold too large since an appreciable portion of the activity was probably carried by partially inactivated enzyme. The new method gives 1700 ± 100 cal corresponding to 6 % effective collisions of enzyme and substrate. Minimum values for the energy of activation in the reactions of hemoglobin and oxygen and peroxidase and hydrogen peroxide are somewhat smaller than this. Further studies with catalase may give similarly small values if catalase inactivation is completely eliminated. if all collisions were effective, a maximum velocity constant of $\frac{3.5 \times 10^7}{0.06}$ $= 6 \times 10^8$ would be obtained.

A recalculation of the theoretical reaction velocity on the basis of these data shows that the smallest reasonable theoretical value considerably exceeds the observed rate and may be several orders of magnitude greater. This indicates a lack of data on orientation effects that may obtain in the union of enzyme and substrate and a certain indefiniteness of the theory of collisions in solutions.

Moelwyn-Hughes [34] in discussing a chain mechanism for catalase action found it difficult to reconcile a chain mechanism with an experimental value of the reaction velocity which was not in excess of the theoretical value — a chain mechanism is clearly indicated where the experimental rate clearly exceeds that possible on the basis of collisions of enzyme and substrate. Many other more obvious characteristics of the catalase reaction rule against a chain reaction:

1. The simple first-order kinetics.
2. The lack of any measurable induction period in the disappearance of substrate.

3. The apparently normal inhibition characteristics of the catalase reaction; there is agreement between the spectrophotometric and the activity data for the inhibition of catalase activity by cyanide (35).
4. Catalase has no comparable sensitivity towards »chain breakers».

On the other hand there is no evidence comparable with that obtained with peroxidase [27] to show that the rate of oxygen production is equal to the rate of disappearance of hydrogen peroxide.

Since the reaction of catalase and hydrogen peroxide follows a bimolecular course over such wide ranges of enzyme and substrate concentrations, the complex mechanisms, chain theories, Michaelis theories, ferri-ferro cycle theories, do not find support in these data. In fact the only direct evidence available that the activity of uninhibited catalase involves complexity is the existence of the intermediate compound — a topic which is to be considered elsewhere.

At pH = 3.5 the activity of catalase extrapolated back to the first few seconds of the reaction appears to be roughly equal to that at pH = 7. The principal effect of the acid solutions is to hasten the inactivation of catalase by its substrate. The latter effect also appears to be caused by acetate at pH = 7.0. The results obtained with formate where there is very little inactivation of catalase during the titration substantiate the data of Agner and Theorell [28] on anion inhibition of catalase activity.

The theory of Michaelis and Peckstein [36] that catalase activity was closely related to its amphoteric properties was greatly weakened by Agner and Theorell's finding [28] that these effects were largely due to anion inhibition and has been given a coupe de grace by these data which show that the residual pH effect found by Agner and Theorell was largely due to enzyme inactivation during the course of the reaction. The inactivation of catalase can be increased by excessively dilute enzyme, high substrate concentration, low pH, presence of acetate, etc., shaking, exposure to large surface areas, etc. and the type of inactivation seems similar in these cases. It is felt that these are factors which influence the conversion of catalase to a less active form and the factors may not in themselves represent specific effects. Although catalase has been termed »a rather rugged enzyme»[18], its maximal activity towards hydrogen peroxide is very fragile in dilute solutions.

It appears that the relative activities of the liver and blood catalase preparations are very nearly equal to the relative protohematin content when determined by methods which involve splitting the hemin from the catalase (hemochromogen, CO-hemochromogen, and pyridine hemochromogen). On the other hand, magnetometric measurements of the amount of horse-liver catalase hematin that can combine with cyanide give a result which is about 10 %

less than the ratio of the activities. In either case the data support the conclusion of Lemberg and Legge that the activity increases as the bile pigment content decreases. These data are not sufficiently extensive to prove that all hematins participate equally in the destruction of substrate.

If Kat. F. of horse-liver catalase is calculated as a »complete» catalase as recommended by Keilin and Hartree[10] the values of Lemberg and Legge[29], Sumner *et al.*[32], Keilin and Hartree[10] and Bonnichsen[15] all lie between 52,000 and 60,000 — a remarkably good agreement in view of the different methods of preparation, determination of hematin iron or bile pigment content, and extrapolation of the Kat. F. determination. This method gives a Kat. F. or erythrocyte catalase of 61,000. Over a small range, the Kat. F. (or better, the k_1) of a horse liver catalase may be calculated from this last figure by multiplying by the relative hematin iron content.

When both velocity constants are calculated on the basis of catalase molarity the value of k_1 for horse-blood catalase (3.5×10^7 liter $\times$ mole^{-1} $\times$ second^{-1}) at 22° C does indeed bear a striking resemblance to the rate of formation of the peroxidatic intermediate compound (3×10^7 liter $\times$ mole^{-1} $\times$ second^{-1})[1] and thus the statement that the latter compound forms at a rate sufficient to carry the entire catalatic activity is justifiable. It is, however, unlikely that only one hematin carries the entire activity because the data above indicate that all the catalase hematins may participate in the destruction of hydrogen peroxide. A determination of the exact relationship between the intermediate compound and the catalatic activity awaits further tests.

SUMMARY AND CONCLUSIONS

1. A modified method for the determination of catalase activity is proposed in which the enzyme concentration is increased about 50 fold over that used previously.

2. With this method the first-order reaction velocity constant is nearly independent of time, linear with enzyme concentration, independent of substrate concentration at least up to 0.1 M, and only slightly affected by temperature.

3. In order to avoid confusion with activities determined by older methods and to institute proper units, the activity is calculated from the equation $k_1 = \frac{2.3 \log_e \frac{x_0}{x}}{et}$ (see Eq. 2) instead of in terms of Kat. F. $k_1 = 3.5 \times 10^7$ and 3.0×10^7 liter $\times$ mole^{-1} $\times$ second^{-1} for horse-blood and liver catalases respectively at 22° C.

These values correspond to a Kat. F. of 6.1 and 5.2×10^4 at 0° C. For these two catalases the activity test is used for determining the enzyme concentration and the purity is expressed as the percentage of the enzyme in the total dry weight. For the purification of catalases of unknown properties, this titration procedure is recommended and the results are calculated on a dry weight basis.

4. The velocity constants for the rate of destruction of hydrogen peroxide by catalase and for the formation of the intermediate compound are very nearly equal when both values are calculated on the basis of catalase molarity.

5. The peroxidatic intermediate compound of catalase and hydrogen peroxide does not govern the velocity of the catalatic reaction in the manner required by the Michaelis theory since the value of k_1 is independent of substrate concentration up to at least 0.1 M. The previous demonstrations of a Michaelis compound of $K_m \approx 0.025$ M are concluded to be the result of enzyme inactivation. The Michaelis theory is not, therefore, an explanation of catalase action. A mechanism postulating Michaelis compounds requires that their breakdown constants be in excess of 10^8 s^{-1} and that any oxidation-reduction reactions be extremely rapid.

6. The energy of activation of the reaction of horse blood catalase and hydrogen peroxide by these methods is 1700 ± 100 calories.

7. The fraction of effective collisions of catalase and hydrogen peroxide is large and approaches that estimated for the reactions of hemoglobin and oxygen and peroxidase and hydrogen peroxide.

8. The reaction velocity for catalase and hydrogen peroxide is considerably less than that calculated on the basis of the collision theory.

9. The simplicity of the catalase kinetics obviate the need for a chain mechanism and there are several reasons against a chain mechanism for the destruction of substrate by catalase.

10. The inactivation of catalase by acid solutions and by acetate ion is not rapid but takes an appreciable time for completion. The extent of the inactivation is greatly increased by the presence of substrate. In the initial phases of the reaction the catalase activity is nearly constant from pH = 7 to pH = 3.5.

11. Excessive catalase dilution, large substrate concentration, low pH, rough treatment, the presence of acetate ion, etc. are factors which may accelerate the inactivation of catalase during the decomposition of hydrogen peroxide and may influence the conversion of catalase to a less active form.

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The Effect of Mercuric Bromide on the Hydrolysis of Acetobromoglucose in Acetone

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In an earlier investigation [1] it was found that the reaction between mercuric bromide, water and α-acetobromoglucose is of the second order, depending on the concentrations of mercuric bromide and acetobromoglucose and that β-2,3,4,6-tetraacetylglucose is the product of hydrolysis. Reactions between mercuric salts, water and R. Hal have earlier been studied by Nicolet and Stevens [2], Roberts and Hammet [3], Read and Taylor [4], and Gand [5]. Nicolet and Stevens find that the hydrolysis is catalysed by mercuric bromide, which seems to be deactivated by the reaction $HgBr_2 + 2Br \rightarrow HgBr_4^{2-}$.

Roberts and Hammet suppose that a carbenium ion is an intermediate in their reaction between benzylchloride, mercuric nitrate and water. The reaction is of the second order but is disturbed by the mercuric chloride formation and by a new substance C_7H_7OHgCl. Read and Taylor on the other side have found that the reaction $RBr + H_2O \rightarrow ROH + HBr$ [R = $-CH_3$, $-O_2H_5$, $-CH(CH_3)_2$ and $-C(CH_3)_3$.] is accelerated by mercuric bromide, but they did not get satisfactory rate constants unless they introduced an initial concentration of hydrogen bromide.

As the reaction α-acetobromoglucose + $HgBr_2$ + $H_2O \rightarrow$ β-2,3,4,6-tetraacetylglucose + $HHgBr_3$ differs from reactions of similar type it has been examined more closely. The reaction was followed in a polarimeter, so to interpret the data the mutarotation of β-2,3,4,6-tetraacetylglucose in acid medium had to be investigated (Table 1 and 2).

With Arrhenius equation one gets the activation energy 28700 cal. $k = 10^{19,6} \cdot e^{-28700/RT}$.

Table 2 gives the variation of k with the concentrations of water and hydrogen bromide.

Table 1. Mutarotation of β-2,3,4,6-tetraacetylglucose in acid solutions of various acidity and temperature.

1 mmole β-2,3,4,6-tetraacetylglucose, 1 mmole mercuric bromide, hydrogen bromide, acetone with 1% water ad 20 ml.

t° C	mmole HBr	10^3 k min^{-1}
15	1	5.5 ± 0.2
20	0.5	5.0 ± 0.2
20	1	12.6 ± 0.3
25	1	31.1 ± 0.3
30	1	67 ± 3

Table 2. Mutarotation of β-2,3,4,6-tetraacetylglucose in acid solutions of various water concentrations.

20.0° C · 0.5000g β-2,3,4,6-tetraacetylglucose, hydrogen bromide, water and acetone ad 20 ml.

mmole HBr	% H_2O	10^3 k min^{-1}
0.641	2	13.3 ± 0.1
1.30	1	122 ± 1
1.27	2	28.9 ± 0.5
1.28	4	7.98 ± 0.2
2.56	2	96.0 ± 1

The velocity increases with the hydrogen bromide concentration but the relation is not linear. Water is a negative catalyst as would be expected. As a base it neutralizes the acid that catalyses the reaction.

Table 3. Variation of the concentrations of acetobromoglucose and mercuric bromide. 20.0° C. Mercuric bromide, α-acetobromoglucose and acetone with 1% water ad 20 ml.

mmole $HgBr_2$	mmole abg.	k liter · $mole^{-1}$ · min^{-1}
1.00	1.50	3.52 ± 0.05
1.00	1.00	3.44 ± 0.04
1.50	1.00	3.63 ± 0.05

Table 4. Effect of the temperature upon the second order reaction. 1 mmole mercuric bromide, 1 mmole α-acetobromoglucose, acetone with 1 % water ad 20 ml.

t° C	k liter · mole^{-1} · min^{-1}
15	2.48 ± 0.02
20	3.44 ± 0.04
25	4.76 ± 0.04
30	6.70 ± 0.05

With Arrhenius equation one gets the activation energy 11600 cal · $k = 10^{9,22} \cdot e^{-11000/RT}$.

From the results in Table 3 it is clear that the reaction is of the first order with regard to both acetobromoglucose and mercuric bromide. That the mercuric bromide that is consumed reacts to $HHgBr_3$ has already been proved [1] in two runs where the same amounts of acetobromoglucose reacted firstly with 0.567 mmole mercuric bromide, secondly with 1.013 mmole mercuric bromide + 0.451 mmole hydrogen bromide. The initial concentration of mercuric bromide, not bound as $HHgBr_3$, was the same in the two runs and the velocity of the reaction was also the same.

A few runs have been made at higher water concentrations (2 and 4 %). Here the mutarotation is slower and the corrections are smaller (Table 5).

Table 5. Effect of water concentration upon the second order reaction. 1 mmole accetobromoglucose, 1 mmole mercuric bromide, water and acetone ad 20 ml 20.0° C.

% H_2O	k liter · mole^{-1} · min^{-1}
1	3.44 ± 0.04
2	4.19 ± 0.04
4	4.61 ± 0.04

The velocity increases slightly with the water concentration.

One acetolysis of α-acetobromoglucose has also been made in order to see if this reaction too proceeds with Walden's inversion. α-Acetobromoglucose was acetolysed in a mixture of acetic anhydride, acetic acid and sulfuric acid. This mixture is not stable, so an isomerisation of β-pentaacetylglucose to α/β-pentaacetylglucose had to be run at the same time with the same solution. A kinetic analysis proves that the α-acetobromoglucose first gives β-pentaace-

tylglucose, which then reacts to α/β-pentaacetylglucose. The experimental data and calculations based upon two theories (1 and 2 below) are given in Table 6.

Table 6. Acetolysis of acetobromoglucose.

(The table gives only a part of the observed values.) 0.500 g acetobromoglucose, 20 ml of a mixture of 50 ml acetic anhydride, 20 ml acetic acid and 3 ml sulfuric acid, 20.0° C. α-pentaacetylglucose → α/β-pentaacetylglucose . $k = 0.0107\ min^{-1}$.

t min	α found	α calc.	
		1	2
0	9.21		
30	7.27	7.27	7.42
60	6.29	6.32	6.33
90	5.73	5.77	5.65
120	5.39	5.43	5.25
180	5.00	5.04	4.85
240	4.84	4.83	4.70
1200	4.62		

1. It is assumed that the reaction proceeds:
α-acetobromoglucose $\xrightarrow[k_1 = 0.0373]{}$ β-pentaacetylglucose $\xrightarrow[k_2 = 0.0107]{}$ α/β-pentaacetylglucose.

2. It is assumed that the reaction proceeds: —α-acetobromoglucose $\xrightarrow[k = 0.0165]{}$ α/β-pentaacetylglucose. (The best value.)

As the values calculated according to 1 show a maximum deviation of only 0.04, and those according to 2 deviate consistently over the entire range of values, it is clear that the acetolysis involves Walden's inversion.

The effect upon configuration by hydrolysis of compounds R · Hal, catalysed by mercuric salts, has not been observed earlier. The similar reaction, catalysed by silver salts, has however been studied in detail [6]. Here one generally finds inversion and a little racemisation.

The steric course of the reactions catalysed by silver salts is similar to that of the homogenous, unimolecular substitution (S_N1), so it is natural to suppose a similar mechanism. The rôle of the silver salts should be to facilate the dissociation of the halogen ion. In the reaction studied here the rôle of the mercuric bromide is clear. A molecule $HgBr_2$ combines with a Br^- from the acetobromoglucose, and gives $HgBr_3^-$, which under the reaction conditions cannot react futher. The resulting carbenium ion then reacts rapidly with water

and gives 2,3,4,6-tetraacetylglucose and H^+. Unfortunately it is impossible to give a value for the optical purity of the 2,3,4,6-tetraacetylglucose formed, but from the data it is evident that it is rather pure, at least 90 % or higher.

The investigation of the effect of mercuric bromide on hydrolysis of R · Hal will be continued with β-*n*-octylbromide. The intention is to follow the hydrolysis with both polarimetrical and analytical methods.

EXPERIMENTAL

The acetobromoglucose was prepared by a method of Freudenberg [7] and recrystallized from ethyl ether.

The β-2,3,4,6-tetraacetylglucose was prepared by a method in Org. Syntheses [8].

The mercuric bromide was Merck's pro analysi. The acetone was dried over potassium carbonate and distilled. All reactions were made in polarimeter tubes with mantles, through which water from a thermostat circulated. The temperature of the thermostat fluctuated 0.1° C from the mean value, the fluctuations in the tube were smaller. In a typical run (Table 1). 1 mmole β-2,3,4,6-tetraacetylglucose was dissolved in 20 ml of a solution of 1 mmole mercuric bromide and 1 mmole hydrogen bromide in acetone with 1 % water. This solution was preheated in the thermostat to 25° C and as quickly as possible the solution was transfered to a polarimeter tube, 20 cm long. The rotation was determined at definite intervals.

t min	α	$10^2 \cdot \frac{1}{t} \log \frac{\alpha\infty - \alpha_0}{\alpha\infty - \alpha}$
0	0.20	
5	0.53	1.32
10	0.82	1.35
15	1.01	1.24
20	1.26	1.33
30	1.60	1.34
40	1.88	1.40
50	2.07	1.42
60	2.16	1.35
70	2.27	1.38
80	2.34	1.39
90	2.38	1.36
120	2.46	1.33
1200	2.52	

Mean value 1.35 ± 0.01

$K = (3.11 \pm 0.03)\ 10^{-2}$

In another typical run 1 mmole acetobromoglucose was dissolved in 10 ml acetone at 25° C and put in the thermostat. After 15 minutes 10 ml of a similar preheated solution

of 1 mmole mercuric bromide in acetone with 2 % water was added. As quickly as possible the solution was transfered to the polarimeter tube and the rotation determined at definite intervals.

(Only a part of the observed values is given here.)

t min	a	$a_0 - a$	$(a_0 - a)$ corr	x %	$\frac{1}{t} \cdot \frac{x}{a(a-x)}$
0	7.60	0			
2	5.29	2.31	2.32	31.9	4.69
4	3.98	3.62	3.67	48.9	4.79
6	3.31	4.29	4.39	58.6	4.72
8	2.86	4.74	4.90	65.4	4.73
10	2.53	5.07	5.31	70.8	4.85
12	2.40	5.20	5.52	73.6	4.65
14	2.24	5.36	5.76	76.8	4.73
16	2.14	5.46	5.95	79.4	4.82
18	2.07	5.53	6.10	81.3	4.83
20	2.02	5.58	6.24	83.2	4.95
25	1.93				
50	2.12				
1200	2.80				

Mean value 4.76 ± 0.04

The values were determined with 1 min intervals. In order to correct for the mutarotation of 2,3,4,6-tetraacetylglucose, as a first approximation the mutarotation was disregarded and the fraction β-2,3,4,6-tetraacetylglucose formed under each interval calculated.

From the velocity constant for the mutarotation it was calculated how much β-2,3,4,6-tetraacetylglucose had reacted to α/β-2,3,4,6-tetraacetylglucose and what this corresponded to in terms of optical rotation. In this calculation it was assumed that the velocity of the mutarotation varied in a linear way with respect to the concentration of $HHgBr_3$, which is the same as the overall concentration of 2,3,4,6-tetraacetylglucose. Another assumption is that pure β-compound is formed by the hydrolysis. This assumption is justified by the consistency of the calculated reaction velocity constants. A repetition of this approximating calculation only led to corrections within the precision of the experiment.

SUMMARY

The rate of the reaction between mercuric bromide, α-acetobromoglucose and water has been determined in acetone at various relative concentrations and temperatures. The reaction was found to be of the first order with respect to both mercuric bromide and acetobromoglucose and was found to involve Walden's inversion. The concentration of water did not influence the velocity very much. The parameters of the Arrhenius' equation for the reaction were

determined. In order to interprete the data for this reaction, the mutarotation of 2,3,4,6-tetraacetylglucose in acid solutions of acetone-water has been studied. The acetolysis of acetobromoglucose with sulfuric acid has been shown to involve Walden's inversion.

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Zur Bildung von Diacetyl aus Brenztraubensäure mit Acetaldehyd und Acetoin als Zwischenprodukte bei bakteriellen Gärungen

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Die Bildungsreaktionen von Diacetyl, Acetoin (Acetylmethylcarbinol) und 2,3-Butylenglykol sind seit dem Beginn dieses Jahrhunderts und zumal, nachdem van Niel, Kluyver und Derx [1] sowie Schmalfuss und Barthmeyer [2] im Jahre 1929 das Diacetyl als den eigentlichen Aromastoff der Butter nachwiesen, Gegenstand eifriger Forschung gewesen. Die meisten Autoren, die sich mit dem Studium jener Reaktionen befasst haben, sind sich darüber einig, dass die Reaktion über Brenztraubensäure geht. Da aber das Diacetyl leicht zu Acetoin und dieses weiter zu 2,3-Butylenglykol reduziert wird und die Oxydation in der entgegengesetzten Reihenfolge stattfindet, ist es schwierig gewesen zu entscheiden, welcher von diesen Stoffen primär entsteht. — In der vorliegenden Arbeit wird für Diacetyl, Acetoin und 2,3-Butylenglykol der Einfachheit halber und weil keine anderen vier Kohlenstoffatome enthaltenden Substanzen hier Behandlung finden, die gemeinsame Bezeichnung C_4-Verbindungen gebraucht.

Neuberg und Mitarbeiter [3–12] nehmen an, dass das Acetoin durch Acyloinkondensation auf die Weise entsteht, dass durch Einwirkung der Hefecarboxylase aus Brenztraubensäure Kohlendioxyd abgespaltet wird unter Bildung von Acetaldehyd, der sich *in statu nascendi* durch Einwirkung eines besonderen Enzyms, der Carboligase, mit dem stabilen (auch zugesetzten) Acetaldehyd verbindet. Das primär entstandene Acetoin würde dann zu Diacetyl oxydiert und zu 2,3-Butylenglykol reduziert. Dirscherl und Mitarb. [13–17], Langenbeck und Mitarb. [18–22], Tanko und Munk [23] sowie Silverman und Werkman [24] haben indessen die Beteiligung der Carboligase an diesen Vorgängen bezweifelt.

Van Beynum und Pette [25, 26] sowie Sjöström [27] vermuten Acetaldehyd als Zwischenprodukt bei der Bildung der fraglichen Stoffe durch Einwirkung der Aromabakterien der Butter. Sein experimenteller Nachweis in den Gärlösungen der erwähnten Organismen ist den Autoren allerdings nicht gelungen. Sie halten die biologische Oxydation des

Acetoins zu Diacetyl nicht für möglich, sondern sind der Ansicht, dass der Acetaldehyd direkt zu Diacetyl oxydiert wird.

Virtanen und Tarnanen [28], Virtanen [29] und Virtanen und Nikkilä [30, 31] haben in gewissen Ausnahmefällen bedeutende Mengen Acetaldehyd in Säureweckerkulturen gefunden, dagegen nicht unter normalen Bedingungen. Der Acetaldehyd entsteht als die Folge der Tätigkeit besonderer sog. Malzaromabakterien, und das Malzaroma wird auch eben durch den in der Lösung angehäuften Acetaldehyd hervorgerufen [30, 31]. Die eigentlichen Aromabakterien bilden dagegen keinen Acetaldehyd. Die Autoren sind der Meinung, dass als erstes Kondensationsprodukt Acetoin entsteht, das dann weiter zu Diacetyl oxydiert wird.

Nach Storgårds [32] geht das Acetoin bei den Aromabakterien ohne Acetaldehyd als Zwischenprodukt aus zwei Molekülen Brenztraubensäure hervor. An dieser Reaktion nimmt nach Virtanen [33] nebst Silverman und Werkman [24] ein von der Hefecarboxylase verschiedenes Enzym teil.

Auch Green, Westerfeld, Vennesland und Knox [34] sind der Meinung, dass aus je zwei Molekülen Brenztraubensäure ein Molekül Acetoin gebildet wird. Sie nehmen aber immerhin an, dass bei der Reaktion zuerst Acetaldehyd entsteht, der sich dann mit dem einen Brenztraubensäuremolekül zu einem Zwischenprodukt kondensiert, aus welchem erst durch Abspaltung eines neuen CO_2-Moleküls das Acetoin hervorgeht.

Werkman und Mitarb. [35, 36], haben sowohl aus Bakterien und Hefe als aus Herzmuskel des Schweins Enzympräparate hergestellt, die die Fähigkeit besassen, Acetaldehyd zu Acetoin zu verarbeiten. Werkman ist auch der Meinung, dass die Bildung der C_4-Verbindungen aus der Brenztraubensäure auf verschiedenen Wegen erfolgen kann.

Bång [37, 38] hat eine Theorie vorgelegt, welcher gemäss die Aromabakterien die Brenztraubensäure ohne zwischengeschaltete Acetaldehydphase direkt zu Diacetyl verarbeiten. Das primär entstandene Diacetyl entzieht dem Alkohol Wasserstoff und wird zu Acetoin und weiter zu 2,3-Butylenglykol reduziert.

Auch Martius [39] vermutet, dass das Diacetyl primär gebildet wird. Nach ihm entsteht aber aus der Brenztraubensäure durch Abspaltung von Wasserstoff und Kohlendioxyd zunächst ein ketonartiges Radikal ($CH_2 = CO$), welches unter anaeroben Bedingungen als Wasserstoffakzeptor fungiert und zu Acetaldehyd reduziert wird. Das Radikal kann sich ferner auch mit dem Acetaldehyd verbinden, wobei Diacetyl gebildet wird, welches Wasserstoff aufnimmt und zu Acetoin nebst 2,3-Butylenglykol reduziert wird. Bei der nach der Martiusschen Radikaltheorie erfolgenden Diacetylbildung wäre gemäss Suomalainen und Jännes [40] ein vom Thiamin gebildetes Oxydoreduktionssystem (vgl. Myrbäck, Vallin und Magnell [41]) tätig.

EXPERIMENTELLER TEIL

Das Bakterienmaterial und Ansetzen der Gärungen

Die Diacetyl- bzw. Acetoinbildung wurde an sowohl aus malzaromatischem als aus normalem Säureweckern isolierten Aromabakterien untersucht. Das erstere, das sog. Malzaromabakterium (Stamm M), unterscheidet sich von den normalen Aromabakterien des Säureweckers vornehmlich dadurch, dass es Carboxylase enthält und vermittels derselben aus Brenztraubensäure Acetal-

dehyd bildet (vgl. Virtanen und Nikkilä [30, 31]). Das aus normalem Säurewecker isolierte Bakterium brachte die Milch zum Koagulieren und dürfte in erster Linie bei den Aromabakterien vom Typ des *Streptococcus paracitrovorus* (Hammer) unterzubringen sein.

Zur Herstellung von Zellmasse für die Gärversuche wurden die Malzaromabakterien einen bis anderthalb Tage lang bei 37° C in einer Nährlösung von folgender Zusammensetzung gezüchtet:

5 l geklärte Molke (nach Karström [42])
13 g Pepton
90 » Presshefe
25 » Citronensäure

Die Züchtung des *Streptococcus paracitrovorus* erfolgte in der von Hammer [43] empfohlenen und nach Storgårds [32] bereiteten, Tomatenauszug enthaltenden Nährlösung bei 28° C.

Die entstandenen Säuren wurden während der Kultivierung durch Zusatz von 10 %iger NaOH-Lösung neutralisiert, wodurch sich die Reaktion der Nährlösung dauernd ungefähr bei ihrem ursprünglichen Wert (pH 6,2) hielt.

Die Bakterienzellen wurden durch Zentrifugieren von der Nährlösung abgeschieden und zweimal mit physiologischer Kochsalzlösung gewaschen.

Die so erhaltene Bakterienmasse wurde sodann entweder möglichst homogen in Wasser suspendiert oder bei Zimmertemperatur auf Tontellern getrocknet und trocken gemahlen, oder es wurde aus ihr nach Kalnitsky, Utter und Werkman [44] eine zellfreie Enzymlösung hergestellt.

Die Gärversuche erfolgten in $M/20$ Phosphatpufferlösungen. Während derselben wurde die Azidität der Gärlösungen durch Zusatz von 0,5 N NaOH-Lösung oder durch Anwendung von $CaCO_3$ als Neutralisationsmittel auf möglichst konstanter Höhe gehalten. Eine genügende Homogenität der Lösungen wurde durch mechanisches Schütteln oder mit Hilfe eines Mischers erzielt. Die aeroben Gärversuche erfolgten in mit Rückflusskühlern ausgerüsteten, etwa zur Hälfte gefüllten Erlenmeyerkolben zu 500 ml. Die anaeroben Versuchsbedingungen wurden durch Verjagen der Luft mittels Stickstoff aus den Gärkolben erzielt. Gärzeit, pH und sonstige Einzelheiten erhellen im Zusammenhang mit den einzelnen Versuchen. Am Ende der Versuche wurden die Gärungen in den verschiedenen parallelen Kolben gleichzeitig durch Erhitzen unter Rückflusskühlern abgebrochen.

Die Analysenmethoden

Der Gehalt der zu untersuchenden Gärlösungen an unvergorenem Zucker wurde im neutralisierten Schenck-Filtrat nach Bertrand ermittelt.

Die Citronensäure wurde nach Kometiani [45], die Brenztraubensäure nach Straub [46] bestimmt.

Die Milchsäure wurde gemäss der in diesem Laboratorium üblichen Methode im Schenck-Filtrat, aus welchem die Kohlehydrate nach Salkowsky und van Slyke besei-

tigt worden waren, nach dem Prinzip von Friedemann, Cotonio, Shaffer und Kendall[47] mit einem von Lieb und Zacherl[48] konstruierten Apparat bestimmt.

In demselben Apparat erfolgte auch die quantitative Bestimmung des Acetaldehyds so, dass letzterer in $Na_2S_2O_3$-Lösung überdestilliert und dann in dieser jodometrisch bestimmt wurde. Sein qualitativer Nachweis fand nach den Anweisungen von Rimini[49] statt. In diesem Zusammenhang wurde auch festgestellt, dass die von Block und Bolling[50] angewandte Methode zur Bestimmung von Acetaldehyd mittels *p*-Hydroxydiphenyl entgegen den Angaben der Autoren nicht völlig spezifisch ist, denn auch das Diacetyl reagiert in diesem Versuch ähnlich wie der Acetaldehyd.

Das Diacetyl wurde nach White, Krampitz und Werkman[51] photometrisch bestimmt. Acetoin und 2,3-Butylenglykol wurden nach Kniphorst und Kruisheer[52] mit $FeCl_3$ zu Diacetyl oxydiert und danach wie oben bestimmt.

Gärversuche mit lebenden Malzaromabakterien

Das pH-Optimum der Diacetyl- bzw. Acetoinbildung

Es ist schon lange bekannt gewesen, dass saure Reaktion die Acetoin- bzw. Diacetylbildung im Säurewecker begünstigt. Viele Forscher haben diese Tatsache auch im Laboratorium festgestellt. Da das pH-Optimum dieser Bildungsprozesse bei den verschiedenen Bakterien verschieden sein kann, war es für die Ausführung der folgenden Versuche wichtig, diese Umstände auch bei dem Malzaromabakterium kennen zu lernen.

Zu diesem Zweck wurden Gärungen mit der gleichen Bakteriensuspension in Glucose-Phosphatlösungen mit verschiedenem pH unter aeroben Bedingungen ausgeführt und die während 20stündiger Gärzeit gebildeten Mengen von C_4-Verbindungen (Diacetyl, Acetoin und 2,3-Butylenglykol) bestimmt. Fig. 1 zeigt die Resultate.

Man ersieht, dass das pH-Optimum der Bildung von C_4-Verbindungen beim Malzaromabakterium etwa bei pH 5,4 liegt.

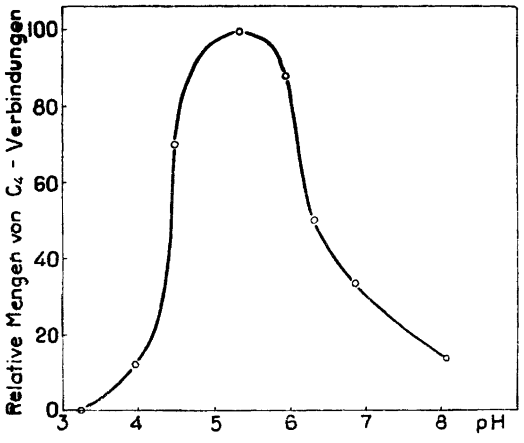

Fig. 1. pH-Abhängigkeit der Bildung der C_4-Verbindungen.

Brenztraubensäure, Brenztraubensäure + Acetaldehyd und Acetaldehyd als Substrat

In den nachfolgenden Versuchsreihen, deren Resultate in Tabelle 1 wiedergegeben sind, wurde die Bildung der C_4-Verbindungen in parallelen aeroben Gärversuchen untersucht, in denen entweder Brenztraubensäure allein (I), Brenztraubensäure und allmählich zugesetzter Acetaldehyd (II) oder Acetaldehyd allein (III) als Substrat dienten.

Tabelle 1. Aerober Gärversuch mit dem Malzaromabakterium und mit Brenztraubensäure (I), Brenztraubensäure + Acetaldehyd (II) und Acetaldehyd (III) als Substrat. Gärzeit 20 Stunden, Temperatur 40° C, pH 5,6.

			I	II	III
Brenztraubensäure	zugesetzt,	mg	500	500	—
Acetaldehyd (allmählich)	»	»	—	500	500
Brenztraubensäure	vergoren,	»	470,1	470,2	—
Acetaldehyd	gebildet,	mg	15,0	—	—
Diacetyl	»	»	1,21	0,74	0,74
Acetoin	»	»	5,68	16,12	3,43
2,3-Butylenglykol	»	»	1,82	2,21	0
C_4-Verbindungen zusammen	»	»	8,71	19,07	4,17

Wie aus den Versuchsresultaten zu ersehen ist, wird aus Brenztraubensäure durch Einwirkung des Malzaromabakteriums Acetaldehyd gebildet. Das genannte Bakterium enthält also Carboxylase. Der Acetaldehyd wird bei der Reaktion nicht vollständig verbraucht, sondern häuft sich in der Lösung an. In dieser Versuchsreihe wurden beim Abbruch des Versuches in der Gärlösung I etwa 3,2 % Acetaldehyd, auf die vergorene Brenztraubensäure bezogen, gefunden. Das Malzaromabakterium hat aus Brenztraubensäure Diacetyl, Acetoin und 2,3-Butylenglykol gebildet; davon ist die Menge des Acetoins am grössten. C_4-Verbindungen insgesamt wurden in der Gärlösung I zu 1,8 % der vergorenen Brenztraubensäure gebildet; in der Gärlösung II, die neben Brenztraubensäure zugesetzten Acetaldehyd enthielt, beläuft sich der entsprechende Wert auf 4,1 %, also auf mehr als den doppelten Betrag im Vergleich zur Gärlösung I. Der zugesetzte Acetaldehyd hat in der Gärlösung II eine Erhöhung der Acetoinmenge verursacht, die durch seine Einwirkung etwa auf das 3fache gestiegen ist. In bezug auf die Diacetyl- und 2,3-Butylenglykolmengen sind die Unterschiede zwischen den Gärlösungen I und II relativ gering.

Die Geschwindigkeit der Brenztraubensäuregärung scheint durch den Acetaldehydzusatz nicht beeinflusst worden zu sein, denn die Mengen der vergorenen Brenztraubensäure sind sich in beiden Versuchen (I und II) gleich.

In der Gärlösung III diente als ausschliessliches Substrat Acetaldehyd, der der Lösung im Verlauf des Versuches allmählich zugesetzt wurde. Das Malzaromabakterium ist, wie aus den Resultaten hervorgeht, imstande gewesen, den zugesetzten Acetaldehyd ohne Gegenwart von jeglichen anderen Substraten zu C_4-Verbindungen zu synthetisieren.

Die Resultate der obigen Versuchsreihe berechtigen uns zu dem Schluss, dass die Bildung der C_4-Verbindungen aus Brenztraubensäure beim Malzaromabakterium über Acetaldehyd erfolgt. Der Befund, dass der zugesetzte Acetaldehyd namentlich gerade zu einer Erhöhung der Acetoinmenge geführt hat (II), deutet auf die primäre Bildung des letzteren hin.

Die Abfangung des Acetaldehyds während der Gärung

Neuberg hat gezeigt, dass man bei Hefegärungen den naszierenden Acetaldehyd durch geeignete Abfangmittel fixieren und dadurch den Verlauf der Reaktionen beeinflussen kann. Sofern die oben geäusserte Annahme bezüglich des Auftretens von Acetaldehyd als Zwischenprodukt bei der Bildung der C_4-Verbindungen durch das Malzaromabakterium richtig ist, müsste es theoretisch möglich sein, auf jene Bildungsreaktionen durch Abfangen des Acetaldehyds bei den betreffenden Gärungen einzuwirken.

Tabelle 2 zeigt die Resultate eines mit dem Malzaromabakterium ausgeführten Versuches, in welchem Citronensäure als Substrat und (in der Gärlösung II) $CaSO_3$ als Abfangmittel für Acetaldehyd diente. Als Neutralisationsmittel kam $CaCO_3$ zur Anwendung. Die Homogenität der Lösungen wurde während des Versuches durch mechanische Mischung aufrechterhalten.

Tabelle 2. Aerober Gärversuch mit dem Malzaromabakterium, Citronensäure als Substrat. Zur Fixierung des bei der Gärung gebildeten Acetaldehyds wurde der Gärlösung II $CaSO_3$ zugesetzt. Gärzeit 48 Stunden, Temperatur 40° C, pH 6,0.

			I	II
Citronensäure (neutr.)	zugesetzt,	mg	6000	6000
$CaSO_3$ als Abfangmittel	»	g	—	4
Citronensäure	vergoren,	mg	4575,0	4269,0
Acetaldehyd	gebildet,	mg	7,6	39,9
Diacetyl	»	»	13,7	12,9
Acetoin	»	»	178,8	66,7
2,3-Butylenklykol	»	»	333,0	180,0
C_4-Verbindungen zusammen	»	»	525,6	259,6

Aus den Resultaten ist zu ersehen, dass die Fixierung des naszierenden Acetaldehyds einen sehr deutlichen Einfluss auf die Mengen der gebildeten C_4-Verbindungen sowie die des unter den Endprodukten gefundenen Acetaldehyds gehabt hat. In der Gärlösung ohne $CaSO_3$ wurden 0,17 % und in der mit $CaSO_3$ 0,93 % vom vergorenen Substrat Acetaldehyd gefunden. Für die C_4-Verbindungen ergeben sich als entsprechende Werte 11,5 % und 6,1 %. Diese Ergebnisse erweisen deutlich, dass beim Malzaromabakterium der Abbau der Citronensäure über Brenztraubensäure und Acetaldehyd zu den C_4-Verbindungen geht.

Acetoin als primäres Kondensationsprodukt

Die oben mitgeteilten Versuchsresultate deuten darauf hin, dass das Acetoin vor dem Diacetyl und dem 2,3-Butylenglykol gebildet wird. Zur Bestätigung dieser Annahme wurden in der folgenden Versuchsreihe die aus der Brenztraubensäure gebildeten C_4-Verbindungen in kurzen Zeitabständen bestimmt. Gleichzeitig wurden die Veränderungen des Redoxpotentials elektrometrisch verfolgt. Tabelle 4 zeigt die Ergebnisse; die Veränderungen des Redoxpotentials während der Gärungen sind in Fig. 2 dargestellt.

Tabelle 4. Gärversuch mit Malzaromabakteriensuspension, Brenztraubensäure als Substrat. Bestimmungen der Brenztraubensäure und der C_4-Verbindungen erfolgten 2, 5, 12 und 25 Stunden nach Beginn des Versuches. Temperatur 40° C, pH 5,5.

		Aerob	Anaerob
Nach 2stündiger Gärung:			
Brenztraubensäure	vergoren, mg	77,7	50,5
Diacetyl	gebildet, »	0	0
Acetoin	» »	10,73	2,40
2,3-Butylenglykol	» »	0	0
C_4-Verbindungen zusammen	» »	10,73	2,40
Nach 5stündiger Gärung:			
Brenztraubensäure	vergoren, mg	118,6	104,6
Diacetyl	gebildet, »	1,79	0,60
Acetoin	» »	11,92	4,21
2,3-Butylenglykol	» »	0,60	1,80
C_4-Verbindungen zusammen	» »	14,31	6,61
Nach 12stündiger Gärung:			
Brenztraubensäure	vergoren, mg	178,2	176,7
Diacetyl	gebildet, »	1,79	1,21
Acetoin	» »	11,32	5,71
2,3-Butylenglykol	» »	2,98	3,31
C_4-Verbindungen zusammen	» »	16,09	10,22
Nach 25stündiger Gärung:			
Brenztraubensäure	vergoren, mg	274,6	327,0
Diacetyl	gebildet, »	1,79	1,20
Acetoin	» »	6,56	3,55
2,3-Butylenglykol	» »	16,39	9,44
C_4-Verbindungen zusammen	» »	24,74	14,19
Milchsäure	» »	38,46	75,69

Man ersieht deutlich, dass die Bildung der C_4-Verbindungen durch aerobe Bedingungen stark begünstigt wird. Beim Abbruch des Versuches (nach 25 Stunden) wurden in der aeroben Gärlösung insgesamt 24,74 mg C_4-Verbindungen gefunden, was 9,0 % der vergorenen Brenztraubensäure ausmacht, während die entsprechenden Werte in der anaeroben Gärlösung nur 14,19 mg und 4,4 % betragen.

Das Redoxpotential der aeroben Gärlösung (Kurve I in Fig. 2) hat sich während der

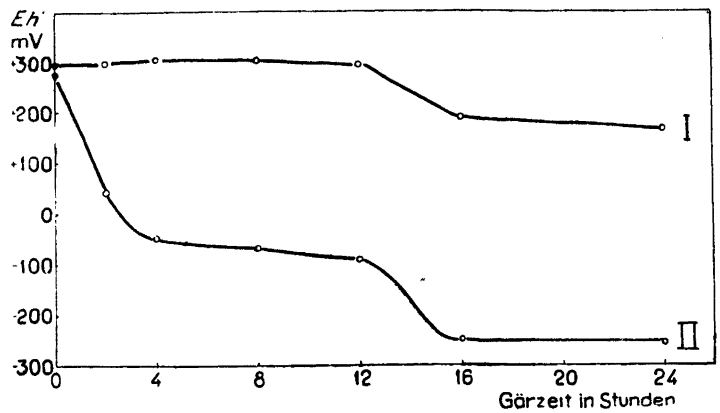

Fig. 2. Redoxpotentialkurven der in Tabelle 4 dargestelten Gärversuche. I Aerob. II Anaerob.

12 ersten Stunden der Gärung auf der ursprünglichen Höhe (etwa bei + 300 mV) gehalten. Danach ist ein ziemlich schroffer Fall um etwa 100—130 mV eingetreten.

Bei der anaeroben Gärlösung zeigt das Redoxpotential (Kurve II in Fig. 2) während der ersten 4 Stunden einen Fall um etwa 350 mV. Danach wird die Kurve flacher. Nach 12 Stunden setzt ein neuer schroffer Fall um etwa 150 mV ein. Beim Abbruch des Versuches (nach 25 Stunden) belief sich die Potentialdifferenz zwischen der aeroben und der anaeroben Gärlösung auf etwa 400 mV.

Von C_4-Verbindungen wurde nach zweistündiger Gärung sowohl in der aeroben als in der anaeroben Gärlösung nur Acetoin gefunden. Dieser Befund spricht stark zugunsten der Auffassung, dass bei diesen Reaktionen das Acetoin vor dem Diacetyl und dem 2,3-Butylenglykol entsteht. Im weiteren Verlauf der Gärung hat sich die Menge des Acetoins in beiden Gärlösungen vermehrt, bis nach 12 Stunden ein bedeutender Rückgang zu verzeichnen ist. Letzteres in Verbindung mit einer gleichzeitigen bedeutenden Zunahme des 2,3-Butylenglykols und der Potentialfall der Gärlösungen nach der 12. Stunde erweisen, dass sowohl unter aeroben als anaeroben Bedingungen eine Reduktion von Acetoin zu 2,3-Butylenglykol stattgefunden hat.

Diacetyl wurde nach 5 Stunden in beiden Gärlösungen, jedoch bedeutend weniger als Acetoin gefunden. Danach ist seine Menge in der aeroben Gärlösung bis zum Abbruch des Versuches unverändert geblieben, in der anaeroben ist sie dagegen bis zur 12. Gärstunde, dann aber nicht mehr gestiegen.

Die Resultate dieser Versuchsreihe erweisen deutlich, dass von den C_4-Verbindungen das Acetoin primär gebildet wird, wonach es weiter zu Diacetyl oxydiert und zu 2,3-Butylenglykol reduziert werden kann.

Gäversuche mit getrockneten Malzaromabakterien

Die getrocknete Bakterienmasse hat sich in diesen Versuchen nicht als fähig erwiesen, Glucose zu vergären, wohl aber wurden Brenztraubensäure und Acetaldehyd angegriffen. Tabelle 3 enthält die Resultate eines Versuches, bei dem Acetaldehyd als Substrat diente.

Tabelle 3. Aerober Gärversuch mit getrockneter Bakterienmasse bei Gegenwart von Toluol und mit Acetaldehyd als Substrat. Gärzeit 84 Stunden, Temperatur 40° C, pH 5,2.

Acetaldehyd	zugesetzt, mg	150
Diacetyl	gebildet, »	0,16
Acetoin	» »	0,54
2,3-Butylenglykol	» »	1,76
C_4-Verbindungen zusammen	» »	2,46

Die Werte lassen also erkennen, dass die getrockneten Malzaromabakterien ein Enzym oder ein Enzymsystem enthalten, das bei Gegenwart von Toluol aus Acetaldehyd C_4-Verbindungen zu synthetisieren vermag. Die Resultate des Versuches bestärken also die Auffassung, dass der Acetaldehyd als Zwischenprodukt bei der Bildung der C_4-Verbindungen durch das in Frage stehende Bakterium auftritt.

Gärversuche mit zellfreier Enzymlösung aus Malzaromabakterien

Die Oxydation des Acetoins zu Diacetyl und Reduktion zu 2,3-Butylenglykol

Folgender, in Tabelle 5 dargestellter Versuch wurde mit zellreier Enzymlösung[44] in einer Phosphatpufferlösung ausgeführt, die als Substrat Acetoin + Diacetyl enthielt.

Tabelle 5. Aerober Gärversuch mit zellfreier Enzymlösung des Malzaromabakteriums (I) nebst Kontrolle (II). Substrate: Acetoin + Diacetyl. Gärzeit 20 Stunden, Temperatur 40° C, pH 5,6.

		I	II
Acetoin	zugesetzt, mg	40,8	40,8
Diacetyl	» »	4,5	4,5
Enzymlösung	» ml	7,5	—
Während des Versuches:			
Diacetyl	vermehrt, mg	1,15	0,05
Acetoin	vermindert »	4,70	0,90
2,3-Butylenglykol	gebildet »	0,44	0

Die zellfreie Enzymlösung des Malzaromabakteriums hat in diesem Versuch Acetoin zu Diacetyl oxydiert und zu 2,3-Butylenglykol reduziert.

Die Abnahme des Acetoins während des Versuches hat jedoch die zusammengerechneten Mengen von Diacetyl und 2,3-Butylenglykol übertroffen. Das Acetoin ist demnach in der Gärlösung auch noch von andersartigen Veränderungen betroffen worden, mög-

licherweise ist es über Diacetyl durch Einwirkung der Diacetylmutase (vgl. Green, Stumpf und Zarudnaya [53]) zu Essigsäure oxydiert worden.

Während des Versuches ist durch Einwirkung des Luftsauerstoffs in der Kontrollgärlösung (II) eine kleine Menge Acetoin zu Diacetyl (0,05 mg) oxydiert worden.

Gärversuche mit *Streptococcus paracitrovorus*

Die nachstehende Versuchsreihe wurde mit einer Bakteriensuspension von *Streptococcus paracitrovorus* unter aeroben Bedingungen mit Glucose (I) und Glucose + Acetaldehyd (II) als Substrat durchgeführt. Tab. 6 zeigt die Ergebnisse.

Tabelle 6. Aerober Gärversuch mit Bakteriensuspension von Streptococcus paracitrovorus. Substrate: Glucose (I) und Glucose + Acetaldehyd (II). Gärzeit 20 Stunden, Temperatur 28° C, pH 5,5.

			I	II
Glucose	zugesetzt,	mg	3000	3000
Acetaldehyd (allmählich)	»	»	—	302
Glucose	vergoren	»	1005,0	738,0
Diacetyl	gebildet,	mg	2,76	0,55
Acetoin	»	»	7,31	9,25
2,3-Butylenglykol	»	»	3,48	4,00
C_4-Verbindungen zusammen	»	»	13,55	13,80

In der Gärlösung mit Glucose als ausschliessliches Substrat (I) wurden an C_4-Verbindungen 13,55 mg oder 1,4 % der vergorenen Glucose gebildet. In der Gärlösung, die ausserdem Acetaldehyd zugesetzt enthielt (II), betrugen die entsprechenden Mengen 13,80 mg oder 1,9 %. Der Acetaldehydzusatz hat also die Acetoinbildung aus der Glucose gefördert.

In Tabelle 7 sind die Resultate einer Versuchsreihe zusammengefasst, in welcher Brenztraubensäure (I), Brenztraubensäure + Acetaldehyd (II) und Acetaldehyd (III) als Substrat dienten. Der Acetaldehyd wurde den Gärlösungen während des Versuches allmählich zugesetzt.

Tabelle 7. Aerober Gärversuch mit Bakteriensuspension von Streptococcus paracitrovorus. Substrate: Brenztraubensäure (I), Brenztraubensäure + Acetaldehyd (II) und Acetaldehyd (III). Gärzeit 20 Stunden, Temperatur 28° C, pH 5,6.

			I	II	III
Brenztraubensäure	zugesetzt,	mg	500	500	—
Acetaldehyd (allmählich)	»	»	—	578	578
Brenztraubensäure	vergoren,	»	238,0	264,4	—
Diacetyl	gebildet	mg	2,39	0,58	0
Acetoin	»	»	4,78	17,56	0
2,3-Butylenglykol	»	»	2,39	2,89	0
C_4-Verbindungen zusammen	»	»	9,56	21,03	0

Wie man aus den angeführten Werten ersieht, sind aus der Brenztraubensäure durch die Einwirkung von *Streptococcus paracitrovorus* C_4-Verbindungen entstanden. Acetaldehydbildung hat sich in den Gärlösungen dieses Bakteriums nicht nachweisen lassen. Dagegen hat der der gärenden Brenztraubensäure zugesetzte Acetaldehyd die Bildung der C_4-Verbindungen aus der Brenztraubensäure bedeutend stimuliert. Die Gesamtmenge der aus Brenztraubensäure als alleiniges Substrat hervorgegangenen C_4-Verbindungen entspricht nämlich 4,0 % der vergorenen Brenztraubensäure. In der Gärlösung II, in welcher der Brenztraubensäure noch Acetaldehyd zugesetzt wurde, beträgt dieser Prozentwert dagegen 7,9. Die Acetoinbildung aus der Brenztraubensäure ist also durch den Acetaldehydzusatz ganz erheblich gefördert worden. In der Gärlösung, in welcher lediglich Acetaldehyd als Substrat diente (III), liessen sich C_4-Verbindungen überhaupt nicht nachweisen.

BESPRECHUNG DER ERGEBNISSE

Man ist sich im allgemeinen darüber einig, dass bei den biologischen Bildungsreaktionen von Diacetyl, Acetoin (Acetylmethylcarbinol) und 2,3-Butylenglykol Brenztraubensäure als Zwischenprodukt auftritt. Die Meinungsverschiedenheiten betreffen in erster Linie die Frage, welche von den genannten C_4-Verbindungen primär entsteht, um dann über Oxydations- und Reduktionsvorgänge in die anderen überzugehen, und auf welchen Wegen sich diese Reaktionen abspielen.

Neuberg betrachtet das Acetoin als primäres Kondensationsprodukt, an dessen Bildung bei der Hefe zwei verschiedene Enzyme beteiligt sind. Aus der Brenztraubensäure wird zunächst durch Einwirkung der Carboxylase Acetaldehyd gebildet, dessen Kondensation zu Acetoin dann von einem zweiten Enzym, der Carboligase, geleitet wird.

Das Vorhandensein der Carboligase bei diesen Reaktionen ist insbesondere von Dirscherl und Langenbeck bezweifelt worden. Ihnen gemäss würden diese Vorgänge allein schon durch die Anwesenheit der Carboxylase möglich gemacht.

Nach den Untersuchungen von Storgårds enthalten die Aromabakterien keine Carboxylase, und nach Virtanen wird in diesem Fall aus zwei Brenztraubensäuremolekülen ohne Acetaldehyd als Zwischenprodukt ein Molekül Acetoin gebildet. Dieselbe Reaktion haben auch Silverman und Werkman in zellfreier Enzymlösung von *Aerobacter aerogenes* festgestellt.

Martius ist der Ansicht, dass aus der Brenztraubensäure durch Abspaltung von Wasserstoff und Kohlendioxyd ein ketonartiges Radikal entsteht, welches sich mit dem Acetaldehyd zu Diacetyl verbindet.

In der vorliegenden Arbeit ist die Bildung von Acetoin bzw. Diacetyl sowohl bei einem Bakterium (bei dem sog. Malzaromabakterium), das mit Hilfe von Carboxylase Brenztraubensäure zu Acetaldehyd verarbeitet, als bei

einem Bakterium *(Streptococcus paracitrovorus)*, dem das genannte Enzym fehlt, untersucht worden.

Sowohl in den aeroben als in den anaeroben Gärlösungen beider Bakterien wurde im allgemeinen mehr Acetoin als Diacetyl und 2,3-Butylenglykol gebildet. Bei sehr kurzen Gärzeiten konnte durchgehends nur Acetoin festgestellt werden, während sich Diacetyl und 2,3-Butylenglykol erst im weiteren Verlauf der Gärung eingefunden haben (Tabelle 4). Diese Befunde sprechen anscheinend ausserordentlich stark dafür, dass das Acetoin bei bakteriellen Gärungen vor dem Diacetyl und dem 2,3-Butylenglykol gebildet wird.

Das Acetoin wird leicht auf rein chemischem Wege zu Diacetyl oxydiert und zu 2,3-Butylenglykol reduziert; in Gärlösungen werden jene Veränderungen indessen von Enzymen geleitet (Tabelle 5).

Beide in der vorliegenden Arbeit untersuchten Bakterien vermögen Glucose und Brenztraubensäure bei der Bildung von Acetoin zu verwerten. Aus Citronensäure bildet das Malzaromabakterium bedeutend mehr Acetoin als aus Glucose, bei *Streptococcus paracitrovorus* dagegen ist die Acetoinbildung aus Citronensäure fraglich (vgl. Nikkilä [54]). Die beiden Bakterien unterscheiden sich voneinander auch darin, dass das Malzaromabakterium, das also Carboxylase enthält, aus dem zugesetzten Acetaldehyd Acetoin zu synthetisieren vermag, während *Streptococcus paracitrovorus* dieses Vermögen abgeht (Tabellen 1, 3 und 7). Das Auftreten von Acetaldehyd als Zwischenprodukt bei der Acetoinbildung ist somit von der Bakterienart abhängig. Ein Zusatz von Acetaldehyd zu den mit Glucose oder Brenztraubensäure beschickten Gärlösungen der beiden Bakterien hat doch in hohem Masse fördernd auf die Acetoinbildung eingewirkt (Tabellen 1, 6 und 7).

Das Malzaromabakterium ist demnach mit einem Enzym ausgerüstet, welches den Zusammenschluss von zwei Acetaldehydmolekülen zu Acetoin katalysiert. Ob bei dieser Reaktion die Carboxylase allein oder neben ihr auch die von Neuberg genannte Carboligase wirkt, kann nicht mit Sicherheit entschieden werden.

Das in *Streptococcus paracitrovorus* enthaltene, von der Carboxylase abweichende Enzym (Virtanen) vermag Brenztraubensäure, aber nicht Acetaldehyd allein zur Bildung von Acetoin zu verwerten. Die fördernde Einwirkung von Zusatz des Acetaldehyds auf die Acetoinbildung aus Brenztraubensäure beruht möglicherweise darauf, dass ein Acetaldehydmolekül an die Stelle eines Brenztraubensäuremoleküls treten kann.

ZUSAMMENFASSUNG

Es wurde die Bildung von Diacetyl, Acetoin (Acetylmethylcarbinol) und 2,3-Butylenglykol sowohl bei einem Bakterium (dem sog. Malzaromabakterium), das mittels der Carboxylase aus Brenztraubensäure Acetaldehyd bildet, als bei einem Bakterium *(Streptococcus paracitrovorus)*, dem das genannte Enzym fehlt, untersucht. Das erstgenannte Bakterium wurde bei den Gärversuchen ausser als Suspension auch als Trockenpräparat und zellfreie Enzymlösung, *Streptococcus paracitrovorus* jedoch nur als Suspension verwendet.

Es wurde gezeigt, dass das Acetoin bei bakteriellen Gärungen vor dem Diacetyl und dem 2,3-Butylenglykol entsteht (Tabelle 4). Das primär gebildete Acetoin kann sodann zu Diacetyl oxydiert oder zu 2,3-Butylenglykol reduziert werden (Tabelle 5).

Zusatz von Acetaldehyd zu den Gärlösungen mit Glucose und Brenztraubensäure hat die bakterielle Acetoinbildung bedeutend gefördert (Tabellen 1, 6 und 7; vgl. auch Nikkilä [54]).

Es konnte ferner gezeigt werden, dass Acetaldehyd als Zwischenprodukt bei den Bildungsreaktionen des Acetoins bei dem Carboxylase enthaltenden Malzaromabakterium, nicht aber bei dem jenes Enzyms entbehrenden *Streptococcus paracitrovorus* auftritt.

Auch hat sich ergeben, dass bei bakteriellen Gärungen ohne Ca-Ion keine Bildung von C_4-Verbindungen stattfindet.

Zum Schluss spreche ich dem Vorstand dieses Laboratoriums, Herrn Professor Dr. A. I. Virtanen, der mir bei meiner Arbeit mit wertvollen Ratschlägen und Anleitungen beigestanden hat, meinen besten Dank aus.

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Dextran and its Use as a Plasmasubstitute *

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As is well known, we possess in blood and plasma therapeutic agents for the treatment of shock, *e. g.* after severe losses of blood, contusions and extensive burns. The use of blood and plasma for the purpose of infusion is, however, accompanied by certain disadvantages. In order to obtain sufficient blood and plasma there is needed, among other things, a large and complicated organization with Blood Donation Centres. It is troublesome to keep blood or to separate out plasma. The cost of blood and plasma is therefore high. In the event of war or other catastrophies it is quite impossible to meet the demand for blood and plasma. Blood and plasma are very delicate means for infusion; they do not bear sterilization by heating. Therefore there always exists a certain risk that one will transport certain infectious diseases with the infusion. Both blood- and plasma-infusions, at times, give rise to undesirable reactions. With blood-infusions one must pay attention to the patient's bloodgroup.

It is therefore natural that physiologists and chemists have tried to find solutions which would be able to replace the expensive and delicate blood or plasma for transfusions.

Even during the First World War Bayliss tried to use solutions of gum-arabic for the purpose of infusion. Later, yet other substances such as gelatin, polyvinylalcohol, pectin, polyvinylpyrrolidon etc. have been tested for this purpose. The infusion of these colloids has however been accompanied by certain inconveniances. Some of the substances tested have given undesirable reactions, some have antigenic properties, and some could not be broken down by the organism, hence they are accumulated, especially in the liver.

Before I pass on to give an account of the attempts of Grönwall and myself to obtain a plasmasubstitute from dextran [1—5], I think it is necessary for

* Read at the sixth Scandinavian Meeting of Chemists in Lund, August 29th, 1947.

me to state some of the conditions a colloid should fulfill, in order that it should be applicable as a plasmasubstitute.

For shock after not only bleeding but also contusions and burns, it is necessary by means of infusion of liquids to increase the volume of the circulating blood, so that the blood pressure is kept up to the normal value. This can not be done in a satisfactory way with crystalloid solutions, since substances of low molecular weight quickly leave the bloodstream. There is required instead infusion solutions containing colloids, which produce the same colloid-osmotic-pressure as the plasma proteins. An assumption, that the colloids should produce this pressure and keep the water in the blood vessels, is that they should have such a large molecular size that they do not pass out through the walls of the blood vessels but remain in the blood. The molecular weight of the colloid should consequently not be too small. The molecular weight however should not be too large since the colloid-osmotic-pressure then becomes too small. The molecular weight should be of the order of magnitude of 100,000. It is not necessary for the substance to bee monodisperse. However, it can be thought to be desirable that the distribution of the molecular weight should have a convenient appearance.

The colloid must be able to be injected intravenously in large quantities and on repeated occasions without being accompanied by any undesirable reactions. It must consequently be quite untoxic and not have antigenic properties. It should of course be able to be given to all, independent of blood-group and such like.

The solution must not be too viscous. Preferably the viscosity should be of the same order of magnitude as for plasma.

The substance should be of such a nature that the body is able to get rid of it at a suitable speed, so that it does not remain in the blood too long or accumulate in the organs.

The colloid solution should be able to be sterilized by means of heating at 120° C so that one can be absolutely sure that neither bacteria nor virus infection is transferred. The sterilized solutions should be very stable so that the flasks containing the prepared solutions can be kept for a long time at room-temperature without special precautions.

Finally the substance should be able to be prepared on a large scale and the price should be lower than that for dried plasma.

It is not so difficult to find colloids which only fulfill some of the conditions set forth. But if one shall find a colloid which fulfills all the conditions set forth at the same time, one must surely have a little luck.

Fig. 1. Electron-microscope photograph of dextran molecules. 30,000 ×.

It has now appeared that by means of partial hydrolysis of the polysaccharide dextran we can prepare a substance which fulfills the requirements indicated above for a plasma substitute.

Dextran was discovered during the last century by the German sugar-chemist Scheibler. He found that in sugar-refining there sometimes appeared a mucous substance which caused a great deal of trouble, among other things that thereby the sugar juices filtered slowly. Already during the last century it was shown that dextran is a polysaccharide which on hydrolysis gives glucose. It was also shown that dextran is formed in sugar juices which are infected with the bacterium *Leuconostoc mesenteroides*.

It was also during work with sugar juice that we came into contact with dextran for the first time. Since 1941 in Tiselius' laboratories we have carried out researches on the colloids of sugar beet in close collaboration with the Swedish sugar-industry. During these studies we happened to get a sugar beet juice infected with *Leuconostoc mesenteroides*. We obtained some dextran, on which we carried out some researches. Among other things we showed that the molecular weight of dextran was unusually high, of the order of magnitude of several millions. An exact value could not be given however, since the

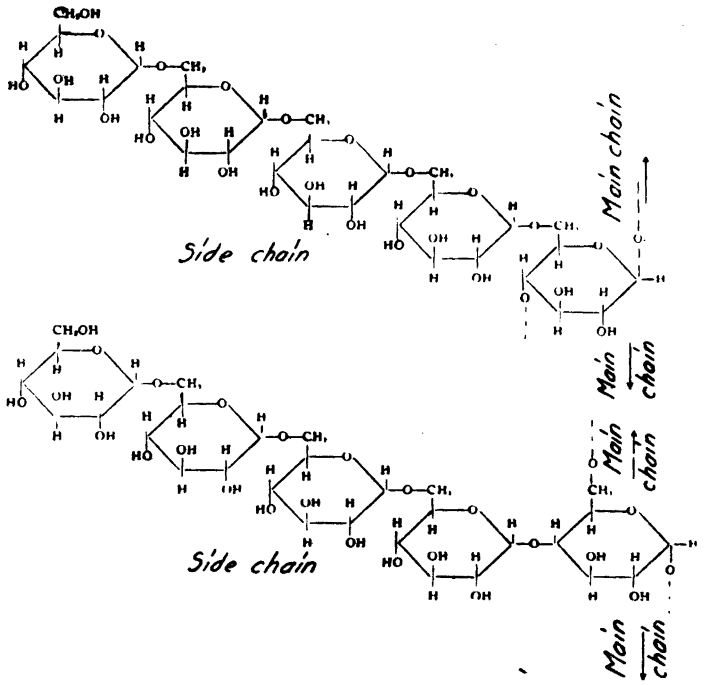

Fig. 2. Formula of dextran.

dextran did not have a single molecular weight. Later however, in collaboration with Halling and Kinell the dextran has been fractionated into more distinct fractions for which molecular weight determinations etc. are being carried out [6]. Quite early we could show that dextran has a long molecule. This appears even from electron microscope pictures of dextran molecules which I took together with Kai Siegbahn during 1943 [7,8] (Fig. 1). Magnification of the image is 30,000 ×. The thickness of the threads at the narrowest place is 30—100 Å units. 50 Å units is equivalent to about 10 glucose units. Here and there one can see knots on the threads at regular distances.

A molecular breadth of about 50 Å units can be explained with the formula for dextran which Levi, Hawkins and Hibbert suggested on the basis of methylation experiments [9] (Fig. 2).

They consider that dextran consists of long main chains with short side chains. Mainly the bindings are 1—6 but 1—4 bindings also occur. What in the electron microscope is visible as a thread should consequently be a main chain with short side chains.

Dextran is not broken down by the usual amylases.

Among the foreign works on dextran which are carried out during later years, the researches on the enzymatic synthesis of dextran from saccharose are quite the most interesting. Hehre could show that one could obtain from cultures of *Leuconostoc mesenteroides* a bacteria-free filtrate which contained an enzyme which could convert saccharose to dextran according to the formula:

$$n \text{ saccharose} = \underset{\text{dextran}}{(\text{glucose} - H_2O)_n} + n \text{ fructose}$$

Hehre has shown that, in distinction from the synthesis of starch, glucose-1-phosphate is not an intermediate product here [10].

After this very short survey of the chemistry of dextran we return to the problem of producing a plasma substitute from dextran. This work was started with Grönwall in January 1943 at the Institutes of Biochemistry and Physical Chemistry in Uppsala.

It was very quickly shown by experiments with animals that the viscous aqueous solutions which are obtained from the natural dextran could not be injected intravenously. But when the dextran was partially hydrolyzed to a molecular weight of the same order of magnitude as that of the proteins of blood, we could inject the test animals with large quantities without any injurious results. The hydrolysis of dextran is carried out by the aid of hydrochloric acid and is stopped when a product of suitable molecular weight has been obtained. The dextran can be precipitated from the water solution by the addition of alcohol. It must of course be purified very carefully. The solution must be so pure that one can, for example, after severe burns, when large losses of plasma take place, inject up to ten liters intravenously during some days into one and the same patient.

The viscosity and the colloid-osmotic-pressure for the 6 % solutions used are of the same order of magnitude as for plasma. Besides the partially hydrolyzed dextran the solutions also contain 0.9 % of sodium chloride. The solutions can be heated at 120° C and the sterilized solutions are very stable.

If a normal infusion-dose of dextran be injected intravenously, for example, into a dog or a man, the concentration of dextran in the blood sinks to 0 in about 5 days. The time that the dextran stays in the blood seems to be ideal. A small part of the dextran, namely the fraction which has the smallest molecular size, is filtered out through the kidneys into the urine. Even after repeated large infusions no accumulations in the organs can be detected. Animals had for a long time been injected with large quantities of dextran. Some time after the last injection the animals were killed and examined histologically by Gellerstedt, without any sign of injury or accumulation

being observable. We were neither able to detect any dextran by means of polarimetric determinations nor by means of Hint and Thorsén's recently produced dextran determination method, which admit the detection of small quantities of dextran even in the presence of glycogen. Since the quantities of the dextran injected had been very great, one must form the conclusion that dextran is slowly broken down in the body. A support for this presumption is also given by some hormone investigations which I have performed. If one gives dextran-injected rabbits thyroid gland preparation, the dextran disappears out of the blood more quickly than normally. The most probable interpretation of this result is that when the metabolism is increased by means of the thyroid gland hormone, then the dextran also breaks down more quickly [11].

After dextran infusion the sedimentation-rate of the erythrocytes is increased. The original dextran produces a greater sedimentation than the partially hydrolyzed dextran. The sedimentation produced is less and less the more one breaks down the dextran. Thorsén and Hint have confirmed this by accurate tests with dextran fractions of different molecular size. This proved that the change of the sedimentation-rate of the erythrocytes was primarily caused by the largest dextran molecules in the therapeutically used dextran solution.

The therapeutic effect was studied by our animal tests for shocks caused by bleeding, histamine and contusion which we produced in rabbits and cats in narcosis. A good effect on blood pressure, heart activity and breathing could always be recorded (Fig. 3). The figure shows a blood pressure curve from such a test with a rabbit. Along the y-axis is the animal's blood pressure, the x-axis is a time-axis. Before the test the rabbit's blood pressure was 100 mm Hg. In deep narcosis the animal's hind legs were crushed. (Repeated crushings at I, II and III.) The blood pressure dropped by this means to about 30 mm and stayed at this value. After some minutes 20 ml of dextran solution were injected intravenously (at IV) after which the blood pressure quickly rose to the normal value (V).

Since the animal tests passed over favourably, we dared to begin clinical tests firstly on a very limited scale. Since these tests gave promising results, an extensive clinical test was begun under Bohmansson's guidance. In these tests, Thorsén and Wilander and, as burn-specialist, Rosenqvist took part [12]. Besides the work already published Thorsén is now compiling extensive clinical material.

It is obvious that it was necessary that the clinical tests should be carried out with the greatest care and therefore the tests took a large time. Now, however, about 2,000 l of dextran solution have been injected into about a thousand patients, hence the conclusions which were drawn from the tests could be regarded as well founded.

Fig. 3. Blood pressure curve from a test with a rabbit.

Since this is a meeting of chemists and not a congress of physicians and since the time is limited I cannot now, unfortunately, give an account of all the interesting details in these clinical tests. I shall restrict myself to say that the result of shock-treatment by dextran is good and that when required one can inject several liters of dextran solution without injurious secondary reactions. The dextran concentration in plasma can rise to several per cent, in which case the concentration of protein can be appreciably lower than normal. The concentration of the colloids seems to adjust itself so that the colloid-osmotic pressure of the blood becomes normal. A part of the blood-protein can consequently be temporarily replaced by dextran without inconvenience. Afterwards plasma protein is newly formed at the same time as the dextran disappears.

In his thesis on the treatment of extensive burns Rosenqvist judges the effect of dextran as of the same value as that of plasma [13]. At the Scandinavian Surgical Congress in June 1947 the clinical results were presented by Thorsén [14] and Bohmansson [15], after which dextran could be released for commerce.

Thorsén maintained that the shock prophylaxis which is often used in operations is not fully satisfactory. After operations latent shock is present in so many cases that colloidal and not crystalloidal solutions should be infused. This implies that dextran can be calculated to get a use not only for accidents and extensive burns or operations where shock has already entered but also in the daily routine of surgical departments to prevent shock in operations.

Bohmansson summed up the result of the clinical tests with these words: »Dextran is a safe and very good plasma substitute for the treatment of shock and for the prevention of shock.»

Raw dextran is now prepared by *Svenska Sockerfabriks Aktiebolaget* and hydrolyzation, purification and preparation of the infusion solutions are carried out by *A.B. Pharmacia* in Stockholm.

Finally I should also desire to state that it would not have been possible to carry through this work without the fine collaboration between the scientific institutions in Uppsala, the industries concerned — *i. e. A.B. Pharmacia* and *Sockerbolaget* — and the doctors who performed the clinical tests.

SUMMARY

A brief survey is given of some of the conditions which a colloid should fulfill, in order that it shuld be applicable as a plasma substitute. Some properties of the polysaccharide dextran are described. By means of partial hydrolysis of dextran it is possible to prepare a substance which fulfills the requirements indicated for a plasma substitute.

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Serum Protein Fractionation According to Henriques and Klausen's Method and with Methanol

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According to Sørensen's investigations [1], pure protein solutions may be considered systems of reversibly connected components in which the single dissociable factors are in a state of mutual equilibrium. In biological fluids, such as, for example, serum, several of these systems of components must be supposed to be active (Geill [2]) and to form *inter se* more or less firmly united complexes, the state of equilibrium of which may be displaced in case of changes of the composition of the solvent. Owing to this overlapping it is therefore possible, when a single protein is precipitated from such a solution, partly that only a small portion of the protein in question is precipitated, partly that the solution still contains small quantities of the substance united with the other components contained in the system and also that the precipitate contains small quantities of these components.

Under practical conditions this proves to be so. On comparison with electrophoresis Tiselius [3] thus found that after a 55 % saturation of serum with ammonium sulphate the filtrate still contained about 25 % of the globulin fraction. Similar observations have been made by Svensson [4] and by Cohn and collaborators [5] who, after precipitation in horse serum with a 2.05 molar solution of ammonium sulphate, found that the filtrate contained small quantities of globulin. After precipitation with 22.5 % sodium sulphate solution the filtrate contained about one-fourth of the beta-globulin and three-fourths of the alpha-globulin (Guttman and collaborators [6]). According to Taylor and Keys [7] the sodium sulphate precipitation gave 5.2± 3 % more nitrogen in the albumin fraction than stated by the electrophoresis values. On a direct comparison between the methods of precipitation of Howe [8] and of Henriques and Klausen [9] small but unquestionable differences were

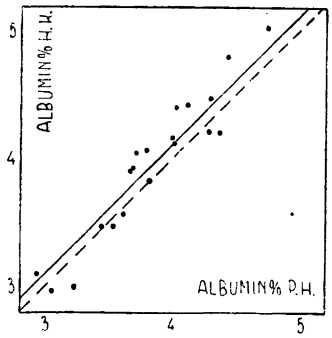

Fig. 1. Relations between the albumin per cents according to Henriques and Klausen and according to Pillemer and Hutchinson.

recently found by Bing, Næser, Rasch and Røjel[10], Howe's method giving slightly higher albumin values than that of Henriques and Klausen.

Whilst in case of precipitation the serum proteins occur as a more or less continuous system with mutual overlapping of the individual fractions, this does not seem to be the case in electrophoresis, in which the fractions can be defined with far greater accuracy. At present, therefore, electrophoresis is believed to afford the most correct picture of the proportion between the individual serum protein fractions. As mentioned above, several authors

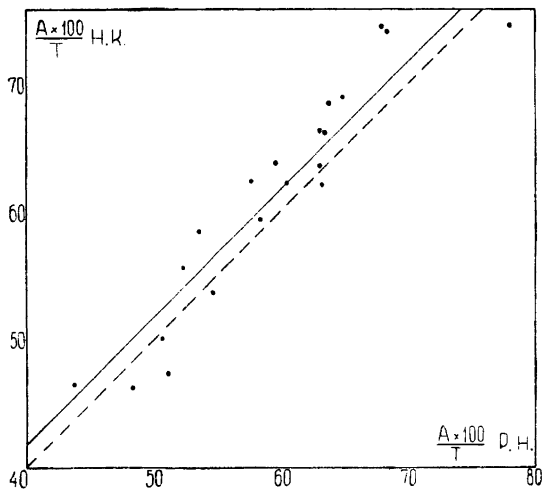

Fig. 2. Relation between the relative albumin per cents according to Henriques and Klausen and according to Pillemer and Hutchinson.

Table 1. Total serum protein, albumin and globulin in grammes per 100 ml. Relative albumin %.

No.	Total protein T	Pillemer and Hutchinson			Henriques and Klausen		
		Alb. A	Glob.	$\frac{A \times 100}{T}$	Alb. A	Glob.	$\frac{A \times 100}{T}$
1	5.45	3.44	2.01	63.1	3.46	1.99	63.5
2	6.30	3.99	2.31	63.4	4.16	2.14	66.1
3	6.33	3.05	3.28	48.2	2.96	3.37	46.8
4	6.75	4.29	2.46	63.5	4.48	2.27	66.4
5	6.46	3.53	2.93	54.7	3.47	2.99	53.7
6	7.05	3.68	3.37	52.2	3.92	4.13	55.6
7	6.92	3.70	3.22	53.5	4.04	2.88	58.5
8	7.14	3.61	3.53	50.6	3.57	3.57	50.0
9	6.36	3.79	2.57	59.6	4.06	2.30	63.8
10	6.78	4.28	2.50	63.3	4.21	2.57	62.1
11	6.45	4.11	2.34	63.8	4.42	2.03	68.5
12	6.70	2.93	3.77	43.7	3.10	3.60	46.3
13	5.61	4.37	1.24	78.0	4.20	1.41	74.8
14	6.52	3.81	2.71	58.4	3.87	2.65	59.4
15	6.63	4.01	2.62	60.5	4.12	2.51	62.2
16	5.90	4.02	1.88	68.2	4.40	1.50	74.6
17	6.47	4.42	2.05	68.3	4.80	1.67	74.2
18	6.33	3.65	2.68	57.6	3.93	2.40	62.2
19	6.32	3.22	3.10	51.0	2.99	3.33	47.3
20	7.29	4.73	2.56	65.0	5.03	2.26	68.9
		76.63			79.19		

$$\frac{79.19 - 76.63}{20} = 0.13\ \% \qquad s = \pm \sqrt{\frac{0.6998}{20 - 1}} = \pm\ 0.192$$

$$t_{0.01} = \frac{0.13}{0.0425} = 3.06 > 2.86 \quad \bar{x} - \bar{y} = 0.13 > 0.0425 \times 3$$

consequently use that method as their starting point in comparisons between the different methods of fractionation.

The separation between albumin and globulin may, however, also be effected in another manner, *inter alia* by precipitation with alcohol at —5° (Cohn [5]) or by precipitation with methanol at 0° (Pillemer and Hutchinson [11]). The two last-named authors found an extremely good correspondence between the albumin/globulin quotient determined by means of electrophoresis and determined by means of precipitation with methanol in normal individuals.

If, on the other hand, the albumin/globulin quotient was determined in the same sera according to Howe's method, considerable differences were found.

The present publication shows a comparison between Pillemer and Hutchinson's methanol method and Henriques and Klausen's method, which is the one most frequently used in this country, as both a possible difference and its magnitude will be of interest. The results will appear from Table 1 and from Figures 1 and 2.

The precipitation of globulin is effected according to Pillemer and Hutchinson's method at 0° in the course of 15 minutes with constant mechanical stirring with cooled reagents. According to Henriques and Klausen's method the globulin is precipitated under standard conditions by semisaturation with ammonium sulphate at constant pH and total protein concentration (Bing[12]). A duplicate analysis of the total protein and of the albumin was made in each serum according to the two methods and, to control the correctness of the nitrogen analyses, an analysis was made of the nitrogen in an amino-acetic acid solution of known concentration. All the patients are over 60 years, except no. 4, a 29-year-old woman.

It will be seen that Henriques and Klausen's method has a tendency to give slightly higher albumin values, on an average 0.13 % more albumin per analysis, than the methanol precipitation. (The deviation on the differences is 0.192 (19 degrees of freedom), the standard deviation on the mean error 0.0018, $t_{0.01} = 3.06 > 2.86, \bar{x} \frac{\varsigma}{\varsigma} \bar{y} = 0.13 > 0.0425 \times 3$). As the material only comprises 20 analyses, too great importance can hardly be attached to the statistical information. If the albumin values arrived at by means of Pillemer and Hutchinson's method can be considered a fairly reliable expression of the albumin values that would have been found by means of electrophoresis, it is still probable that with Henriques and Klausen's method the values will only show a slight discrepancy. This will hardly play any decisive rôle in the practical employment of the ammonium sulphate method of Henriques and Klausen.

SUMMARY

Using different methods of serum protein fractionation small differences always will be found. In this investigation the methanol fractionation of Pillemer and Hutchinson and the ammonium sulfate fractionation of Henriques and Klausen were performed on twenty sera in order to learn how great this difference might be. It was found that the ammonium sulfate method gave a little higher albumin values than the methanol method. As the difference between the albumin per cents of the two albumin series was 0.13 at a mean, and the mean error 0.0425, the difference between the two methods was

significant. The discrepancy is very small, however, and will be of no significance for the practical use of the method of Henriques and Klausen in clinical chemistry.

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Inactivation of Thrombin by Means of Tetranitromethane *

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In view of the inhibitory effect of tetranitromethane (TNM) on the diphteria toxin-antitoxin precipitin reaction found by Ehrenberg, Fischer and Löfgren [1, 2] the influence of TNM on the clotting of fibrinogen was investigated.

Fibrinogen prepared from ox plasma (Bordet) as described by Astrup and Darling [3] was used. Ox thrombin (B) prepared after Astrup and Darling [4,5] was obtained from *Løvens kemiske Fabrik*, Copenhagen. Buffer: *N*/15 phosphate, pH 6.8.

The following solutions were prepared:

Th_1: 1 ml thrombin solution (100 units/ml) + 4 ml buffer.

Th_2: 1 ml thrombin solution (100 units/ml) + 4 ml buffer saturated with TNM.

Fi_1: 5 ml fibrinogen solution + 10 ml buffer.

Fi_2: 5 ml fibrinogen solution + 10 ml buffer saturated with TNM.

After standing for half an hour at room temperature Th_2 had developed a faint yellow colour, while Fi_2 was intense yellow. The following clotting times were found (in seconds):

0.2 ml Th_1 + 1 ml Fi_1: 45, 48; 0.2 ml Th_2 + 1 ml Fi_1: 155, 205.
0.2 ml Th_1 + 1 ml Fi_2: 55, 65; 0.2 ml Th_2 + 1 ml Fi_2: >240.
0.5 ml Th_1 + 1 ml Fi_2: 29, 30; 0.5 ml Th_2 + 1 ml Fi_2: 170, 210.

It is seen that the presence of TNM in the fibrinogen solution only increases the clotting time to a slight extent, while the thrombin solution containing TNM has lost most of its activity. TNM thus seems to inactivate thrombin while it is without influence on the clotting ability of fibrinogen even

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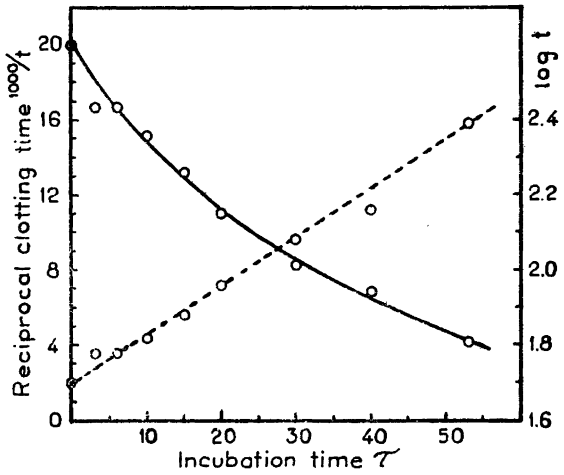

Fig. 1. Inactivation of thrombin with TNM at 20°. Abscissa: Incubation time τ. Ordinates: Full curve: Reciprocal clotting time $t \times 10^3$. Dotted curve: log t.

if the yellow colour is more intense in the latter case. This was confirmed in the following experiments:

To 2 ml thrombin solution (100 units per ml) was added 4 ml buffer saturated with TNM. 0.2 ml was removed after standing at room temperature for varying periods of time, and the clotting time of 1 ml fibrinogen solution (Fi_1) determined. Table 1 shows the results. As the reciprocal clotting times are a rather accurate measure of the potency of the thrombin solution these values are calculated and the corresponding curve drawn in Fig. 1.

When TNM is present in excess the inactivation can be described as a first order reaction:

Table 1. Inactivation of thrombin by TNM.

Incubation time τ min	Clotting time t s	$1000/t$	log t
0	50	20	1.699
3	60	16.7	1.778
6	60	16.7	1.778
10	66	15.2	1.820
15	76	13.2	1.881
20	91	11.0	1.959
30	120	8.3	2.079
40	145	6.9	2.161
53	245	4.1	2.389

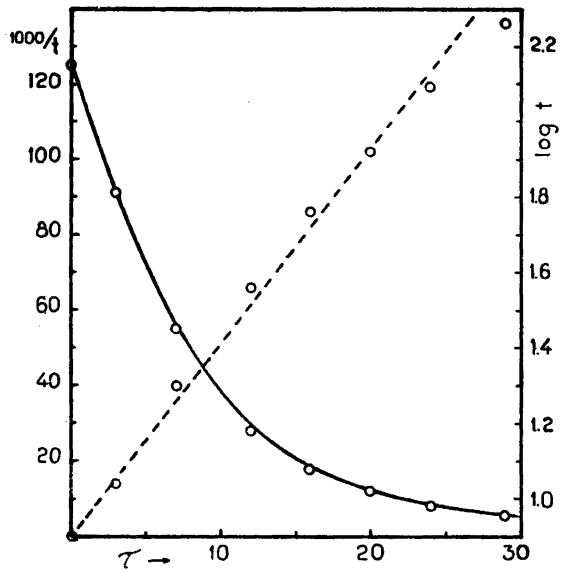

Fig. 2. Inactivation of thrombin with TNM at 37°, compare Fig.1.

$$dx = k \cdot (a—x) \cdot d\tau \tag{1}$$

where a is the original concentration of thrombin and x the amount inactivated at time τ. After integration equation (2) is obtained as $x = 0$ for $\tau = 0$:

$$\log \frac{a}{a—x} = \frac{k}{2.303} \cdot \tau \tag{2}$$

As the amount of unchanged thrombin $(a—x)$ is proportional to the reciprocal of the clotting time t:

$$t \cdot (a—x) = \text{const.} \tag{3}$$

and hence:

$$\log t = \frac{k}{2.303} \cdot \tau + \text{const.} \tag{4}$$

Thus there should be direct proportionality between $\log t$ and τ. In Fig. 1 is drawn a curve with the reaction time τ as abscissa and the logarithm of the clotting time t as ordinate. The curve is a straight line. The inactivation therefore proceeds as a first order reaction. At 37° the inactivation proceeds with a considerably increased velocity, an example is shown in Fig. 2.

Table 2. Incubation of fibrinogen with TNM. τ = incubation time (min); t = clotting time (s).

Room temperature			37°		
τ	control t	TNM t	τ	control t	TNM t
3	10	8	0	5	6
6	9	10	4	7	8
12	9	11	8	5	10
25	8	12	16	—	11
40	8	11	24	—	12
68	9	11	45	7	14
			60	7	17

From the curves the following velocity constants for the reaction and the periods of half-life ($\tau_{½}$) may be calculated:

20°: $k = 0.0005$ s^{-1}; $\tau_{½} = 23$ min.
37°: $k = 0.002$ s^{-1}; $\tau_{½} = 5.8$ min.

Similar values were found in other experiments.

From the Arrhenius equation (5) it is now possible to calculate the activation energy E of the reaction:

$$E = \frac{R \cdot T_1 \cdot T_2}{T_2 - T_1} \cdot \ln \frac{k_2}{k_1} \qquad (5)$$

Here R is the gas constant (1.987 cal per degree per mole), T_1 and the absolute temperatures and k_1 and k_2 the corresponding velocity constants. By inserting the above mentioned values we get:

$$E \cong 15.000 \text{ cal}$$

The activation energy thus found is considerably lower than the activation energy found for the heat inactivation and denaturation of enzymes and proteins, compare Sizer [6], in which, however, the heat of dissociation was found by Steinhardt [7] to furnish a large part of the activation energy as calculated in the usual manner, see La Mer [8].

A fibrinogen solution is only very slowly changed in the presence of TNM as evident from the following experiments.

Fi_1: 5 ml fibrinogen solution, 5 ml buffer, 5 ml H_2O.

Fi_2: 5 ml fibrinogen solution, 5 ml buffer saturated with TNM, 5 ml H_2O.

These mixtures are placed at room temperature and one ml clotted by adding to 0.2 ml of a thrombin solution containing 25 units per ml. A similar experiment was performed at 37°. The results are tabulated in Table 2. Even at 37° the ability of fibrinogen to clot is decreased only to a slight extent.

The inactivation of thrombin by TMN resembles the inactivation by the natural antithrombins found in plasma and serum and by trypsin, Glazko and Ferguson [9], Astrup and Darling [10], Volkert [11], processes which also proceeds much faster at 37° than at room temperature and with almost the same velocity as we found. But as the môde of inactivation is known neither for the natural antithrombins nor for tetranitromethane it is too early to speculate about the details of this process.

The only other enzymatic reaction so far studied in the presence of TNM is the action of amylase on starch, Ehrenberg *et al.* [1] In this reaction TNM had no effect. Thus it is probable that TNM is a reagent acting specifically on certain enzymes and not on others.

SUMMARY

In the presence of tetranitromethane the inactivation of thrombin proceeds as a first order reaction. The activation energy E is about 15.000 cal. Fibrinogen is not changed.

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Adsorption Experiments with Gramicidin and Related Substances

The Heterogeneity of Tyrocidine

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The experiments described here were begun with a view to finding out whether adsorption chromatographic methods could be of use as an additional criterion of homogeneity for the antibacterial peptides gramicidin, tyrocidine and 'gramicidin S'. It was also hoped that they might throw light on the nature of tyrothricin, the crude material from which gramicidin and tyrocidine are isolated, since it is not known what other substances it may contain. Hotchkiss [1] suggests that the non-crystallizing residue of tyrothricin consists of substances not greatly differing from gramicidin and tyrocidine, and the results of the present work tend to support his conclusion.

The main evidence of homogeneity previously available for gramicidin and tyrocidine was the constancy of their physical and chemical properties on repeated crystallization from one or two different solvents (*cf.* Hotchkiss [1], Synge [2]). In the case of gramicidin, physical and chemical characterization of a fraction purified chromatographically had also suggested homogeneity (Gordon, Martin and Synge [3, 4]). For gramicidin S, in addition to constancy of properties on recrystallization, the simple picture revealed by study of the products of complete and partial acid hydrolysis suggested homogeneity (Synge [5]; Consden, Gordon, Martin and Synge [6]). Nevertheless, particularly in view of experience in the ergot alkaloid series (Stoll and Hofmann [7]; Stoll, Hofmann and Becker [8]), it still seemed possible that the products might consist of two or more different peptides crystallizing together in proportions unchanged or not markedly changed by recrystallization. Further tests of homogeneity were therefore desirable, and adsorption analysis, particularly 'front analysis' (Tiselius [9, 10, 11, 12, 13]), seemed promising for the purpose. At the

same time it was hoped that experience of the adsorption behaviour of these fairly well-defined peptides having molecular weights of the order of a few thousand might be a useful orientation for future work with other compounds in this class, since the range of methods available for purifying and characterizing such substances is at present very limited.

MATERIALS

Gramicidin. Specimen R3 (Synge[2]) and a specimen supplied by the Wallerstein Company, New York, during 1945.

Tyrocidine hydrochloride. A specimen, thrice recrystallized, prepared in the same way and from the same material as that studied by Gordon, Martin and Synge[4] and a specimen supplied by the Wallerstein Company during 1945.

Gramicidin S (hydrochloride). Specimen I (Synge[5]) and the specimen studied by Consden *et al.*[6]

Tyrothricin. A 'de-fatted' specimen supplied by the Wallerstein Company during 1945.

No significant differences were observed at any time between the behaviour of the different specimens of the same peptide.

METHODS

Adsorption experiments. Most of the adsorption runs were made with optical control in the interferometric apparatus of Tiselius and Claesson[14] (see Claesson[15]). The general procedure was as given by Claesson[15]. Of the pure solvents available in bulk only the lower alcohols were satisfactory solvents for all the materials studied. Anhydrous ethanol was accordingly used as solvent unless otherwise stated. Before use the solvent was de-aerated by shaking *in vacuo* for a few minutes at room temperature. Heating was usually required for effecting solution; the solutions were cooled before use, and invariably remained clear. The adsorbent was packed in ordinary 'front analysis' filters, which were supplied with liquid from syringes of the type described by Claesson[15]. Where elution or displacement analysis was employed, a measured volume of the solution for analysis was forced into an ordinary 'front analysis' filter (previously washed with fresh solvent) from a micro-burette by means of compressed air. The filter was then without delay connected to a syringe filled with solvent or displacing solution, the whole fitted to the interferometer and operated as described by Claesson. All the experiments were done at 25°.

All retention volumes, and sometimes the volumes on the abscissae of front analysis plots are given 'corrected' — *i. e.* the volume of solvent initially present in the pores of the filter has been *subtracted from*, and the volume of solvent (1.2 ml) that had to flow at the beginning of a run to fill the outlet tube has been *added to* the volume present in the receiver at the moment the optical reading was taken. The volume between the interferometer cuvette and the end of the outlet tube was 1.5 ml. This means that, if

mixing in the outlet tube is ignored, the optical reading at any moment corresponds to the material flowing into the receiver when 1.5 ml more has entered it. Accordingly a cut is shown on the diagrams opposite the optical reading taken when the receiver contained 1.5 ml less than when the cut was made.

The refractive index increments($\Delta\mu_0 10^5$) extrapolated for 1 % *w/v* solutions in ethanol of the substances studied were found to be for gramicidin S, 165; tyrocidine hydrochloride, 180; tyrothricin, 183; and gramicidin, 197.

The charcoal specimens used as adsorbents were from batches of material prepared, used, and fully described by Claesson[15].

Partition chromatographic identification of amino-acids was done by the filter-paper methods of Consden, Gordon and Martin[16]. Filter-paper of the grade 'OB' manufactured by Munktell, Grycksbo, Sweden was employed.

Tryptophan colour reaction. An amount of peptide corresponding to not more than 0.1 mg of tryptophan was dissolved in 2.5 ml acetic acid, and 2.5 ml 10 *N* HCl containing 0.1 % *w/v* *p*-dimethylaminobenzaldehyde was added. The resulting mixture was kept in a dark place at room temperature for the colour to develop (*cf.* Synge[2]).

Tyrosine was determined in acid hydrolysates by the colorimetric method of Arnow[17].

Ethanolamine and NH_3 determinations were done in Conway units on solutions made from HCl-acetic acid hydrolysates of the peptides that had been evaporated to dryness[18].

Optical Rotations were determined in a 1 dm tube on material that had been dried to constant weight in a vacuum desiccator at room temperature.

Evaporation was done *in vacuo* below 40° unless otherwise stated.

RESULTS

Front analyses were done as the first stage of investigating all the materials studied, and it was only when evidence had been obtained in this way of the heterogeneity of tyrocidine that elution and displacement experiments were done with it. Accordingly the work with front analysis of the various substances is described first, and there follow accounts of the fractionation of tyrocidine by other adsorption methods and of the characterisation of the fractions isolated from tyrocidine.

Front analysis work

Choice of adsorbents. Alumina Cγ 'according to Brockmann' (Savory & Moore, London) showed some adsorption of tyrocidine and gramicidin S. The diagram with gramicidin S (0.1 % *w/v*) showed a large step followed by a small one at retention volumes 8 and 14 ml per g adsorbent respectively. On testing

the effluent with $AgNO_3$ it was seen that Cl^- was absent from the first step material, but present in the second. Thus with this adsorbent ion exchange effects seem to exist in addition to 'ordinary' adsorption, which complicate interpretation of the data.

Tyrocidine hydrochloride, analysed in the same way, gave about the same degree of adsorption but no well-defined fronts — not a surprising result if ion-exchange effects are superimposed on the behaviour of the mixture of peptides that tyrocidine appears to be. Gramicidin showed very little adsorption on the alumina. Tyrocidine showed little adsorption on two samples of silica differing in their adsorptive capacity for *N*-2 : 4-dinitrophenyl-amino-acids.

With charcoals no significant interference by ion exchange effects was detected. Tested with 0.23 % *w/v* solutions of tyrothricin, the charcoals *Carboraffin* (unwashed)., *Eponit 3n*, *Norit* SA 30 and *Carbo activ* III (*cf.* Claesson [15]) gave front analysis diagrams all having the same general form, but differing in the retention volumes for the different components. Carbo activ III, although not the most strongly adsorbing of the charcoals tested, showed the greatest relative differences for the different components, and was therefore used in all the work described below.

Gramicidin. Front analysis on Carbo activ III showed a single step. The retention volume for a 0.1 % *w/v* solution was 35 ml and for a 0.2 % *w/v* solution 20 ml per g adsorbent. In the latter experiment the properties of the material coming through the filter at different stages were compared. Fractions corresponding roughly to the first, second and third 20 mg portions of material to emerge from a filter packed with 0.45 g of charcoal were collected separately. No significant differences between the fractions, or divergences from the data for unfractionated gramicidin were noted. $[\alpha]_D^{18-19°}$ lay in the range + 7.8° to + 8.6° (94 % *w/v* aqueous ethanol, c = 1.5) (*cf.* Synge [2]). No noticeable difference appeared in the colours given by the different fractions in the tryptophan reaction. The fractions were hydrolysed for 24 h at 110° with 0.4 ml glacial acetic acid and 1.5 ml 6 *N* HCl. Ethanolamine N in the hydrolysates lay in the range 3.8—4.7 % of total N (*cf.* Synge [18]). No obvious differences in the amino-acid composition were observed when paper-strip chromatograms (*n*-butanol-H_2O) were run on the hydrolysates of the different fractions.

Gramicidin S (hydrochloride). Front analysis on Carbo activ III showed a single step. The retention volume for a 0.1 % *w/v* solution was 7.5 ml per g adsorbent. In view of the physical and chemical data indicating that gramicidin S is a homogeneous substance (see below) it did not appear necessary to subject the effluent from the filter to further analysis.

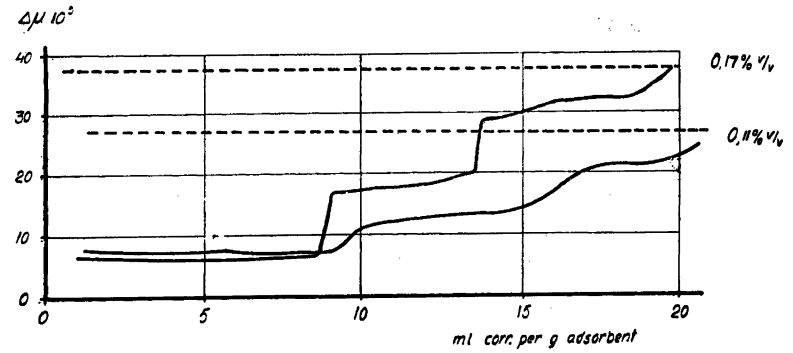

Fig. 1. Front analysis of tyrocidine hydrochloride solutions in ethanol on Carbo Activ III (dotted lines correspond to the solutions used).

Tyrocidine hydrochloride. Front analysis on Carbo activ III immediately showed a number of components to be present. Fig. 1 shows typical front analysis diagrams for 0.11 % and 0.17 % *w/v* solutions. From the increasing fluorescence of the effluent solution in ultra-violet light it was immediately suspected that the successive components had differing tryptophan contents. This was confirmed by the Ehrlich colour reaction — 'first-step' was found to give no colour reaction, 'second-step' material gave a rapidly developing pink colour, becoming blue after 3—4 days, whereas an equal weight of the material issuing from the filter after the rather ill-defined third step (like the original tyrocidine) gave an initial slightly less strong pink colour darkening in 24—48 h to a considerably deeper blue-purple. Further analytical data on the fractions obtained by front analysis are given below, together with those on fractions obtained by elution and displacement development.

In order to test whether the fractions might be artefacts formed from originally homogeneous material by some chemical reaction mediated by the charcoal itself, all the material that had issued from the filter during a front analysis was collected, evaporated to dryness and subjected to front analysis in a filter of the same size packed with fresh charcoal. The resulting front analysis diagram had the same form as previously, except that, as expected, the relative heights of the first and second steps were somewhat increased. It is difficult to imagine how this result could have been obtained if the different fractions were due to alteration of an originally homogeneous material by the charcoal itself, unless such alteration occurred rapidly as soon as the tyrocidine came into contact with the charcoal.

The effect on the form of the front analysis diagram of adding increasing proportions of water to the solvent was investigated. Fig. 2 shows a family

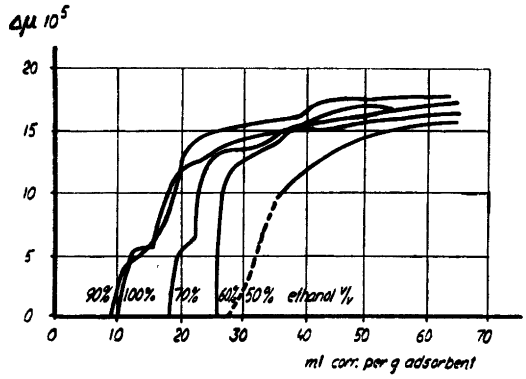

Fig. 2. Front analysis of tyrocidine hydrochloride (0.1 % w/v) on Carbo Activ III in different ethanol-water mixtures.

of plots for solvents of different water content. While the retention volumes increase with increasing water content, the selectivity of the adsorbent for the different components diminishes. It should be noted that tyrocidine hydrochloride crystals are more readily soluble in aqueous than in absolute alcohol, and that their speed of solution appears, for the range of concentrations studied here, to increase with the water content of the ethanol.

Tyrothricin. Fig. 3 shows a typical front analysis of tyrothricin on Carbo activ III. The diagram is consistent with what might be expected for a mixture of (say) 85 % of tyrocidine hydrochloride and 15 % of gramicidin; the step (at about 55 ml) that should correspond with gramicidin is not very sharp or

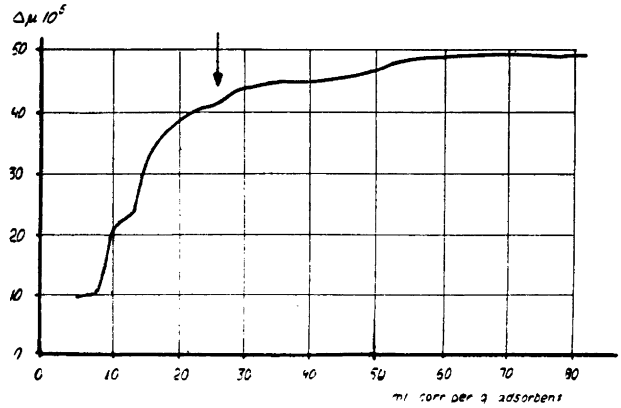

Fig 3. Front analysis of tyrothricin (0.23 % w/v) on Carbo Activ III in ethanol.

large, but this is a usual phenomenon with the later components on front analysis diagrams.

The material issuing from the filter before and after the cutting point (26 ml) indicated by the arrow on the diagram was evaporated to dryness, hydrolysed, and examined for amino-acids by two-dimensional partition chromatography. As expected, the amino-acids peculiar to gramicidin (glycine and alanine) were detected in the second but not in the first fraction. All the known constituent amino-acids of tyrocidine (valine, leucine, phenylalanine, tyrosine, proline, ornithine, glutamic acid, aspartic acid and tryptophan (colour reaction only) were recognised in both fractions, but it is of interest that no indication was obtained of the presence of other amino-acids. This suggests that any peptides other than gramicidin and tyrocidine that are present in tyrothricin must have a somewhat similar amino-acid composition.

Work with elution and displacement development

Experiments were carried out with a view to isolating the more strongly adsorbed components of tyrocidine in a pure state — since front analysis could only be used for isolating the least adsorbed component (I) that gave no colour with Ehrlich's reagent.

Recognition of the components. For this work the colour reaction with Ehrlich's reagent proved particularly valuable, since it soon became evident that, consistent with the data obtained by study of the fractions resulting from front analysis (see above), the component of tyrocidine corresponding to the second step (II) gave rapidly a bright pink colour with the reagent. Thus fractions rich in this component showed, in the early stages of the reaction, a *pink* colour about twice as intense as that obtained with an equal weight of unfractionated tyrocidine. This pink colour changed slowly to blue but only after 3—4 days. On the other hand fractions rich in the still more strongly adsorbed component(s) III gave very little colour during the first few hours of the reaction: with these a *pure blue* colour developed rather slowly, reaching after one day an apparent intensity slightly greater than that of the purple-blue colour given by an equal weight of unfractionated tyrocidine.

In view of the continual intensification of the colour, quantitative comparisons were unreliable, but it can be stated that fractions rich in II which were submitted to further study gave at least as much pink colour in the early stages of the reaction as double the weight of unfractionated tyrocidine, while fractions rich in III gave only as much pink colour in the early stages of the reac-

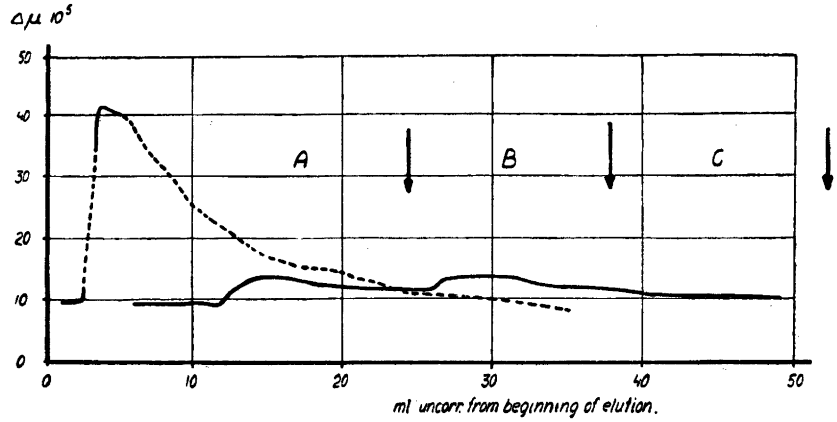

Fig. 4. Elution development of tyrocidine hydrochloride (ethanol: Carbo Activ III).
——— *Filter contains 2.12 g charcoal; pressed in 15 mg tyrocidine hydrochloride in 7 ml ethanol.*
- - - - - - - - *Filter contains 1.91 g charcoal; pressed in 52 mg tyrocidine hydrochloride in 3.3 ml ethanol.*

tion as would be obtained with 1/10 the weight of fractions rich in II. It seems likely that contamination of the components II and III in the corresponding fractions isolated did not exceed 20 %. There was of course no difficulty in preparing fractions embodying component I quite free from components II and III.

Fractionating procedures. For concentrating component II simple elution development with ethanol proved satisfactory, provided the chromatograms were not overloaded. Fig. 4 shows the optical diagram obtained in such an experiment. Cut A (containing 18 % of the total N of the tyrocidine) proved to be component I, nearly free from component II, which became apparent in cut B (16 % of the N); cut C (12 % of the N) appeared to consist of highly concentrated component II with little or no III (pure pink colour after 24 h).

If the chromatogram was more heavily loaded a single peak was obtained in the diagram (dotted line in Fig. 4), and this material was found on analysis to consist of components I and II very poorly resolved. There was no indication that component III was being eluted. It was thus evident that some displacing agent would be required for removing component III from the charcoal (*cf.* Tiselius [22]). Various possible agents were tried, namely picric acid, gramicidin, acetyl-DL-tryptophan and, at the suggestion of Dr. S. Claesson, stearic acid. All of these substances are weight for weight more

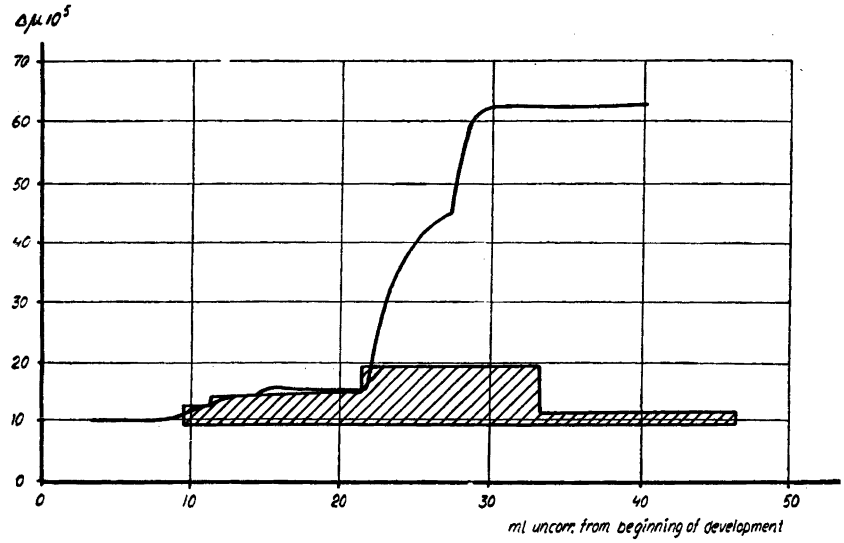

Fig. 5. Displacement development of 14 mg tyrocidine hydrochloride with 0.5 % w/v stearic acid in ethanol (see text). (The hatched area shows the content of tyrocidine-N in the various cuts).

strongly adsorbed on charcoal from ethanol solution than any of the components of tyrocidine.

14 mg tyrocidine HCl (in 7 ml ethanol) was pressed into a 1250 π mm^3 filter packed with 2.0—2.2 g Carbo activ III; development was then effected, under optical control, with pure ethanol or solutions in ethanol of stearic acid (0.5 % *w/v*: retention volume 13 ml/g), picric acid (0.5 % *w/v*: retention volume 14 ml/g), acetyl-DL-tryptophan (0.3 % *w/v*:retention volume 16 ml/g) or gramicidin (0.25 % *w/v*: retention volume 17 ml/g). The optical data showed clearly that only with stearic acid was any considerable displacement occurring. The amount of tyrocidine was determined in successive portions of the effluent during development, up to a total of 45 ml. In the runs with pure ethanol and with stearic acid, this was done by N (Kjeldahl) determination. With gramicidin and acetyltryptophan development, the tyrocidine in the effluent was determined by virtue of its amide-N content, the NH_3 being liberated by hydrolysis of the evaporated fractions in 1 : 1 *w/v* acetic acid: 10 *N* HCl in sealed evacuated tubes for 10 days at 38° (under these conditions gramicidin and acetyltryptophan liberate no NH_3).

The results showed that in the first 45 ml of effluent during development by simple elution, gramicidin displacement or acetyltryptophan displacement, only 30—40 % of the tyrocidine was recovered. On the other hand, 73 %

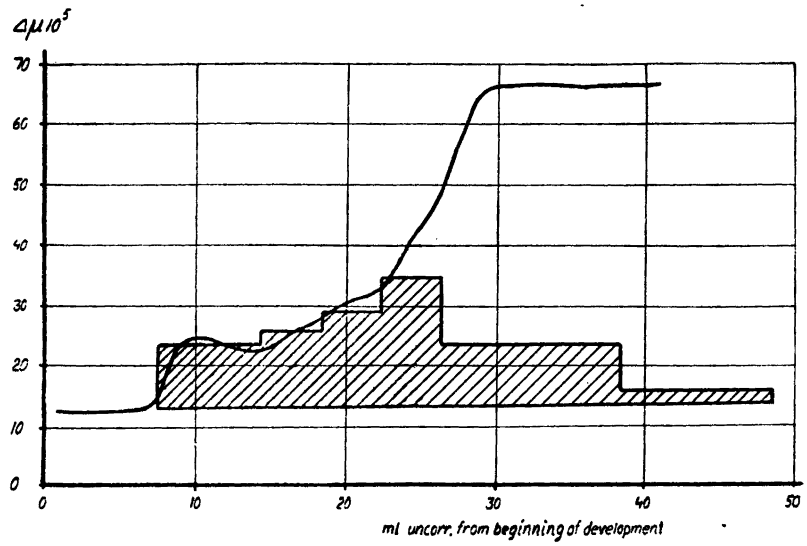

Fig. 6. Same experiment as illustrated in Fig. 5, but with 43 mg tyrocidine hydrochloride (see text).

was recovered in the stearic acid displacement. Fig. 5 shows the optical curve plotted together with the quantities of tyrocidine based on the N determinations. Fig. 6 shows a similar plot for an experiment in which 43 mg of tyrocidine hydrochloride was displaced in the same way. Here 79 % of the tyrocidine issued in the first 45 ml. In each case the initial flat portion of the curve was found to be due to component I, while components II and III were isolated in a state of mutual contamination from fractions in the region of the stearic acid front. Material issuing later, with the stearic acid, appeared to be almost purely component III, but the quantity was small. For preparative purposes it was therefore better to elute as much as possible of components I and II with pure solvent, and then to displace component III from the column with stearic acid solution. It proved easy to separate the mixture of peptide and stearic acid resulting from evaporation of the effluent by extracting this residue with boiling light petroleum (40—60°), dissolving the insoluble material in 50 % *w/v* aqueous ethanol, and filtering off traces of stearic acid which remained undissolved. On evaporating the filtrate, the peptide was obtained free from stearic acid and apparently almost free from component II.

In a typical preparation, 52 mg tyrocidine hydrochloride (1 % *w/v* in ethanol) was pressed into a filter packed with 2.0 g Carbo activ III. Elution was effected with 50 ml ethanol, and the effluent discarded. 60 ml 0.5 % *w/v*

stearic acid in ethanol was then passed, and the effluent was collected. On working it up as above, the yield was 10 mg.

Characterization of the components of tyrocidine

Apart from their colour reaction with Ehrlich's reagent and differing fluorescence, which have been noted above, no striking difference could be detected in the properties of the components.

In the preliminary stages of the work fractions obtained by front analysis were characterized with the results shown in Table 1.

Table 1. Characterization of fractions from front analysis of tyrocidine.

Fraction from front analysis	Components	$[\alpha]_D^{18°}$ (95 % *w/v*) aqueous ethanol, c = 1.1)	as % of total N of fraction	
			Tyrosine — N*	Amide — N*
'First step'	I	— 93°	6.6	13.9
'Second step'	I + II	— 104°	6.7	13.9
'Final' (same as unfractionated tyrocidine)	I + II + III	— 103°	7.7	14.6

Subsequently fractions were available in which components I, II and III had been substantially separated from one another. Like the original tyrocidine, these fractions showed little tendency to crystallise when concentrated in ethanol solution, amorphous gums resulting. Samples rich in each of the components were separately hydrolysed with acid, and the constituent amino-acids were examined by two-dimensional partition chromatography. Tryptophan appeared to be destroyed (the hydrolysates of fractions rich in tryptophan had more humin) but the other 8 amino-acids were all present in each hydrolysate, nor did their relative proportions seem to be greatly different in the different fractions. Diffusion constants were also determined for the different fractions, with the results shown in a following paper (Pedersen and Synge [19]).

DISCUSSION

The experiments described here permit one to hope that adsorption chromatography may be of value in work with other peptides having molecular

* Determined after hydrolysis in 1 : 4 *w/v* acetic acid — 6 *N* HCl in sealed evacuated tubes for 24 h at 110°.

weights of the order of a few thousand both for testing homogeneity and effecting isolations. The method of front analysis gave no indication of heterogeneity in gramicidin S, a peptide probably having a molecular weight of 1100—1200 (Pedersen and Synge [19]) for which there exists some evidence of homogeneity obtained by crystallographic and structural chemical studies (Crowfoot and Schmidt [20]; Consden, Gordon, Martin and Synge [6]). On the other hand with tyrocidine (molecular weight perhaps 2,500) front analysis revealed heterogeneity, proving in this case to be a more sensitive criterion than the commonly accepted one of constancy of properties on recrystallization. None of the other available information on tyrocidine is inconsistent with its being a mixture of components, and the evidence now presented may help to explain the controversy as to its tryptophan content (reviewed by Hotchkiss [1]). It was further possible, by elution development and displacement development to separate the different components of tyrocidine from one another. As concerns gramicidin (mol. wt. perhaps 4,000) neither the front analysis data nor the other information available suggest heterogeneity, although a mixture of peptides similar as to content of tryptophan, but differing in respect of the other amino-acids might well give a single step in front analysis.

If the peptides here studied are arranged in order of increasing adsorption on charcoal (gramicidin S, the 3 components of tyrocidine, gramicidin) it is seen that the extent of adsorption is correlated with the content of aromatic amino-acid residues, and particularly of tryptophan. This is in agreement with adsorption data for free amino-acids (Tiselius [13]; Tiselius, Drake and Hagdahl [21]). Neither the front analysis nor the displacement development results with acetyltryptophan and gramicidin suggest that there are sufficiently strong mutual displacement effects between the different peptides to give useful separations by the procedure employed in the carbohydrate series (Tiselius [22, 23]; Tiselius and Hahn [24]). The rather strong displacing effect of stearic acid may prove useful in other work involving the adsorption of peptides on charcoal.

When the front analysis first revealed the existence of an apparently tryptophan-free component (I) of tyrocidine which was similar in adsorption behaviour to gramicidin S, it was tempting to suppose that this substance might actually be gramicidin S, and that tyrocidine was simply a mixture of gramicidin S with other peptides crystallizing together in stoichiometric proportions. However, the amino-acid composition, optical rotation, diffusion constant and poor crystallizing power of component I all rule out this possibility, and it is evident that the relationship between gramicidin S and tyrocidine is a more subtle one. The amino-acid composition and the diffusion constants

of the different tyrocidine components reveal no striking differences between them, except as concerns the content and reactivity of the supposed tryptophan residues. It is perhaps most satisfactory to regard the different components as being the same peptide structure containing one or more loci at which may exist a tryptophan residue or else its precursors or successors in some biosynthetic process. However, before such a view could be substantiated or any clear answer given to the problem of the tryptophan of tyrocidine, it would be necessary to study the different components of tyrocidine by methods less equivocal than those based on colorimetry, fluorimetry or microbiological assay.

SUMMARY

1. The adsorption behaviour of gramicidin, tyrocidine, tyrothricin and gramicidin S has been studied by front analysis, mostly on charcoal chromatograms using ethanol as solvent.

2. Tyrocidine, which proved heterogeneous, has been further fractionated by elution and displacement development.

3. The resulting fractions have been characterized; the different components of tyrocidine appear to differ chiefly in their content of tryptophan.

4. The data are considered in relation to the information they yield both as to the peptides investigated and as to the possibilities of applying adsorption methods to other peptides having molecular weights of the order of a few thousand.

One of us (R. L. M. S.) took part in this work while on study leave from the Lister Institute of Preventive Medicine, London, England.

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Equilibrium in Ammoniacal Solution of Silver Nitrate

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When silver ions are added to ammonia solution, the chief reaction is

$$Ag^+ + 2\ NH_3 = Ag(NH_3)_2^+ \qquad (1)$$

After this reaction is complete, further addition of silver nitrate results in the formation of a precipitate. The experiments and computation show, however, that when the stoichiometric concentration is chosen sufficiently small the precipitation does not yet begin at the inflection point of the titration curve. A homogeneous equilibrium therefore exists at the inflection point and it is thus possible to study quantitatively this equilibrium by determination of the maximum slope of the titration curve.

Application of the mass action law to the homogeneous equilibrium in ammoniacal solution of silver nitrate gives

$$[Ag^+]\ [NH_3] / [AgNH_3^+] = K_1 \qquad (2)$$

$$[AgNH_3^+]\ [NH_3] / [Ag(NH_3)_2^+] = K_2 \qquad (3)$$

$$[NH_4^+]\ [O\bar{H}] / [NH_3] = K_B \qquad (4)$$

According to Bjerrum [1] complexes richer in ammonia than diammine are not present in analytically demonstrable quantities. In addition we have the stoichiometric equation

$$c_{NH_3} = [NH_3] + [NH_4^+] + [AgNH_3^+] + 2\ [Ag(NH_3)_2^+] \qquad (5)$$

and

$$c_{Ag} = [Ag^+] + [AgNH_3^+] + [Ag(NH_3)_2^+] \qquad (6)$$

where c_{NH_3} is the stoichiometric normality of ammonia and c_{Ag} that of silver nitrate [2]. From electroneutrality of the solution it follows

$$c_{Ag} + [\overline{OH}] = [Ag^+] + [AgNH_3^+] + [Ag(NH_3)_2^+] + [NH_4^+] \quad (7)$$

Introducing equation (6) into (7) the result is

$$[NH_4^+] = [OH^-] \quad (8)$$

From equations (4) and (8) we find that

$$[NH_4^+] = \sqrt{K_B}\ [NH_3]^{\frac{1}{2}} \quad (9)$$

Eliminating $[Ag(NH_3)_2^+]$ between (5) and (6) gives

$$c_{NH_3} = 2\ c_{Ag} - 2\ [Ag^+] + [NH_4^+] - [AgNH_3^+] + [NH_3] \quad (10)$$

By introducing equations (1) and (9) into (10) we find

$$c_{NH_3} = 2\ c_{A_b} - 2\ [Ag^+] + \sqrt{K_B}\ [NH_3]^{\frac{1}{2}} + [NH_3] - [Ag^+]\ [NH_3]\ /\ K_1 \quad (11)$$

If equation (2) and (3) are solved for $[AgNH_3^+]$ and $[Ag(NH_3)_2^+]$ as well as substituted in equation (6) there results

$$c_{Ag} = [Ag^+] + \frac{[Ag^+]}{K_1}[NH_3] + \frac{[Ag^+]}{K_1K_2}[NH_3]^2 \quad (12)$$

Solving with respect to $[NH_3]$ yields

$$[NH_3] = -K_2/2 + \sqrt{K_2^2/4 - K_1K_2 + K_1K_2\ c_{Ag}/[Ag^+]} \quad (13)$$

or

$$[NH_3] = Ax^{-\frac{1}{2}}\ (1-\delta)^{\frac{1}{2}} - K_2/2 \quad (14)$$

in which

$$A = \sqrt{K_1K_2c_{Ag}},\quad x = [Ag^+] \text{ and } \delta = (1-K_2/4K_1)x/c_{Ag} \quad (15)$$

Approximative expression for $[NH_3]$ are

$$[NH_3] = Ax^{-\frac{1}{2}}\ (1-\delta)^{\frac{1}{2}} \quad (16)$$

and

$$[NH_3] = Ax^{-\frac{1}{2}} \quad (17)$$

Introducing expression (14) into equation (11) gives

$$c_{NH_3} = 2\ c_{Ag} - K_2/2 - 2\ (1-K_2/4K_1)\ x + Ax^{-\frac{1}{2}}\ (1-\delta)^{\frac{1}{2}} - A'\ x^{\frac{1}{2}}\ (1-\delta)^{\frac{1}{2}} + Bx^{-\frac{1}{4}}\ (1-\delta)^{\frac{1}{4}} \quad (18)$$

in which

$$A' = A/K_1 \text{ and } B = \sqrt{AK_B} \tag{19}$$

The term $Bx^{-\frac{1}{2}}\ (1-\delta)^{\frac{1}{2}}$ is obtained using the approximative expression (16). This term is namely small as compared with other terms and the error made is therefore negligible. Differentiating (18) with respect to $px = -\log x$ yields

$$P = 2.303\Big\{\ 2\ (1-K_2/4K_1)\ x + 1/2\ Ax^{-\frac{1}{2}}\ (1-\delta)^{-\frac{1}{2}} + 1/2\ A'x^{\frac{1}{2}}\ (1-\delta)^{-\frac{1}{2}}\ (1-2\delta) + \\ + 1/4\ Bx^{-\frac{1}{2}}\ (1-\delta)^{-\frac{3}{2}}\Big\} \tag{20}$$

Equation (20) is valid by titration with ammonia solution at which c_{Ag} remains constant. By titration with silver nitrate solution the case becomes more complicated. This is not dealt with in this paper. Some titrations carried out in this manner, however, give the same value for the constant K_1K_2 as the titrations performed in the reverse direction. Differentiating once more with respect to px and equating to zero gives after rearranging

$$x = (1/8\ AF_1 - 1/8\ A'x\ F_2 + 1/32\ Bx^{-\frac{1}{2}}\ F_3)^{\frac{2}{3}}\ (1-K_2/4K_1)^{-\frac{2}{3}} \tag{21}$$

in which

$$F_1 = (1-2\delta)\ (1-\delta)^{-\frac{3}{2}} \tag{22}$$

$$F_2 = (1-6\delta + 4\delta^2)\ (1-\delta)^{-\frac{3}{2}} \tag{23}$$

$$F_3 = (1-4\delta)\ (1-\delta)^{-\frac{7}{4}} \tag{24}$$

From equation (21) the silver ion concentration at inflection point can be calculated. With the aid of the experimental value of the quantity P at the inflection point and the values of K_1, K_B and c_{Ag} the constant K_1K_2 can be computed from equations (20) and (21). It is then convenient to use equation (20) in the form

$$A = \Big\{\ 0.434\ P - 2\ (1-K_2/4K_1)\ x - 1/4\ Bx^{-\frac{1}{2}}\ (1-\delta)^{-\frac{3}{2}}\Big\}\ \frac{2x^{\frac{1}{2}}\ (1-\delta)^{\frac{1}{2}}}{1 + x/4K_1\ (1-2\delta)} \tag{25}$$

For computations the constant K_1 is obtained according to Bjerrum [1] from

$$pK_1 = 3.315 - 0.01\ \mu \tag{26}$$

and K_B according to Kilpi [3] from

$$pK_B = 4.733 - \frac{\sqrt{\mu}}{1 + 1.8\ \sqrt{\mu}} + 0.036\ \mu \tag{27}$$

If approximative equation (16) or (17) is introduced into equation (11) equations are obtained which follow also from (18), (20) and (21) by substituting $K_1/4K_2 = 0$ in the first case and in addition $\delta = 0$ in the latter case. By using these equations we obtain a value for pK_1K_2 which is in the first case about 0.02 and in the latter case about 0.035 smaller than that obtained with the aid of equations (21) and (25).

EXPERIMENTAL

The titrations were performed in a water thermostat at $25 \pm 0.01°$. As indicator electrode a silver iodide electrode was used. Tank nitrogen, carbon dioxide free, was bubbled through solution. The titration vessel employed was similar to that used earlier [4]. The K type potentiometer of Leeds & Northrup was used for measurement of the electromotive force.

Kahlbaum's chemicals for analysis were used. Carbonate-free ammonia solution was prepared by passing ammonia gas, obtained by heating strong ammonia water containing a little calcium hydroxide, into water.

For further experimental details, especially for the calculation of the minimum value of the quantity P from potentiometric data, the reader is referred to the earlier papers of the author [4].

RESULTS

A separate titration was carried out as follows. First 50 ml water or potassium nitrate solution and the main part of the 0.305 N ammonia solution were added in the titration vessel. Now during vigorous stirring 5 ml of 0.05 N silver nitrate was slowly dropped in. When the potential of the indicator electrode became constant, the fine titration for determination of the maximum slope of the titration curve was performed with the above 0.305 N ammonia solution. The data of a typical tiration are given in Table 1, where the denotations are:

C_{NH_3} and C_{Ag} respectively, the normality of ammonia solution and of silver nitrate solution respectively,

a_{NH_3} and a_{Ag} respectively, the amount of the ammonia and of the silver nitrate solution respectively, added up to the inflection point in ml,

Δa the mean increment of ammonia solution in ml,

ΔE the maximum jump of the potential as interpolated from the e. m. f. values in mV,

V the volume of the titrated solution at the inflection point in ml,

P the reciprocal value of the maximum slope of the titration curve.

Table 1. A typical titration of silver nitrate solution with ammonia solution.

t	a	E	ΔE
0	1.501	270.8	
			9.4
6	1.607	261.4	
			10.7
12	1.713	250.7	
			11.7
18	1.819	239.0	
			11.5
24	1.925	227.5	
			10.3
30	2.031	217.2	

$C_{NH_3} = 0.305$. $C_{Ag} = 0.0500$.
$a_{NH_3} = 1.801$. $a_{Ag} = 5.00$.
$c_{NH_3} = 9.69 \cdot 10^{-3}$. $c_{Ag} = 4.40 \cdot 10^{-3}$
$c_{NH_3} - 2\ c_{Ag} = \begin{cases} 0.89 \cdot 10^{-3} \text{ (found)} \\ 0.91 \cdot 10^{-3} \text{ (calc)} \end{cases}$

$\Delta a = 0.106$. $\Delta E = 11.81$. $V = 56.8$. $P = 2.85 \cdot 10^{-3}$.

Table 2 includes the data of the titrations, some of which are carried out with added potassium nitrate. Silver nitrate concentration is the same in all titrations. The numerical values of the constant K_1K_2, the ion concentrations at inflection point calculated from the data of Table 2 as well as the corresponding values of the constants K_1 and K_B are given in Tables 3 and 4. From Tables 3 and 4 it appears that the constant K_1K_2 is within the limit of experimental errors independent of ionic strength. For this constant at the ionic strength zero is thus obtained

$$pK_1^\circ K_2^\circ = 7.21$$

which is the average value of the values in Tables 3 and 4.

Table 2. Computation of maximum slope of the titration curve from potentiometric data.

μ	V	Δa	ΔE	$P \cdot 10^3$	$c_{Ag} \cdot 10^3$	$c_{NH_3} \cdot 10^3$	$(c_{NH_3}-2c_{Ag}) \cdot 10^3$	
							found	calc.
0.0044	56.81	0.106	11.83	2.84	4.40	9.73	0.93	0.82
0.0044	56.79	0.107	12.07	2.80	4.40	9.61	0.81	0.82
0.0044	56.80	0.106	12.15	2.77	4.40	9.69	0.89	0.82
0.0044	56.80	0.104	11.80	2.79	4.40	9.68	0.88	0.82
1.00	56.75	0.106	12.20	2.76	4.40	9.70	0.90	0.85
1.00	56.81	0.107	12.05	2.82	4.40	9.65	0.85	0.85
1.00	56.82	0.105	11.90	2.80	4.40	9.64	0.84	0.85
1.00	56.79	0.106	12.10	2.78	4.40	9.67	0.87	0.85

Table 3. Equilibrium constants and ion concentrations at inflection point when $\mu = 0.0044$ and $P = 2.80 \cdot 10^3$.

K_1	$4.85 \cdot 10^{-4}$	$[Ag^+]$	$1.42 \cdot 10^{-4}$	$[OH^-]$	$1.67 \cdot 10^{-4}$
K_B	$2.12 \cdot 10^{-5}$	$[AgNH_3^+]$	$3.80 \cdot 10^{-4}$	$[NH_4^+]$	$1.67 \cdot 10^{-4}$
K_1K_2	$6.26 \cdot 10^{-8}$	$[Ag(NH_3)_2^+]$	$3.90 \cdot 10^{-3}$	$[NH_3]$	$1.31 \cdot 10^{-3}$

Table 4. Equilibrium constants and ion concentrations at inflection point when $\mu = 1.00$ and $P = 2.79 \cdot 10^{-3}$.

K_1	$4.95 \cdot 10^{-4}$	$[Ag^+]$	$1.43 \cdot 10^{-4}$	$[OH^-]$	$2.14 \cdot 10^{-4}$
K_B	$3.87 \cdot 10^{-5}$	$[AgNH_3^+]$	$3.76 \cdot 10^{-4}$	$[NH_4^+]$	$2.14 \cdot 10^{-4}$
K_1K_2	$6.20 \cdot 10^{-8}$	$[Ag(NH_3)_2^+]$	$3.91 \cdot 10^{-3}$	$[NH_3]$	$1.30 \cdot 10^{-3}$

DISCUSSION

From the values of Table 3 it follows that the product $[Ag^+]\,[OH^-] = 2.37 \cdot 10^{-8}$ at inflection point when $\mu = 0.0044$. In this ionic strength the solubility product of silver oxide has the value $3.13 \cdot 10^{-8}$ according to the measurements by the present author [5]. As regards Table 4 $[Ag^+][O\overline{H}] = 3.06 \cdot 10^{-8}$ and the solubility product is $8.26 \cdot 10^{-8}$. Thus the equilibrium in both cases is homogeneous, even if in the first instance the beginning of precipitation is near.

Inflection point and equivalent point differ from each other. According to calculations and measurements the difference $c_{NH_3} - 2c_{Ag}$ is about $1 \cdot 10^{-3}$. The silver ion concentration at equivalent point can be calculated according to equation (18), from which we obtain after substituting $c_{NH_3} = 2\ c_{Ag}$ and rearranging

$$[Ag^+] = \left\{ \frac{A\ (1-\delta)^{\frac{1}{2}} - A'x\ (1-\delta)^{\frac{1}{2}} + Bx\ (1-\delta)^{\frac{1}{4}} - K_2/2\ x^{\frac{1}{2}}}{2\ (1-K_2/4K_1)} \right\}^{\frac{2}{3}} \qquad (28)$$

The corresponding value of $[OH^-]$ can be calculated from equations (13) and (9) when equation (8) is taken into account. In the above experimentally studied cases we obtain for $[Ag^+]$, $[O\overline{H}]$ and $[Ag^+]\,[O\overline{H}]$ the following values at equivalent point

μ	$[Ag^+]$	$[O\overline{H}]$	$[Ag^+]\,[O\overline{H}]$
0.0044	$2.75 \cdot 10^{-4}$	$1.40 \cdot 10^{-4}$	$3.85 \cdot 10^{-8}$
1.00	$2.83 \cdot 10^{-4}$	$1.86 \cdot 10^{-4}$	$5.32 \cdot 10^{-8}$

In the first case the product $[Ag^+]\,[\overline{OH}]$ is thus greater than the solubility product. No precipitation at equivalent point is, however, observed. In pAg units the difference between inflection and equivalent point is in both cases about 0.3. The corresponding pH difference is about 0.07. Thus the potential jump of the silver electrode is about four times greater than the corresponding potential jump of the glass electrode. This state of things follows also from the approximative equation

$$[\overline{OH}] = B\,[Ag^+]^{-\frac{1}{4}} \qquad (29)$$

which is obtained from (8), (9) and (17).

When the concentration of $AgNH_3^+$ is not taken into consideration, we obtain for pK_1K_2 a value which is 0.2 smaller than the value calculated above.

The value $pK_1^\circ K_2^\circ = 7.21$ obtained in the present paper is in good agreement with the values in the literature, especially with the value $pK_1^\circ K_2^\circ = 7.23$ obtained by Bjerrum [1].

SUMMARY

Equilibrium in ammoniacal silver nitrate solution is studied potentiometrically. At the inflection point which occurs when one mole silver nitrate per two moles ammonia is added, the equilibrium is homogeneous, if the solution is sufficiently dilute.

The maximum slope of the titration curve is determined and the equilibrium constant is calculated from experimental values. The values obtained agree satisfactorily with the previous values in the literature.

The influence of potassium nitrate additions on the equilibrium constant lies within the limit of experimental errors.

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The Iron-Binding Protein of Swine Serum *

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In 1925 Fontès and Thivolle [1] showed that horse serum includes an iron-containing fraction. Subsequently Barkan [2] and Warburg [3] were able to confirm this observation. Barkan showed that at the pH of the blood the iron in the serum (»serum iron») was non-dialyzable. Barkan [4] further found that in native serum the serum iron is not ultrafiltrable. Starkenstein and Harvalik [5] enriched serum with iron; and on fractionation of such serum with ammonium sulphate (50 % saturation) the added iron was found in the globulin fraction. On salting of serum with ammonium sulphate Barkan & Schales [6] found that the protein-bound serum iron was precipitated at 50 % saturation. Vahlquist [7] investigated the protein-binding of the iron in serum through iron analyses on serum fractions that were separated by electrophoresis. He found that the γ-globulin fraction was poor in iron and that the albumin fraction contained less than one-half of the total serum iron. From these experiments he arrived at the conclusion that the main part of the iron was combined with the α-and β-globulin fractions.

Holmberg and Laurell [8] showed that serum contains a high-molecular component that is able to bind iron firmly. It was found that the amount of this component in the serum was decisive of its iron-binding capacity. These authors found the iron-binding capacity of serum from normal persons to be about 300 γ % Fe.

Schade and Caroline [9] were able by means of a microbiological method to show that normal serum contains an iron-binding component. They were further able to show that on fractionation of the serum after Cohn [10] this component is found in fraction IV : 2, which chiefly is made up of a »β-globulin fraction» poor in lipoid.

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* At the Sixth Meeting of Scandinavian Chemists in August 1947 Laurell gave an account of the studies described in the present paper.

In a survey of the protein components of human plasma Cohn [11] gives some numerical values for »β_1-metal-combining protein» (fraction IV : 7) taken from a paper by Oncley, Scatchard and Brown [12]. The molecular weight for fraction IV : 7, determined by osmotic measurements, was 93,000, and the partial specific volume was 0.725. In his paper Cohn gives an approximate iso-electric point = 5.6; and he also states that the metal-combining protein has been crystallized by Koechlin.

Laurell [13] has given an account of some methods for determination of the iron-binding component content of the serum and for variations of this protein fraction under physiological and pathological conditions. He also shows that the reaction

$$\text{iron} + \text{iron-binding protein} \rightleftharpoons \text{iron-containing protein}$$

is reversible.

Previously the isolation of the serum iron-protein complex has met with unsurmountable difficulties, partly because the available methods for fractionation were not sufficiently precise for this purpose, partly because an adequate knowledge about the binding of iron in the native serum iron-protein complex was wanting. In this respect, the conditions have now improved, partly through the alcohol precipitation method given by Cohn, partly because it now is practicable by means of the analytical methods given by Holmberg and Laurell [8], Schade and Caroline [9] and Laurell [13] to separate in the serum specifically bound iron (firmly combined with the iron-binding protein) from the less firmly bound iron (loosely combined with the serum proteins). (The serum proteins have a great tendency to combine with iron. This iron is not so firmly bound, however, as the iron in the iron-binding protein.) A circumstance that facilitates the fractionation is the phenomenon pointed out by Holmberg and Laurell [8] that when the iron-binding protein takes up iron, the mixture becomes reddish in color. Thus it is possible by measuring the intensity of this color to estimate the effectivity of the fractionation. This observation has also been confirmed by Schade and Caroline [9].

PURIFICATION OF THE IRON-BINDING PROTEIN

Swine serum was used in the experiments on fractionation. It offers two advantages above most other sera. In the first place, it possesses a relatively high iron-binding capacity (about 400 γ %). Furthermore, it is practically free from bilirubin, on which account it is easy through measuring the intensity

of color to follow the iron-containing protein in the course of the fractionation, as this is the entirely dominating colored constituent of swine serum if the withdrawn blood is fairly free from hemolysis.

The first step in the process of purification has consisted in salting-out as follows: A serum was diluted with an equal volume of water. Then as much ferrous salt was added as is required to saturate the iron-binding component with iron. Under thorough stirring, a saturated solution of ammonium sulphate, neutralized with ammonia, was added until the final concentration of the mixture became 60 % saturation. The ammonium sulphate solution was added through a fine glass tube (immersed in the serum). The rate at which the ammonium sulphate was added was regulated so that it took some hours to reach this concentration. The precipitation took place at room temperature, and also the ammonium sulphate solution had been saturated at the same temperature. The stirring was further continued for some hours after the addition of ammonium sulphate had been performed. After this, the mixture was left standing over night before the precipitate was separated. About 90% of the iron-binding component was found to be present in the solution. Some loss of this component is unavoidable as the precipitate is not washed — in order not to increase the volume of the solution unnecessarily.

Then the ammonium sulphate concentration was increased to 75 % saturation, and the mixture was left standing till the following day before the precipitate was separated by filtration. The precipitate consisted chiefly in albumin, the iron-containing protein, a relatively small amount of globulins and hemoglobin.

The precipitate was dissolved in water and dialyzed for two days against running tap-water in order to render the solution salt-free. The dialyzed solution was then mixed with so much sodium acetate-acetic acid buffer with pH 4.8 that the pH of the mixture became 5.2. The buffer was added under continous stirring during which the pH was watched by means of a glass electrode. The specific resistance of the solution was used for control of the salt concentration. The specific resistance of the solution should not be lower than 1000 ohms if a good separation is to be obtained in the following step of the procedure.

The temperature of the solution was then lowered to 0° C. Under continuous stirring, 60 vol. % ethyl alcohol, with a temperature of —5° C, was added slowly through a fine glass tube immersed in the solution. Throughout this part of the procedure it is essential to keep the temperature under +4° C. When the alcohol concentration in the mixture reached 20 vol.%, the addition of alcohol was stopped. A strong precipitation was obtained. The mixture was left standing over night at +4° C and then the precipitate was separated

by filtration at the same temperature in refrigerator. The solution thus obtained was brilliantly red in color.

Then the alcohol concentration in the filtrate was increased to 25 vol. %, and the resulting precipitate was removed by filtration after the mixture had been left standing for 24 hours at from —3° C to —5° C whereafter the precipitate now formed was removed by filtration (it was soluble in physiological salt solution, and it consisted partly in iron-containing protein, partly in impurities). The remaining protein in the mother-liquor consisted chiefly of the iron-containing protein. By lowering the temperature of the mother-liquor to about —15° C the iron-containing protein could be precipitated as a beautiful red sediment which again dissolved readily in neutral solutions. The solution of the fractionated iron-containing protein obtained in this way was freed from alcohol through dialysis against running water. In frozen condition, the solution of the iron-containing protein could be stored without any change taking place in its properties.

A reprecipitation of the iron-containing protein was performed after the schema outlined above in order further to rid it of impurities.

(The optimal fractionation is obtained when the specific resistance in the solution prior to the addition of alcohol is higher than 1000 ohms.)

PROPERTIES OF THE IRON-CONTAINING PROTEIN

The purified iron protein complex may be precipitated neither with 50 % saturated ammonium sulphate nor with saturated sodium sulphate solution. As is evident from the above, the complex is precipitated in mixture with the other serum proteins in the albumin fraction. Nor is the iron-containing protein precipitated on dialysis against distilled water till all the salt is removed. With a view to its aspects of solubility, there is no reason to designate the iron-containing protein as a globulin.

In order to test the purity of the iron-containing protein prepared in the way here described, experiments were carried out with electrophoresis, ultracentrifuging and diffusion.

The electrophoresis experiments were carried out with the apparatus given by Tiselius and optical reading *ad modum* Svensson [14]. For these experiments we employed a buffer containing primary and secondary sodium phosphate with a combined ionic strength of 0.1. Besides, the buffers were 0.1 molar in NaCl, so that the total ionic strength was 0.2. A preliminary experiment with a 1.2 % solution with pH 6.8 showed the substance to be homogeneous electrophoretically. Then a number of experiments were made at 0.0° C with 0.5 %

solutions with pH varying from 5.1 to 7.3. The iso-electric point for the iron-containing protein determined in these phosphate buffers was about 4.4 (extra polated value). Table 1 gives the mobility of the substance and the pH of the solution for the descending side. As will be noticed, the rate of movement was low. (With pH under 5 the iron partly splits off from the protein, on which account we have omitted some values obtained for the mobility in acetate buffers with pH lover than 5.)

The experiments with ultracentrifugation were performed in a Svedberg ultracentrifuge with solutions containing varying percentages of protein dissolved in a buffer made up of 0.025 *M* Na_2HPO_4, 0.025 *M* NaH_2PO_4 and 0.10 *M* NaCl. (60,000 r. p. m. Registration was made by Lamm's scale method.) Also the ultracentrifuge tests showed that the protein was practically pure.

Table 2 gives the values obtained for the sedimentation constant (in Svedberg units). (As to the performance of the ultracentrifuge experiments, the reader is referred to [15].)

From the values recorded in Table 2, the value for concentration 0 is calculated as $s_{20} = 5.8\ S$.

Table 1. Electrophoretical measurements.

pH	Mobility in cm^2/s-volt $\times 10^5$
7.35	2.5
6.85	2.2
6.75	2.4
5.95	1.3
5.45	1.5
5.10	0.8

Table 2. Ultracentrifuge experiments.

Protein concentration in %	Sedimentation constant in Svedberg units
0.80	5.60
0.60	5.86
0.30	5.78
0.26	5.69
0.20	5.68
0.15	5.91
0.08	5.83
0.00 (extrapolated)	5.80

Table 3. Diffusion experiments.

Protein concentration in %	Diffusion constant $D_A \times 10^7$
0.8	5.82
0.5	5.82

Table 4. Iron content of the iron-binding protein.

Preparation no	No of atoms of iron per protein molecule
1	1.5
2	1.3
3	1.3
4	1.4

Also diffusion experiments were carried out with the iron-containing protein dissolved in the same buffer as in the ultracentrifuge tests. In these experiments the concentration varied between 0.5 and 0.8 %. Registration was made by Lamm's scale method. No dependence upon the concentration could be established. The values of the diffusion constant D_A, calculated by the »method of areas» have been summarized in Table 3. The mean value obtained was a diffusion constant $D_A = 5.8 \times 10^{-7}$.

No determination of the partial specific volume has been made. We have reckoned with $V = 0.725$ as given in the articles by Cohn [11] and by Oncley, Scatchard and Brown [12].

By means of these values for the sedimentation constant, diffusion constant and partial specific volume, on employment of Svedberg's formula [15], we thus obtained the molecular weight M = 88,000 for the iron-containing protein here prepared. The »frictional ratio» f/f_0 was calculated to be 1.25. When the molecule is assumed to have the form of a revolution ellipsoid, the ratio between the length and the width of the molecule is 4.9, and the length of the molecule is 170 Å units, while its width is 35 Å units.

The amount of specifically bound iron was determined in four quite different preparations (new serum for each preparation) of the iron containing protein. The results are shown in Table 4. As is seen from the table the iron content varies between 1 and 2 atoms of iron per molecule (mol.wt. = 88,000). It suggests that most probably the iron binding protein can specifically combine with a maximum number of two atoms of iron. The fact that we have not obtained a whole number of iron atoms per molecule can perhaps be explained in different ways. Probably it depends on the facts that a part of the iron has been split off during the preparation and that the ability to combine with iron is easily reduced. (During the preparation the solution has been maintained at pH 5.2.)

SUMMARY

A description is given of the isolation of the iron-binding protein of swine serum. The properties of the thus isolated iron-containing protein are investigated. The molecular weight is estimated as being 88,000, and the iso-electric point as 4.4.

The authors wish to acknowledge their indebtedness to Professors T. Svedberg and A. Tiselius as well as to Docent C. G. Holmberg for their kind permission to carry out this work in their laboratories.

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Preliminary Communications

Isolation of a Phosphorus-rich Substance of High Molecular Weight from *Aspergillus niger*

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While investigating phosphorus metabolism in moulds, Mann made the very interesting observation that extracts from *Aspergillus niger* contained not only pyrophosphate but also metaphosphate [1]. However, he made no determinations of the molecular weight of this metaphosphate. During investigations of the enzymatic breakdown of synthetic polymetaphosphate of very high molecular weight we made preliminary experiments which showed that a fraction of the naturally occurring metaphosphate (from *A. niger* is not dialyzable and therefore may possess a relatively high molecular weight[2].

In 1936 MacFarlane isolated from yeast a nucleic acid preparation which contained 16—18 % P[3]. Later Wiame also isolated from yeast such preparations which contained 17 % P and 8 % N [4, 5].

In our continued experiments we have now shown that it is possible to isolate from *A. niger* a high molecular, non-dialyzable substance with a very high phosphorus-content. This substance does not contain nucleic acid.

For the experiments we have used a culture of *A. niger* v. Tiegh. (no. 594 from the National Collection of Type Cultures maintained in Britain by the Medical Research Council). The mould was cultivated at 28° C on a medium of the following composition: 10 g glucose, 0.2 g K_2HPO_4, 0.4 g $NaNO_3$, 0.05 g $MgSO_4$, $7H_2O$ and 100 ml water. After 8 days' growth the mould was ground in a »Waring

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Blendor» together with active carbon (Norit) in a 2 % solution of sodium carbonate. The extract was then immediately filtered two times through large amounts of active carbon. (The enzyme which breaks down polymetaphosphate has its optimum activity at pH 5—6, which has been demonstrated in our other experiments [6]. It is therefore of importance to keep a rather high pH during the extraction. In the filtration through active carbon the enzyme, which breaks down polymetaphosphate, and of course other organic substances, are removed. At low and high pH the spontaneous breakdown velocity of polymetaphosphate is considerable [2, 7]. It is therefore of importance that the pH of the extract is not made too low or too high.) The clear extract was then dialyzed in a cellophane bag against distilled water for 2 days at 4° C. The extract was then evaporated *in vacuo* at 30° C to about 1/50 of its original volume. The concentrated solution was then filtered again through a small amount of active carbon. Then the solution was dialyzed again one day at 4° C. The solution was then frozen and dried *in vacuo* in the frozen state. A small amount of a white powder was obtained.

This non-dialyzable substance contained 25 % P. (Determined after hydrolysis with sulphuric acid according to Lowry and Lopez [8].) A solution of the substance was investigated in the Beckman spectrophotometer. The characteristic absorption of nucleic acids in ultraviolet could not be observed. (The isolated substance contained less than 0.5 % nucleic acid.) The substance contained 15 % Na (determined by W. Kirsten). The carbohydrate content was less than approximately 5 % determined with the orcinol method. The nitrogen content was low, less than 1 %. A small fraction was heated in a crucible. The ash content was 85 %.

The substance was investigated in the Svedberg ultracentrifuge. The experiments were carried out with 1.5 % and 0.5 % of the substance in a buffer solution with the following composition: 0.025 *M* Na_2HPO_4, 0.025 *M* NaH_2PO_4 and 0.10 *M* NaCl. The »ultracentrifugation diagrams» showed a sedimenting substance, which was polydisperse. Most of the substance could be recovered as sedimenting material under the »peak» of the centrifugation diagram. The calculated sedimentation constants were 2.2 and 2.3 Svedberg units for the 1.5 and 0.5 % solutions.

It seems most probable that the isolated substance is a somewhat impure, high molecular sodium polymetaphosphate, *i. e.* an inorganic colloid.

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2-(Diphenylmethoxymethyl)-imidazoline, a New Potent Antihistamine Agent

RICHARD DAHLBOM AND BERTIL SJOGREN

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Ever since the discovery of the first synthetic therapeutically effective antihistamine agent [1], N-phenyl-N-benzyl-N',

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2-(Diphenylmethoxymethyl)-imidazoline, a New Potent Antihistamine Agent

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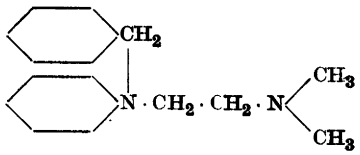
Ever since the discovery of the first synthetic therapeutically effective antihistamine agent [1], N-phenyl-N-benzyl-N',

N'-dimethyl-ethylenediamine (Antergan; I), this field has been made the object of eager investigation. Thus Miescher, Klarer and Urech [2] found that the histaminolytic effect was strongly increased by the exchange of the dimethylaminomethyl group of Antergan for an imidazoline group. The compound thus formed is 2-(N-phenyl-N-benzylaminomethyl) - imidazoline (Antistin; II).



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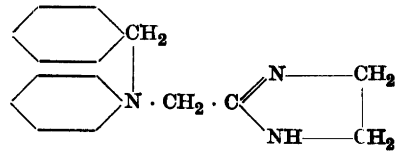

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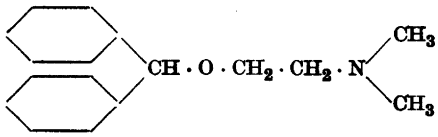
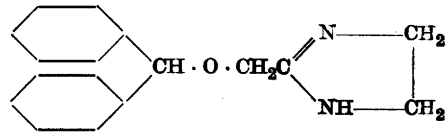


III. *Benadryl.*



IV.

Another compound of a somewhat different type, which has an antihistamine effect comparable with that of Antistin [3, 4], is β-dimethylaminoethyl benzhydryl ether (Benadryl; III).

Also in this laboratory we have been working with synthetic antihistamine compounds since the middle of the year 1946. As the exchange of the dimethylaminomethyl group in Antergan for imidazoline yielded a compound with a strongly increased effect, it seemed to us to be worth while investigating whether a similar substitution in Benadryl could yield a compound with a still better effect than that of any of the compounds mentioned above.

We have succeeded in preparing this compound, 2-(diphenylmethoxymethyl)-imidazoline (IV), as well as a series of allied compounds. We had not intended to publish the results until our experiments were quite completed, but on account of a communication by an Italian author [5], with some pharmacological data concerning compound IV, we feel obliged to publish the mode of synthesis, which is not mentioned in the Italian publication.

Compound IV is prepared by treating the sodium salt of benzohydrol with 2-(chloromethyl)-imidazoline hydrochloride.*

Preliminary pharmacological investigations show that as an antihistamine agent it is at least as strong as Antistin and Benadryl.

Complete chemical and pharmacological data concerning this and a number of allied compounds will be published later.

2-(Diphenylmethoxymethyl)-imidazoline. 18.4 g (0.1 mole) of benzohydrol was dissolved in 75 ml dry toluene, and 2.3 g (0.1 mole) of powdered sodium was added. The mixture was warmed with stirring for two hours at 50° C, and then for a further two hours at 105° C when the sodium metal was gradually consumed. After cooling to roomtemperature, 8.75 g (0.05 mole) of 2-(chloromethyl) - imidazoline hydrochloride [6] was added. The mixture was warmed at 60° C for 1 hour and 15 minutes with

* Swedish patent application has been made.

vigorous stirring. After cooling, the separated sodium chloride was filtered off, and the ice-chilled filtrate was precipitated with a saturated solution of dry hydrogen chloride in toluene, until no more precipitate was obtained. The precipitate, which was white and crystalline, was sucked off and washed with a little petroleum ether. The raw product contained about 20 % NaCl, which was removed by dissolving the hydrochloride in alcohol, filtering off the salt and precipitating the hydrochloride with petroleum ether. The yield was 6.7 g (44 %). After recrystallization from alcohol the pure 2-(diphenylmethoxymethyl)-imidazoline hydrochloride melted at 203—205° C.

$C_{17}H_{18}N_2O \cdot HCl$ (302.8)

Calc.	N 9.25	Cl 11.7
Found	» 9.2	» 11.7

The free base was obtained by dissolving the hydrochloride in water and precipitating with *N* NaOH. This yielded an oil, which soon hardened into white crystals. After recrystallization from alcohol it melted at 102—103° C.

$C_{17}H_{18}N_2O$ (266.3)

Calc.	N 10.5	eq. wt. 266.3
Found	» 10.6	» 267.2 (titr. with 0.1 *N* H_2SO_4 with methyl red as indicator).

With picric acid the base gave a picrate with m. p. 202—204° C.

The hydrochloride was very soluble in water and gave a stable solution. If the solution was acidified with hydrochloric acid it almost immediately disintegrated into benzohydrol and 2-(hydroxymethyl)-imidazoline hydrochloride.

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3. Rieveschl, G., Jr. *U. S. P.* 2421714 (1947).
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5. Cavallini, I. G. *Farm. sci. e tec.* **2** (1947) 89, 94; *C. A.* **41** (1947) 6989.
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Substituted Benzyl Alcohols as Lignin Models

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In the discussions on the nature of the reactive group in lignin responsible for its sulphonation with sulphite cooking acid different model substances have played an important *rôle*. The model substances hitherto studied are subject to two fundamental objections. They either react far to slow or contain groups which, obviously, do not occur in lignin, at least not to such a degree that they can be responsible for the sulphonation reaction. It has recently been shown that it is possible to introduce 1.3 sulphonic acid groups on each »lignin unit» (1 methoxyl) [1].

Unpublish results of Erdtman and Lindgren [2] appear to show that the sulphonation which occurs in technical sulphite cookings and which results in the formation of lignin sulphonic acids with the ratio $SO_3H/OCH_3 = {}^1/_2$ is due to a substitution of hydroxyl groups of outstanding reactivity.

Holmberg [3] showed in an important paper that α-phenethyl alcohol reacts with sulphite cooking acid with formation of α-phenethyl-sulphonic acid $C_6H_5 \cdot CH(OH) \cdot CH_3 \rightarrow C_6H_5 \cdot CH(SO_3H) \cdot CH_3$. Kratzl and Däubner [4] point out that the

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yield of sulphonic acid in this reaction is low and therefore consider this alcohol not to be a suitable lignin model.

Investigations on the action of sulphite cooking acid on substituted chalkones [5] and benzyl ethers [6], however, show that substitution in the benzene ring has a great influence on the reactivity. It appeared to be possible that suitably substituted benzyl alcohols might prove to be better lignin models than α-phenethyl alcohol and benzyl alcohol. For that reason a series of alcohols which appear to be more lignin similar have been subjected to sulphite cookings. Vanillyl alcohol (I), veratryl alcohol (II), apocynol (III) and O-methyl-apocynol (IV) were heated with normal sulphite cooking acid and it was found that they react very quickly and quantitatively to the corresponding sulphonic acids (V—VIII). These alcohols, undoubtedly, are the best lignins models available at present. They tally with lignin not only as regards sulphonation but also in other respects e. g. condensation with reactive phenols such as resorcinol and phloroglucinol under the conditions of the sulphite cooking. It is therefore possible to imitate the inhibition of the sulphite digestion of pine heart wood with these alcohols.

A more complete communication on these reactions and the reactivity of allied compounds will follow shortly.

$4,3\text{-}(HO)(H_3CO)C_6H_3 \cdot CH_2OH \rightarrow 4,3\text{-}(HO)(H_3CO)C_6H_3 \cdot CH_2SO_3H$
I V

$4,3\text{-}(H_3CO)_2C_6H_3 \cdot CH_2OH \rightarrow 4,3\text{-}(H_3CO)_2C_6H_3 \cdot CH_2SO_3H$
II VI

$4,3\text{-}(HO)(H_3CO)C_6H_3 \cdot CH(OH) \cdot CH_3 \rightarrow 4,3\text{-}(HO)(H_3CO)C_6H_3 \cdot CH(SO_3H) \cdot CH_3$
III VII

$4,3\text{-}(H_3CO)_2C_6H_3 \cdot CH(OH) \cdot CH_3 \rightarrow 4,3\text{-}(H_3CO)_2C_6H_3 \cdot CH(SO_3H) \cdot CH_3$
IV VIII

M. p. of pyridin salt of: V 189—190°; VI 149°; VII 164—164.5°; VIII 148°.

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The Acidic and Basic Properties of Oxides

II. The Thermal Decomposition of Pyrosulphates

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In a previous communication [1] dealing with the acid-base properties of the oxides, the influence of the cations on the oxygen bridges of the polyacids was discussed.

It was stated as a general rule that the stability of the oxygen bridges decreases with increasing polarizing power of the cation.

A particularly simple type of reaction which clearly demonstrates this rule is the thermal decomposition of pyrosulphates.

In the pyrosulphate ion two SO_3-groups are connected by a single oxygen bridge

$$[O_3S—O—SO_3]^{2-}$$

and the thermal decomposition consists in a breaking of the bridge

$$[O_3S — O — SO_3]^{2-} = SO_4^{2-} + SO_3$$

An account will now be given of some experimental investigations concerning the influence of the cations on the decomposition equilibrium.

The data on pyrosulphates in the literature are rather scarce.

Weber [2] prepared the pyrosulphates of Na, K, Rb, Cs, Ag and Tl. The pyrosulphates of Na and Ag are obtained directly by heating the sulphate with SO_3 in sealed tubes at 100—150° C. By heating K, Rb, Cs and Tl-sulphates with SO_3 to 100° C, compounds were formed to which were ascribed the formula $Me_2O \cdot 8SO_3$. By heating these compounds further, the normal pyrosulphates were formed.

Baumgarten and Thilo [3] have also prepared a tripyrosulphate $K_3S_3O_{10}$, which is stable in SO_3-atmosphere in the interval 100—150° C.

The data on the thermal decomposition of the pyrosulphates are mainly of a qualitative nature. It is stated that they decompose at temperatures of about red heat.

The equilibrium data of Ishikawa, Masuda and Hagisawa [4] for the reaction

$$2\ NaHSO_{4\ (s)} = Na_2S_2O_{7\ (s)} + H_2O_{(g)}$$

should permit a computation of the change in free energy and the heat of reaction for the decomposition

$$Na_2S_2O_{7\ (s)} = Na_2SO_{4\ (s)} + SO_{3\ (g)}$$

We find

$$\Delta F_{298} = 20.0 \text{ kcal}$$
$$\Delta H = 30.5 \text{ kcal}$$

For the reaction

$$K_2S_2O_{7\ (s)} = K_2SO_{4\ (s)} + SO_{3\ (g)}$$

Berthelot's calorimetric measurements [5] give, however, the value

$$\Delta H = 37{,}900 \text{ kcal}$$

EXPERIMENTS

For the reaction

$$S_2O_7^{2-}{}_{(melt)} = SO_4^{2-}{}_{(melt)} + SO_{3\ (g)}$$

we have

$$K = \frac{a_{SO_4^{2-}} \quad P_{SO_3}}{a_{S_2O_7^{2-}}}$$

Values of

$$K' = \frac{N_{SO_4^{2-}} \quad P_{SO_3}}{N_{S_2O_7^{2-}}}$$

(N = mole fraction) are found by determining the ratio $N_{SO_4^{2-}}/N_{S_2O_7^{2-}}$ in melts in equilibrium with a known SO_3-tension.

The composition of the melt was found by a simple gravimetric method. Starting with a weighed charge of known composition (preferably pure sulphate), the amount of SO_3 expelled or absorbed during adjustment of the equilibrium could be determined by weighing after quick cooling.

During the experiments SO_3-gas of 1 atm was conveyed upwards through a vertical furnace past a platinum crucible containing the weighed sulphate-pyrosulphate charge (Fig. 1). As the experiments were carried out at temperatures where SO_3 is partly decomposed, it must be secured that the decomposition

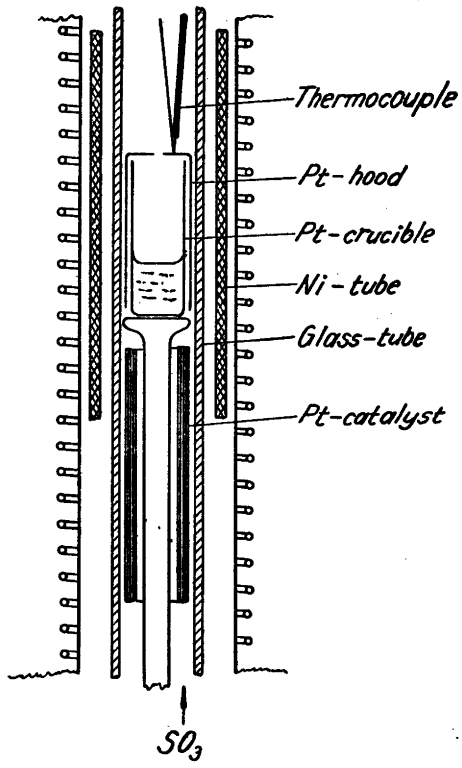


Fig. 1. Apparatus.

$$2\ SO_3 = 2\ SO_2 + O_2$$

is carried to completion. A platinum catalyst was therefore placed in the heating zone immediately beneath the platinum crucible, which was moreover placed under a platinum hood. The flow velocity of SO_3 was kept low. In calculating the SO_3-tension the well known data of Bodenstein and Pohl, Tayler and Lehner, Kapustinsky and Shamovsky were used [6, 7, 8].

Our investigations included the decomposition equilibria for Li, Na, K, Ag and Tl-pyrosulphates. Moreover, a few experiments were carried out in which Na_2SO_4, Li_2SO_4 and $PbSO_4$ were added to the K-system. The results of the former experiments are given in Table 1.

By plotting log K' against $1/T$ we get straight lines within the limits of error. Hence, if the melts may be regarded as ideal mixtures, the slope would represent the heats of decomposition of the pyrosulphates. This assumption is, however, presumably not permissible, and

$$R\frac{d\ \log\ K'}{d\ ^1/_T}$$

Table 1. The equilibrium between SO_3 gas and sulphat-pyrosulphate melts.

System	$t°$ C	mol % $Me_2S_2O_7$	$P_{SO_3(atm)}$	log K'
Ag_2SO_4—$Ag_2S_2O_7$	347	97.72	1	— 1.63
»	395	91.43	1	— 1.03
»	423	82.21	1	— 0.66
»	442	67.05	0.95	— 0.33
»	491	48.93	0.89	— 0.03
Li_2SO_4—$Li_2S_2O_7$	371	99.24	1	— 2.12
»	401	96.85	1	— 1.49
»	417	96.63	1	— 1.46
»	427	94.58	1	— 1.24
Tl_2SO_4—$Tl_2S_2O_7$	554	96.01	0.80	— 1.48
»	585	89.80	0.72	— 1.09
»	623	76.14	0.65	— 0.69
»	661	59.75	0.53	— 0.45
Na_2SO_4—$Na_2S_2O_7$	555	96.10	0.79	— 1.50
»	582	90.61	0.73	— 1.11
»	603	87.28	0.68	— 1.01
»	618	81.62	0.63	— 0.85
»	635	73.33	0.60	— 0.67
»	645	64.80	0.56	— 0.52
»	655	59.39	0.55	— 0.43
K_2SO_4—$K_2S_2O_7$	653	98.77	0.56	— 2.16
»	669	98.26	0.503	— 2.05
»	698	95.76	0.430	— 1.72
»	727	92.12	0.366	— 1.50

may therefore only be taken as a rough measure for ΔH

[Li	32]	kcal
Na	40	»
K	40	»
Ag	26	»
Tl	38	»

The value for Na differs remarkably from the value computed from the data of Ishikawa, Masuda and Hagisawa (30.5 kcal). The value for K however agrees with Berthelot's calorimetric measurement (37.9 kcal).

The experiments with addition of Li_2SO_4, Na_2SO_4 and $PbSO_4$ to $K_2S_2O_7$-K_2SO_4 were carried out at different temperatures, *viz* 662—87° C, corresponding to an original equilibrium ratio $N_{SO_4^{2-}}/N_{S_2O_7^{2-}} = 0.015$—30, and at 779° C,

Table 2. The effect of Na^+, Li^+ or Pb^{2+} on the equilibrium between SO_3 gas and melts of $K_2SO_4 - K_2S_2O_7$.

System	Mol % Li^+ Na^+, $Pb^{2+}/2$	$t°$ C	$N_{SO_4^{2-}} = \frac{N_{SO_4^{2-}}}{N_{SO_4^{2-}} + N_{S_2O_7^{2-}}}$
$Na^+—K^+—SO_4^{2-}—S_2O_7^{2-}$	0	662	1.5
»	7.1	»	2.6
»	17.0	»	6.7
$Li^+—K^+—SO_4^{2-}—S_2O_7^{2-}$	0	687	2.9
»	12.3	»	11.6
»	18.1	»	18.2
»	28.0	»	26.2
»	0	779	25.0
»	9.8	»	36.6
»	17.0	»	56.0
»	23.4	»	71.7
$Pb^{2+}—K^+—SO_4^{2-}—S_2O_7^{2-}$	0	687	2.9
»	3.1	»	7.3
»	8.4	»	18.9

corresponding to an original equilibrium ratio $N_{SO_4^{2-}}/N_{S_2O_7^{2-}} = 0.3$. The results of these experiments are shown in Table 2.

DISCUSSION

In Fig. 2 log K' is plotted against T. It appears from the curve that the stability of the pyrosulphates increases strongly in the order

$$Ag — Li — Na, Tl — K$$

For the inert gas ions the stability increases with the radius of the cation (Li—Na—K). The pyrosulphates of Ag and Tl show, however, that this is not the only factor of importance. $Ag_2S_2O_7$ is far more easily decomposed than $Na_2S_2O_7$, and similarly $Tl_2S_2O_7$ decomposes more easily than $K_2S_2O_7$, despite the fact that the inert gas ions in both cases have the smaller ionic radii. Hence it seems that the polarizing power of the cation is an important factor determining the stability of the oxygen bridge of the pyrosulphate ion. As is well known, the polarizing power is stronger for ions with 18 or 20 outer electrons than for ions with inert gas configuration (octet) of the same radius.

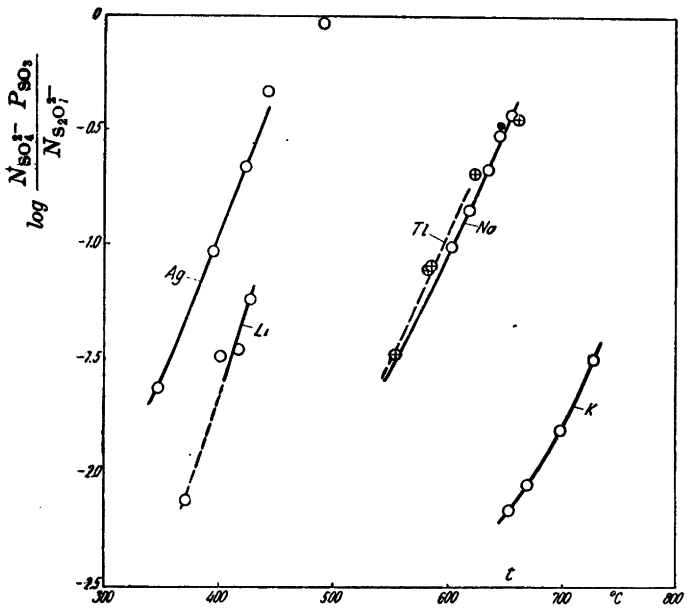


Fig. 2. The variation of log K′ with the temperature.

The preparation of Li-pyrosulphate does not seem to be mentioned in the literature previously. It is, however, readily formed by sulphate in SO_3-atmosphere at temperatures approaching 300° C, and may after cooling be kept unchanged in dry air.

As mentioned above some experiments were carried out in which Na_2SO_4, Li_2SO_4 and $PbSO_4$ were added to the K_2SO_4—$K_2S_2O_7$ mixture, in order to get closer information as to the relations which determine the stability of the pyroion bridge. Fig. 3 shows how the mole fraction of sulphate at equilibrium is increased by the addition (at constant SO_3-tension and at constant temperature). It appears that an addition of Li-,Na-or $PbSO_4$ (beyond a certain limit) causes an increase in the mole fraction of sulphate proportional to the mole fraction of the cation added. The effect increases in the order Na, Li, Pb.

This change corresponds at 687° C to an expulsion of approximately 0.5 mole SO_3 per mole Li and approx. 2 moles SO_3 per mole Pb replacing K in the melt. (At 662° C the corresponding expulsion by exchange of K by Na amounts to 0.2 mole SO_3/Na). The formal explanation of this must be, that each Li^+ takes hold of ½, and each Pb^{2+} of 2 SO_4^{2-} ions, which have to be formed by decomposition of $S_2O_7^{2-}$ whereby SO_3 is expelled. An explanation for this might be, for example, that Pb coordinates 6 oxygen ions from 2 SO_4^{2-}-groups as schematically represented by

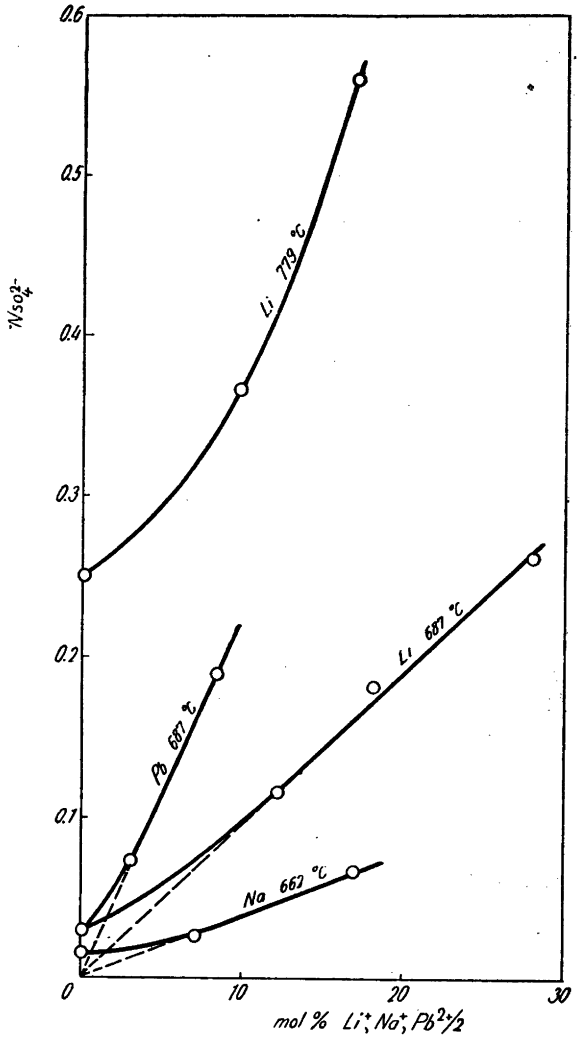
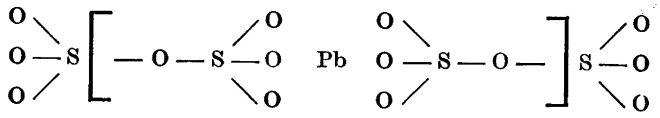


Fig. 3. The variation of the mole fraction of sulphate with addition of Li_2-, Na_2- or $PbSO_4$ to equilibrium melts of K_2SO_4-$K_2S_2O_7$.



The SO_3-group is polarized to such an extent that it can no longer maintain the oxygen bridge. At temperatures a 100° higher (775—780°) the effect of Li is increased to ca. 1 SO_3/Li. This indicates that the coordination effect of Li^+ is a statistical phenomenon.

Lux and Rogler[9] have shown by means of the chromate indicator that the acid character of boron oxide in Na borate melts at 1100° C is strongly increased by addition of various oxides, among others Pb and Li. These effects are undoubtedly of a similar nature to those described in this paper, though their method of investigation lends itself less to quantitative conclusions. Thus it is uncertain to what extent the indicator-acid-base-equilibrium itself is influenced by the cations present. But it seems as if in their case each Pb^{2+}-ion is able to break ca. 6 B-O-B-bridges, while each Li^{+}-ion does not break more than 1—2 bridges. The systems concerned had a high boron oxide content, each BO_3-group was sharing 2—3 oxygen bridges. Hence the primary phenomenon in this case as well appears to be that the Pb^{2+}-ion coordinates 6 oxygen ions (*i. g.* from 2 BO_3-groups which are thereby so strongly polarized that they are unable to form B-O-B-bridges.

The measurements of Lux and Rogler seem to indicate that the number of bridges which are broken per cation added (additions below 20 %) decreases in the sequence $Ba^{2+} \geqq Mg^{2+} \gg Be^{2+}$. This shows clearly that the size of the cation is the decisive factor for the *number* of oxygen bridges to be broken per cation (provided the polarizing power of the cation exceeds a certain amount). The Ba^{2+}-ion is sufficiently large to coordinate 12 oxygen ions, and should therefore be able in principle to break 12 oxygen bridges. The smaller Mg^{2+}-ion can coordinate a maximum of 6, while the Be^{2+}-ion is only able to coordinate 4 oxygen ions.

SUMMARY

1. The equilibrium between SO_4^{2-}—$S_2O_7^{2-}$ melts and SO_3-gas shows that the stability of the pyrosulphates on thermal decomposition according to the equation

$$S_2O_7^{2-} = SO_4^{2-} + SO_3$$

increases in the order

$$Ag — Li — Na,\ Tl — K$$

This shows that the stability of the oxygen bridge

$$S — O — S$$

is highly influenced by the polarizing power of the cation. This effect is also examined by addition of Na_2SO_4, Li_2SO_4 or $PbSO_4$ to K_2SO_4—$K_2S_2O_7$-melts. The results are discussed.

2. The compound $Li_2S_2O_7$ has been prepared. It is formed by heating Li_2SO_4 in SO_3-atmosphere at temperatures below 300° C. The crystals may be kept unchanged in dry air.

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The Acidic and Basic Properties of Oxides

III. Relative Acid-Bace Strenghts of some Polyacids

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In a previous communication [1] the general traits of the acid-base properties of the oxides were outlined. The acid-base function in systems built up of oxides may, according to Lux, be defined as

$$\text{acid} + O^{2-} \rightleftharpoons \text{base}$$

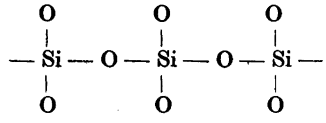
where O^{2-} is an oxygen ion.

The present paper will deal with the acid-base equilibria in systems with polyacids in more detail.

The characteristic feature of the polyacids is the linking of the single groups to large complexes by means of oxygen bridges of the type

$$X - O - X$$

e. g. $[SiO_3^{2-}]_n$ may consist of SiO_4-tetrahedra which are linked together to long chains



or rings where each SiO_4-group has two shared oxygen atoms. $[SiO_{2.5}^-]_n$ forms in a similar way two dimensional nets of SiO_4-tetrahedra, $[BO_2^-]_n$ forms chains or rings of BO_3-groups etc.

These *macromolecular structures* are well known from the lattice investigations of solid compounds. They appear, however, in melts as well, as is indicated for instance by high viscosity and a tendency to form glasses on cooling.

The tendency to form structures with oxygen bridges is especially noticeable in ions of medium ionic potential (*i. e.* charge/radius), as indicated by Zintl and Morawietz [2]. This is illustrated by the crystal structures of the oxides formed by the elements,

Mg, Al, Si, P, S, Cl.

Compare

MgO	SiO_2, (B_2O_3)	SO_3, Cl_2O_7
simple ionic crystal	macromolecular structure	molecular crystal

The transition of a polyacid to its corresponding base consists in a breaking of an oxygen bridge and a simultaneous binding of an oxygen atom.

The acid-base reaction is thus connected with a disintegration of the macromolecular structure. This must presumably take place in steps, as represented schematically by

$$SiO_2 \text{ (space lattice)} \rightarrow SiO_{2,5}^{-} \text{ (nets)} \rightarrow$$
$$SiO_3^{2-} \text{ (chains, rings)} \rightarrow Si_nO_{3n+1}^{(2n+2)-} \text{ (pyro ions etc.)} \rightarrow$$
$$SiO_4^{4-} \text{ (orthosilicate ion)}$$

Hence it seems likely that a correspondingly complicated scheme of reaction has to be applied to explain the acid-base properties of the polyacids.

In the literature only few investigations in this field have been reported, despite the great practical significance in metallurgy and ceramics, glass-technology etc.

Lux and Rogler [3] have investigated acid-base reactions in sodium borate glass by means of the Cr^{VI}/Cr^{III} indicator. These experiments are refered to in a previous communication [1].

There is, however, another type of acid-base reaction, which seems well suited for the study of the acid-base properties of these substances, namely equilibria of the type

$$CO_3^{2-} + \text{acid} \rightleftharpoons CO_2 + \text{base}$$

Several investigations on systems of this kind have already been carried out (*cf.* Niggli [4], Krøger and Fingas [5]). In this connection Niggli's investigations on the systems

$SiO_2 - K_2CO_3 - CO_2$, $SiO_2 - Na_2CO_3 - CO_2$ and
$SiO_2 - Li_2CO_3 - CO_2$,
$TiO_2 - K_2CO_3 - CO_2$ and $TiO_2 - Na_2CO_3 - CO_2$

are of special interest. He investigated homogeneous melts in equilibrium with CO_2 gas at one atmosphere at different temperatures and compositions of the melts. The results thus obtained render possible a discussion of the value of the acid-base constants of the polyacids. The present authors have extended this material by investigating the systems

$B_2O_3 - K_2CO_3 - CO_2$, $B_2O_3 - Na_2CO_3 - CO_2$ and
$B_2O_3 - Li_2CO_3 - CO_2$.

An account will now be given of the experimental methods and the results obtained, after which the experimental material will be discussed.

EXPERIMENTAL

In a platinum crucible alkali carbonate and B_2O_3 or alkali tetraborate were weighed to a total amount of 1—2 g. The mixture was carefully melted over a gas burner, whereby the reaction between carbonate and borate might take place without spraying. The crucible was then placed in the furnace, which consisted of a long, vertical porcelain tube heated by an electric coil. The middle part of the porcelain tube was surrounded by a nickel tube (ca. 25 cm long) in order to equalize the temperature in the reaction zone. A slow stream of CO_2 dried by P_2O_5 was conveyed through the furnace. The crucible was placed in the lower, cold part of the tube and the air replaced by CO_2, whereafter the crucible was raised into the reaction zone, immediately below a Pt-Rh-thermocouple (protected by a pythagoras mantle).

A heating time of about 30 minutes proved to be sufficient to attain equilibrium with the charges used. The equilibrium was then »frozen» by removing the crucible from the reaction zone as quickly as possible. After cooling (in a desiccator) the amount of CO_2 expelled at the equilibrium temperature was determined by weighing the crucible (in a weighing bottle). The greatest source of error in this procedure is the measurement of the temperature in the reaction zone.

The EMF of the thermocouple was measured by compensation to an accuracy of 0.000001 V, which corresponds to 0.1° C. However, this constancy of temperature could not be attained owing to the strong pulsations in the voltage on the heating circuit. Even by using a voltage stabilizer and regulating by hand, an accuracy better than $\pm$ 1° could not be attained.

The experimental results are shown in Table 1, which gives the original composition of the charge and the amount of CO_2 expelled, further the calcu-

Table 1. $M_2CO_3 - B_2O_3$ at 1000° C.

M	M_2CO_3	mol · 10^{+2}		O/B	$-\log N_{CO_3^{2-}}$
		B_2O_3	expelled CO_2		
K	0.796	0.0771	0.0818	2.03	0.085
	»	0.223	0.241	2.04	0.256
	0.805	0.227	0.237	2.02	0.256
	»	0.409	0.432	2.03	0.505
	»	0.502	0.530	2.03	0.670
Na		$Na_2B_4O_7$			
	2.003	0.0127	0.0472	2.68	0.011
	1.370	0.0147	0.0505	2.90	0.019
	»	0.0529	0.1615	2.51	0.070
	»	0.1675	0.424	2.38	0.233
	»	0.314	0.734	2.34	0.474
	»	0.465	0.985	2.28	0.749
	1.612	0.0142	0.0495	2.63	0.016
	»	0.0454	0.144	2.55	0.051
	1.560	0.1107	0.330	2.48	0.135
	0.455	0.0668	0.165	2.37	0.284
Li		B_2O_3			
	1.683	»	1.660	2.65	2.735
	2.345	»	2.005	2.93	0.719
	2.97	»	2.10	2.99	0.426
	1.007	0.363	0.945	2.80	1.105
	1.132	»	1.002	2.88	0.816
	1.310	»	1.043	2.97	0.599
	1.460	»	1.052	2.98	0.460
	1.330	0.460	1.235	2.84	1.033

(N is the mole fraction refering to a mixture of the components $BO_{1.5}$, BO_2^-, etc. and CO_3^{2-}.)

lated degree of the reaction between CO_3^{2-} and polyborate (given as average number of oxygen atoms per borate group).

Some orientating experiments regarding the dependence of the equilibrium on dilution were also carried out. In these the carbonate-borate system was diluted with alkali sulphate. These relations will later be subjected to a more thorough investigation, but in view of the subsequent discussion a few results are given i Table 2.

Table 2. Na_2CO_3—B_2O_3 diluted with sulphate at 1000° C.

mol · 10^{+3}				O/B	-log $N_{CO_3^{2-}}$
Na_2CO_3	Na_2SO_4	$Na_2B_4O_7$	expelled CO_2		
6.80	0	0.145	0.46	2.55	0.038
»	1.63	»	0.46	2.55	0.13
»	7.04	»	0.45	2.53	0.34
7.26	0	1.774	4.10	2.33	0.51
»	10.39	»	3.76	2.28	0.78

(N in this case refers to a mixture of the components $BO_{1.5}$, BO_2^-, etc, CO_3^{2-} and SO_4^{2-}).

DISCUSSION

The acid-base reactions of the polyacids may be regarded as consisting of the real acid-base reaction (*e. g.* the transition of an oxygen ion from one degree of polarization to another) and a polymerization reaction.

For instance, in the case of the acid-base transition ortho-metaborate, the following scheme of reaction may be set up,

$$BO_3^{3-} \rightleftharpoons \tfrac{1}{2}\,(BO_{2.5}^{2-})_2 + \tfrac{1}{2}\,O^{2-} \rightleftharpoons$$

acid-base reaction

$$\frac{1}{n}\,(BO_2^-)_n + \tfrac{1}{2}\,O^{2-}$$

polymerization $(BO_2^-)_n$

$(BO_2^-)_3$ [ring with 3 links]
↓↑
$(BO_2^-)_4$ [» » 4 »]
↓↑
$(BO_2^-)_5$ [» » 5 »]
etc. etc.

Hence the complete computation of the equilibrium demands a thorough knowledge of the distribution of the polymerization in the system.

At present, however, we know very little about these relations.

Our knowledge regarding the deviations from the laws of ideal mixtures is also very limited at present. (The deviations from ideal mixtures in calcium carbonate-alkali carbonate melts will be treated in a subsequent communication.[7])

At the present stage we shall have to be content with semi-quantitative conclusions, but even these include points of interest for the further treatment of the problems.

When trying to set up convenient schemes of reaction two possibilities are at hand.

The polyacid molecule may be regarded as one reactive unit (as a single molecule); then scheme (I) applies,

$$\text{acid} + n\ CO_3^{2-} = (n+1)\ \text{base} + CO_2 \qquad \text{(I)}$$

for example

$$S_2O_7^{2-} + CO_3^{2-} = 2\ SO_4^{2-} + CO_2$$

The use of this scheme demands knowledge of the molecular distribution of the polymerization in the melt. It should be noted that the number of homogeneous reactants remains unchanged as the reaction takes place, hence the equilibrium should not be influenced by dilution as long as polymerization does not have to be considered.

As a simpler alternative each single group in the polyacid molecule may be regarded as a reactive unit. If this holds, scheme (II) applies.

$$2\ \text{acid} + CO_2^{2-} = 2\ \text{base} + CO_2 \qquad \text{(II)}$$

for example,

$$2\ BO_{2.0}^{-} + CO_3^{2-} = 2\ BO_{2.5}^{2-} + CO_2$$

In this case the number of reactants is changed, and the equilibrium must accordingly be influenced by dilution.

For the sake of orientation a few experiments were carried out in which the borate carbonate melt was diluted with sulphate. (This is based on the presumption that the equilibrium

$$SO_4^{2-} + CO_2 \rightleftharpoons CO_3^{2-} + SO_3$$

and even

$$2SO_3 \rightleftharpoons 2\ SO_2 + O_2$$

does not cause any perceptible disturbance. This should be true as $(p_{SO_2} + p_{SO_3})/p_{CO_2}$ is estimated to 10^{-9} at 1000° C).

Table 2 gives the results of two experiments, one carried out in a melt of low polymerized borate (corresp. to $BO_{2.55}$) and the other one in a higher polymerized melt (corresp. to $BO_{2.34}$). The effects of dilution observed seem to indicate that in the region of low polymerization, scheme I has to be employd, while in the region of higher polymerization scheme II is well suitable. This

is in qualitative agreement with what we might expect. The question will, however, be more thoroughly dealt with in a later communication.

With these reservations it is reasonable to use the simple scheme II in the computation of the acid base constants. In the case of the borate system we may write,

$$2\ BO_{1.5} + CO_3^{2-} = 2\ BO_2^- + CO_{2\,(g)}$$
$$2\ BO_2^- + CO_3^{2-} = 2\ BO_{2.5}^{2-} + CO_{2\,(g)}$$
$$\left[2\ BO_{2.5}^{2-} + CO_2^{2-} = 2\ BO_3^{3-} + CO_{2\,(g)}\right]$$

The scheme is formally the same as for the stepwise formation of complexes, (*cf.* also Bjerrum [6]) and may therefore be treated in the same way.

By introducing

$$\frac{N_{BO_{1.5}} \cdot N_{CO_3^{2-}}^{\frac{1}{2}}}{N_{BO_2}} = 1/K_I \text{ etc.}$$

(where N is the mole fraction refering to a mixture of the components $BO_{1.5}$, BO_2^- and CO_3^-).

$$\bar{n}' = \frac{n_O}{n_B} = \frac{1}{2} \cdot \frac{K_I N_{CO_3^{2-}}^{\frac{1}{2}} + 2\,K_I K_{II} N_{CO_3^{2-}} + 3\,K_I K_{II} K_{III} N_{CO_3^{2-}}^{\frac{3}{2}}}{1 + K_I\, N_{CO_3^{2-}}^{\frac{1}{2}} + K_I K_{II} N_{CO_3^{2-}} + K_I K_{II} K_{III} N_{CO}^{\frac{3}{2}-}}$$

where $\bar{n} = \bar{n}' + 3/2$ is the average number of oxygen atoms per boron atom.

By means of this equation the values of K_I, K_{II} (and K_{III}) which satisfy the experimental values of $\bar{n}$, are readily computed by plotting $\bar{n}$ against log $N_{CO_3^{2-}}$ and putting log $K_I \simeq \frac{1}{2}$ log $N_{CO_3^{2-}\,(n\,=\,1.75)}$ etc. [Fig. 1].

The values thus computed for constants of the borate and silicate systems are given in Table 3. (Later investigations, not yet concluded, seems to indicate that Nigglis data on the silicate systems needs some corrections.)

Table 3. Equilibrium constants in alkalicarbonate-borate and -silicate systems.

System	$T°$ C	log K_I	log K_{II}	log K_{III}
Na_2CO_3—B_2O_3	1,000		0.40	
»	900		0.23	
Li_2CO_3—B_2O_3	1,000		(1.4)	0.63
K_2CO_3—SiO_2	998	(1.7)	0.40	(— 1.1)
»	956		0.30	
»	898		0.18	
Na_2CO_3—SiO_2	956			0.35

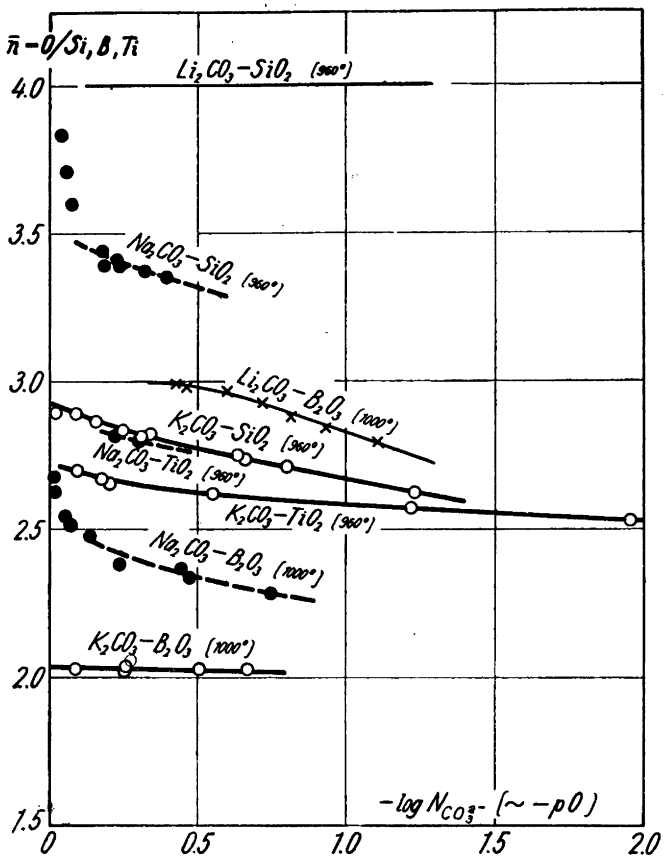


Fig. 1. The average number of O atoms per Si, B or Ti atom as a function of the mole fraction of CO_3^- in the melt. (P_{CO_2} = 1 atm.).

These data illustrate in a striking manner to what extent the values of the constants depend on the cations present. The acid strength of the polyacid decreases strongly in the order,

Li, Na, K

Thus log K_{II} decreases from ca. 1.4 to 0.40 in passing from lithium to sodium borate at 1000° C, and log K_{III} decreases from 0.35 to ca. — 1.1 in passing from sodium to potassium silicate at 956° C.

The temperature variations of the constants permit a computation of the heat of reaction in a homogeneous melts of sodium borate

$$NaBO_2 + \tfrac{1}{2}\, Na_2CO_3 = Na_2BO_{2.5} + CO_{2(g)}$$
$$\Delta H = 12 \text{ kcal}$$

This value may, of course, only be considered as a rough orientation.

SUMMARY

The acid base-equilibria in melts of alkali-carbonate and boron trioxide at 1000° C have been investigated by examining equilibria of the type

$$\text{acid} + CO_3^{2-} \rightleftharpoons \text{base} + CO_2 \quad (1 \text{ atm.})$$

for various compositions of the melt (compare Niggli's studies on the equilibria SiO_2—K_2CO_3, SiO_2—Na_2CO_3 SiO_2—Li_2CO_3, TiO_2—K_2CO_3 and TiO_2—Na_2CO_3). The results show a distinct dependence of the acidity of the polyacids on the cations present and that the acidity decreases in the order

Li, Na, K

The formulation of suitable schemes of reaction for use in the computation of the acid-base constants of the polyacids has been discussed briefly.

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Determination of *l*-Aspartic Acid by Aspartase

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Enzymic methods have recently been applied to quantitative determination of certain amino acids. As far as we have noticed from the literature Virtanen and Laine[1] were the first to use such a method (determination of *l*-aspartic acid by aspartase with dry preparation of *Pseudomonas fluorescens* or *Bacterium propionici*). The method which was strictly specific for this amino acid was employed as a macro method. Its accuracy was at the time not examined in detail. Virtanen and Laine applied somewhat later decarboxylase of lysine *(Bacterium coli)* to detection of lysine[2]. The method was very rough and chiefly employed for mere recognition of lysine.

During the last years Gale[3] has successfully studied the amino acid decarboxylases of hundreds of bacteria and has worked out manometric methods for determination of lysine, tyrosine, arginine, histidine, glutamic acid, and ornithine. The methods are based on the high specificity of amino acid decarboxylases. Schales and Schales[4] have used for determination of glutamic acid an enzyme solution prepared from squash. In the systematic investigations by Gale[5] only the above mentioned six amino acids were decarboxylated by bacteria. Accordingly, application of the accurate and convenient micro method to estimation of other amino acids would not be possible. Virtanen and Laine[6] have, however, earlier observed that *Rhizobium leguminosarium* decarboxylates also *l*-aspartic acid. The reaction is unfortunately slow and therefore not easily applicable as an analytical method. It is now under detailed investigation in this laboratory.

By means of aspartase *l*-aspartic acid can, however, be determined also in relatively small amounts with considerable accuracy. The method is outlined below. The reaction:

$$l\text{-aspartic acid} \rightleftharpoons \text{fumaric acid} + NH_3 * \qquad (1)$$

* It is possible that water participates in the reaction and that the above formula indicates only the final products of the reaction and not its course.

was noted by Quastel and Woolf [7] with resting *B. coli*. Virtanen and Tarnanen brought the respective enzyme, aspartase, into solution from *Pseudomonas fluorescens*. The solution contains regularly also fumarase, the removal of which is difficult. Accordingly, the deamination of aspartic acid by the preparation depends except on the equilibrium of the reaction 1 also on that of the reaction 2:

$$\text{fumaric acid} + H_2O \rightleftharpoons \text{malic acid} \qquad (2)$$

According to the equation of equilibrium:

$$K = \frac{[\text{aspartat} \pm]}{[\text{fumarat} =]\ [NH_4^+]}$$

the deamination of aspartic acid is increased by the reaction 2.

By precipitating the aspartase at pH 4.6 Virtanen and Erkama [8] obtained a preparation which no longer caused the reaction 2. This preparation gave the equilibrium constant $K_{37} = 100$. In spite of the fact that the enzyme preparation did not form malic acid from fumaric acid in a solution without ammonia, it did form in experiments of long duration malic acid from aspartic acid as well as from fumaric acid in the presence of ammonia. Thus it seemed likely that the preparation contained besides aspartase an enzyme which catalyzed the reaction.

$$l\text{-aspartic acid} + H_2O \rightarrow \text{malic acid} + NH_3 \qquad (3)$$

When after the war studies on this problem were again undertaken in this laboratory we did not succeed in liberating aspartase from fumarase by the precipitation described above. For this reason we have for the present been unable to re-examine the reaction 3 and to prepare fumarase-free aspartase for analytical determinations.

Although aspartase is not yet available in a form freed from other enzymes, and consequently, the equilibrium caused by the preparation used in every single case in unknown, it is, however, possible to use aspartase preparations for the quantitative determination of *l*-aspartic acid. It is only necessary to carry out by the side of the actual experiment a parallel one to which has been added approximately the same amount of aspartic acid as is found in the solution to be investigated. The amount of ammonia formed by the enzyme preparation from the known amount of *l*-aspartic acid can thus be determined. The determination must of course regularly include also a parallel experiment with enzyme preparation alone, which enables determination of possible formation of ammonia from the preparation itself.

It should be mentioned that the equilibrium of the reaction is also affected by the magnesium salt content (Jacobsohn and Pereira [9]) of the solution which according to investigations in this laboratory may be attributed to the fact that when a phosphate buffer (pH 7) is used ammonia is partly precipitated as ammonium magnesium phosphate and removed from the solution. Consequently, deamination of aspartic acid is promoted.

EXPERIMENTAL

As enzyme preparations we have in our experiments so far used both finely ground dry preparation of *Pseudomonas fluorescens* and cell-free extract prepared from that. The bacterium grows best in meat extract-pepton nutrient solution. A bacterial mass with highest aspartase activity was obtained by cultivating *Ps. fluorescens* in the following nutrient solution: 1 litre tap water, 8 g meat extract-pepton powder (Bacto Nutrient Broth Dehydrated. Difco Laboratories, U. S. A.), 3 g K_2HPO_4 and 1 g $MgSO_4 \cdot 7H_2O$. The pH was adjusted to pH 7. The solution was heated for 1 hour in an autoclave at 120°, filtered clear while hot, the pH was again adjusted to 7, and sterilized for 15 min at 120° C. Cultivation of the bacterial mass was performed in an aluminium vat containing 200 litres nutrient solution, the layer of the solution was about 5 cm for securing sufficiently aerobic conditions. The vat was sterilized by heating and covered with greaseproof paper. The nutrient solution was inoculated with 10 % of 3 days old culture of *Ps. fluorescens* (nutrient solution the same as above). The heavy inoculation prevented foreign infections during the short culture time (48 h). The temperature of cultivation was about 18° C. The cells were separated from the solution by using a milk separator. The mass was washed twice in the centrifuge tubes with tap water. The salt-containing mass lying at the bottom of the tubes was discarded, only the cream-coloured surface layer was used. It was dried on porous plates. The dry mass was ground in a mortar to a dust-like powder. The mass maintains its full activity for at least half a year, probably for years. The bacterial mass can of course be produced even on a smaller scale by cultivating the bacteria in thin layers in glass flasks.

The cell-free aspartase solution was prepared by suspending 5 g finely ground bacterial mass in 100 ml distilled water with some toluene and by allowing the suspension to stand with occasional shaking for 2 days at 37° C. The suspension was centrifuged and the solution filtered through bacterial filter (Jena 17 G 5 auf 3).

Ammonia was determined in all experiments with the apparatus of Pucher. The method itself was modified so that ammonia was trapped in the receiver by 0.01 *N* H_2SO_4 and the excess of acid was titrated with 0.01 *N* NaOH by using as indicator Tashiro's alcoholic solution of a mixture of methylene blue and methylene red. The solution to be analyzed was made alkaline in the distillation flask by Folin's solution (5 g Na_2CO_3 + 5 g NaCl in 100 ml water).

Tables 1 and 2 give two examples of our determinations of the liberation of ammonia from *l*-aspartic acid by aspartase preparations. The one was carried out with dried bacteria, the other with enzyme solution. The accuracy of the method in parallel experiments is illustrated by the examples.

Experiment with dried bacteria

A sample of 500 mg bacterium powder was placed in each of 6 test tubes, containing 5 ml of 0.067 *M* phosphate buffer (pH 7.0) and 2 ml toluene per tube. The powder was suspended carefully in the solution. To three of the tubes was added to each 10 ml solution with 20.0 mg neutralized *l*-aspartic acid, and to the other three respectively 10 ml distilled water. The tubes were allowed to stand for 40 hours at 37° C with occasional shaking after which they were placed in ice water for interruption of the reaction. The results are given in Table 1.

Table 1. Formation of ammonia from l-aspartic acid by dry preparation of Pseudomonas fluorescens.

Expt.	Aspartic acid, mg	Aspartic acid-N, mg	0.01 *N* H_2SO_4 ml	NH_3-N formed, mg	NH_3-N formed from asp. acid, mg	NH_3-N liberated from asp. acid-N, %	Found asp. acid-N, % of added*
I		—	17.20	2.408	—	—	—
II	—	—	17.46	2.444	—	—	—
III		—	17.38	2.433	—	—	—
	Average			2.428			
IV	20	2.10	26.53	3.714	1.286	62.19	98.6
V	20	2.10	26.96	3.774	1.346	64.09	101.6
VI	20	2.10	26.79	3.751	1.323	63.00	99.9
	Average			3.746		63.09	

Experiment with cell-free enzyme solution

The aspartase-containing solution prepared in the manner described above was pipetted into 9 test tubes, 5 ml to each whereupon 5 ml of 0.067 *M* phosphate buffer (pH 7.0) and 2 ml toluene were added. To three of the tubes was added 20 mg *l*-aspartic acid in 2 ml of water, to three again 60 mg *l*-aspartic acid in 6 ml of water, while three were kept as controls without aspartic acid. Distilled water was added to each of the tubes to make the volume up to 22 ml. The tubes were kept for 7 days at 37° after which they were placed in ice water and ammonia determination was made. The results are given in Table 2.

As can be seen from the tables the parallel experiments with equal amounts of aspartic acid give very concordant results. The accuracy of the ammonia determination decides how small amounts of *l*-aspartic acid can be determined by the method. By using the dry preparation the enzyme concentration can be raised so high that the equilibrium is reached in some hours. It is important that the reaction should have proceeded as far as possible before determination

* Calculated from the mean value of ammonia formed from aspartic acid.

Table 2. Formation of ammonia from l-aspartic acid by the cell-free solution prepared from the dry preparation of Pseudomonas fluorescens.

Expt.	Aspartic acid, mg	Aspartic acid-N, mg	0.01 *N* H_2SO_4 ml	NH_3-N formed, mg	NH_3-N formed from asp. acid, mg	NH_3-N liberated from asp. acid-N, %	Found asp. acid-N, % of added*
I	0	—	7.22	1.011	—	—	—
II	0	—	7.20	1.008	—	—	—
III	0	—	7.17	1.003	—	—	—
	Average			1.007			
IV	20.0	2.10	16.05	2.247	1.240	59.05	100.0
V	20.0	2.10	16.08	2.251	1.244	59.24	100.3
VI	20.0	2.10	16.02	2.243	1.236	58.86	99.7
	Average			2.247	1.240	59.05	
VII	60.0	6.30	24.08	3.371	2.364	37.52	99.3
VIII	60.0	6.30	24.55	3.437	2.430	38.57	102.1
IX	60.0	6.30	23.94	3.348	2.341	37.22	98.3
	Average			3.385	2.378	37.77	

of ammonia because the quantities of ammonia will then be greater and the results accordingly more accurate. A difference of a few minutes in the duration of the experiment will no longer have any practical meaning and the quantity of aspartic acid in the control experiment needs not to correspond so exactly that in the solution to be investigated as would be necessary if the formation of ammonia were far from the maximum. This is clearly illustrated by Table 2 which shows the liberation of ammonia from 20 mg and 60 mg of aspartic acid when an equal quantity of enzyme solution and the same reaction time were used. In the former case 59.05 % ammonia were split off, in the latter 37.77 %. In the latter case equilibrium was not yet attained.

The most convenient and rapid way of applying the described method to determination of *l*-aspartic acid is to employ finely ground bacterial mass as enzyme preparation. With enzyme solution possibly a somewhat higher accuracy is attained but before the solution can be used it should be concentrated since otherwise the time required is too long for practical analyses. Use of dried bacteria is naturally much more simple.

Aspartase can well be applied to determination of *l*-aspartic acid in protein hydrolysates and in *l*-aspartic acid-containing solutions in general, provided

* Calculated from the mean value of ammonia formed from aspartic acid.

that they do not contain ammonia, fumaric or malic acids because these substances affect the equilibrium. Before determination of aspartic acid they must therefore be removed from the solution to be investigated: ammonia by distillation and the acids by extraction.

Experiments for determination of aspartic acid-N in casein by the method concerned

Two sorts of casein were available. Casein I (according to Hammarsten) was a preparation of the medical firm Orion, Helsinki, its total N-content was exceptionally low, 14.6 % of ash-free dry matter. Casein II (according to Hammarsten) was a preparation of Schering-Kahlbaum and its total N-content was 15.70 % of ash-free dry matter. The lower total N-content of the former casein may be due to the manner of preparation. It has been ascertained in this laboratory that for instance, amide-nitrogen is partly split off from casein in an acid solution without any enzymes [10].

For analyses 5 g of air-dry casein were hydrolyzed by boiling in 25 ml of 37 % HCl for 6 hours. The hydrolysate was diluted and made alkaline with NaOH (indicator phenolphthalein) and the ammonia N was removed *in vacuo* at 40—42° C by simultaneously leading air through the solution until the volume was reduced to about 1/3 of the initial. The solution was neutralized with HCl to pH 7 and made up to 300 ml with water. Toluene was added in order to prevent infections. The hydrolysate thus prepared is fit for use only for two days at the highest. A sample of 5 ml of this hydrolysate was used for each analysis.

Procedure

For experimental vessels we used 25 ml test tubes closed with glass stoppers. An equal quantity of dried *Ps. fluorescens* powder was added to each test tube and besides toluene 1 ml. In order to facilitate the suspending of the test solutions 3 pure glass beads were placed to each tube. In every experiment the total volume of the solution was the same, 21 ml. The experiments 1—3, 10—12, 19—21, 28—30 are controls for determination of the NH_3-N liberating from the bacterial mass. Each contained 160 mg bacterial mass + 10 ml. 0.062 *M* (pH 7) phosphate buffer + 10 ml water. — The experiments 4—6, 13—15, 22—24, and 31—33 show the formation of NH_3-N from the bacterial mass + *l*-aspartic acid. The difference between the experiments with and without aspartic acid indicates that NH_3-N has been formed from aspartic acid. Each test tube contained 160 mg bacterial mass + 10 ml buffer + 5 ml H_2O + 5 ml neutral aspartic acid solution with 0.538 mg *l*-aspartic acid N (5.12 mg aspartic acid). — In the actual analyses, experiments 7—9, 16—18, 25—27, and 34—36, we used a quantity of casein hydrolysate, the aspartic acid content of which was according to the preliminary experiments known to be as nearly equal as possible to that of the control experiments. Since the percent of NH_3-N formed in the controls is known, the amount of aspartic acid N present in the hydrolysate can be calculated on the basis of the NH_3 estimations. Each experiment

contained 160 mg bacterial mass + 10 ml buffer + 5 ml H_2O + 5 ml hydrolysate. In the analyses of casein I the total N in the 5 ml hydrolysate was 10,914 mg and in those of casein II 11,850 mg. The time of aspartase action was in all experiments, both in controls and in actual ones, 120 min. The tubes were kept in a water thermostat with temperature adjusted to 34° C. The tubes were shaken vigorously at every quarter of an hour. Immediately after cessation of the reaction the contents of the tubes were washed to the NH_3-distillation apparatus and NH_3 was determined. Each of the four set of experiments including controls and actual experiments (1—9, 10—18, 19—27, and 28—36) was carried out without interruption. This is necessary for the parallelism of the controls and the analyses.

Table 3. Determination of aspartic acid from the casein hydrolysate by dry preparation of Ps. fluorescens. In experiments I and II employed casein was a preparation of Orion, acc. to Hammarsten, in experiments III and IV of Schering-Kahlbaum acc. to Hammarsten. Duration of experiment 2 h, temperature 34° C.

	Expt. no.	*l*-Aspartic acid N, mg	N in casein hydrolysate mg	NH_3-N formed mg	NH_3-N split off from aspartic acid mg	%	*l*-Aspartic acid N in casein mg	% of total N	Aspartic acid N % of added*
I	1	—	—	0.416	—	—	—	—	—
	2	—	—	0.428	—	—	—	—	—
	3	—	—	0.424	—	—	—	—	—
			Average	0.423					
	4	0.538	—	0.848	0.425	78.99	—	—	100.2
	5	0.538	—	0.848	0.425	78.99	—	—	100.2
	6	0.538	—	0.846	0.423	78.62	—	—	99.8
			Average	0.847	0.424	78.81			
	7	—	10.91	0.951	0.528	78.81	0.670	6.14	
	8	—	10.91	0.965	0.542	78.81	0.688	6.30	
	9	—	10.91	0.947	0.524	78.81	0.665	6.09	
			Average	0.954	0.531	78.81	0.674	6.18	
II	10	—	—	0.441	—	—	—	—	—
	11	—	—	0.455	—	—	—	—	—
	12	—	—	0.447	—	—	—	—	—
			Average	0.448					
	13	0.538	—	0.868	0.420	78.06	—	—	101.5
	14	0.538	—	0.854	0.406	75.46	—	—	98.1
	15	0.538	—	0.864	0.416	77.33	—	—	100.6
			Average	0.862	0.414	76.95			
	16	—	10.91	0.979	0.531	76.95	0.690	6.32	
	17	—	10.91	0.981	0.533	76.95	0.693	6.35	
	18	—	10.91	1.000	0.552?	76.95	0.717	(6.57)	
			Average	0.987	0.539	76.95	0.700	6.33	

* Calculated on the basis of the average decomposition percentage of three decompositions.

	19	—	—	0.421	—	—	—	—	—
	20	—	—	0.426	—	—	—	—	—
	21	—	—	0.421	—	—	—	—	—
			Average	0.423					
III	22	0.538	—	0.839	0.416	77.32	—	—	99.3
	23	0.538	—	0.848	0.425	78.99	—	—	101.5
	24	0.538	—	0.839	0.416	77.32	—	—	99.3
			Average	0.842	0.419	77.88			
	25	—	11.85	0.948	0.525	77.88	0.674	5.69	
	26	—	11.85	0.953	0.530	77.88	0.681	5.75	
	27	—	11.85	0.956	0.533	77.88	0.684	5.77	
			Average	0.952	0.529	77.88	0.680	5.74	
	28	—	—	0.427	—	—	—	—	—
	29	—	—	0.433	—	—	—	—	—
	30	—	—	0.441	—	—	—	—	—
			Average	0.434					
IV	31	0.538	—	0.851	0.417	77.51	—	—	101.5
	32	0.538	—	0.840	0.406	75.46	—	—	98.7
	33	0.538	—	0.843	0.409	76.02	—	—	99.4
			Average	0.845	0.411	76.39			
	34	—	11.85	0.965	0.531	76.39	0.695	5.86	
	35	—	11.85	0.972	0.538	76.39	0.704	5.94	
	36	—	11.85	0.951	0.517	76.39	0.677	5.71	
			Average	0.963	0.529	76.39	0.692	5.84	

In the experiments (Table 3) the splitting off of ammonia has proceeded very far (76-79 % of aspartic acid N). It can be seen from the values that in the parallel experiments of the same set only slight variations occur in the formation of ammonia from added aspartic acid. The same applies to the experiments with casein hydrolysate. Some greater deviations, as for instance, in the experiments 14 and 18 may be ascribed to the fact that the quantity of toluene in the pipette has been slightly smaller or greater than in the other experiments. Therefore it would be best to perform the analysis so rapidly that addition of toluene is unnecessary for preservation of the hydrolysate. Of particular importance to the application of the method is that all members of the same experiment are interrupted after an equally long period.

Other amino acids than *l*-aspartic acid did not split off ammonia under the experimental conditions. Especially *l*-glutamic acid was investigated in this respect.

SUMMARY

Determination of *l*-aspartic acid is possible with considerable accuracy by the use of aspartase preparations. As the formation of ammonia through aspartase is exclusively restricted to *l*-aspartic acid, only determination of ammonia is required.

For enzyme preparations both finely ground dry preparation and cell-free enzyme solution of *Pseudomonas fluorescens* have been used. As the enzyme preparations employed have contained varying amounts of fumarase in addition to aspartase it is not possible to calculate *a priori* the equilibrium of the reaction. Determination of aspartic acid requires therefore three parallels.

1. Enzyme preparation + buffer solution (pH 7) for detection of ammonia formed from the preparation.

2. Enzyme preparation + buffer solution + a known quantity of *l*-aspartic acid. The difference between the experiments 1 and 2 expresses the quantity of ammonia liberated from aspartic acid.

3. Enzyme preparation + buffer solution + solution from which the aspartic acid is to be determined. On the extent of decomposition of aspartic acid in experiment 2 it is possible to calculate the amount of *l*-aspartic acid in the solution to be analyzed by means of the ammonia formed.

Casein (Schering-Kahlbaum, acc. to Hammarsten) with a N-content of 15.70 % of ash-free dry matter was found to contain 5.8 % *l*-aspartic acid N from total N. Another casein preparation with only 14.6 % N contained 6.2—6.3 % *l*-aspartic acid N from total N.

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A Micro Method for Determination of Dextran in Blood

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Grönwall and Ingelman [1, 2, 3, 4], suggested that dextran, when hydrolyzed to a suitable molecular size, could be used as a plasma substitute. Dextran, thus treated, has been tried out clinically by Bohmansson *et al.*[5] and has been found to possess valuable properties for the prevention and treatment of shock and hypoproteinaemic conditions.

Earlier methods for the determination of dextran were based on its optic activity or on the determination of glucose after complete hydrolyzation of dextran [6]. The clinical applicability of these methods is, however, limited owing to the relatively large amounts of blood required and to the complexity of the procedure.

These disadvantages have been overcome in the method described below which is therefore better suited for clinical purposes.

PRINCIPLE

Dextran is precipitated by copper sulphate in the presence of sodium hydroxide, copper being bound up quantitatively but not stoichiometrically. After the precipitate has been centrifuged off, the remaining concentration of copper is determined photometrically.

REAGENTS

1. 0.6 *N* trichloracetic acid.
2. 2.5 *N* sodium hydroxide.
3. Copper reagent. A fresh reagent is prepared for each determination from the following stock solution:

$CuSO_4 \cdot 5H_2O$	3.000	g
Sodium citrate	30.000	g
Dist. water to	1000.0	ml

Copper sulphate and sodium citrate are dissolved separately in a relatively small amount of distilled water, then mixed and filled up to 1000.0 ml with distilled water.

The stock solution is stable for several months if kept in a dark room. In order to prepare the reagent, 20 ml of the stock solution are filled to 100.0 ml with distilled water.

4. 0.2 % sodium diethyl-dithiocarbamate in distilled water.

PROCEDURE

Precipitation of proteins

The blood proteins are precipitated with trichloracetic acid. This is carried out by diluting 0.2 ml of heparinized plasma or whole blood in 4.8 ml of distilled water after which 5 ml of 0.6 *N* trichloracetic acid are added. The mixture is then shaken vigorously and placed in a water bath at 70—80° C for about 5 minutes. The protein sediment is then filtrated off. It is preferable to use a fine filter, *e. g.*, Berzelius 00.

Precipitation of dextran

2.0 ml of 2.5 *N* sodium hydroxide are pipetted in an ordinary 10 ml centrifuge tube (or special glass-stoppered tube). Then 5.0 ml of the filtrate and 2.0 ml of the copper reagent are added. The tube is closed with a stopper and the contents are well mixed. When ordinary centrifuge tubes are used, the stopper is coated with plastic. Without protective coating, the stopper may liberate substances which influence the reaction. The tubes are then placed in an agitating apparatus for 4 hours or more, to allow ample time for the formation of the dextran-copper compound. The resulting sediment is then centrifuged at 2000-3000 r.p.m. for at least 10 minutes. The remaining solution should be clear and the sediment of a homogeneous bright blue colour. A discoloured sediment is probably due to incomplete removal of the proteins.

Colour reaction

6.0 ml of the clear solution are pipetted in a 100 ml volumetric flask which has previously been filled up to 70—80 ml with distilled water. Care should be taken not to pipette away any traces of the precipitate. It is therefore advisable to remove the stopper before centrifuging, as small particles of the precipitate tend to gather around the stopper. The flask is then shaken vigorously and 3.0 ml of the sodium diethyl-dithiocarbamate reagent are added and mixed well immediately. After this the flask is filled up to 100.0 ml with distilled water and then shaken once again.

It is of the utmost importance that the above description is followed meticulously. If the volumetric flask is filled with less water or insufficiently shaken before adding the reagent, the ensuing formation of copper carbamate may become massive enough to cause opalescence which is not revertible and greatly affects the results.

The solution will be clear, of a yellow straw colour and slightly fluorescent. The colour is fairly stable and the reading need not be performed immediately.

Determination

The readings are done in the Zeiss Pulfrich photometer against the blank value of the following mixture: 3.0 ml of the sodium diethyl-dithiocarbamate reagent; 1 ml of 2.5 *N* sodium hydroxide; distilled water to 100.0 ml. This is to compensate for the possible presence of copper in distilled water. 5 or 3 cm cuvettes are used for the readings. The filter is S 47 (470 mμ). When other types of photometers are used, the method should be adapted accordingly.

The percentage of dextran is read off from a curve which has been plotted previously in accordance with known dextran values. For the construction of this curve, multiple determinations are made of the following 6 concentrations: 0.25; 0.5; 1.0; 1.5; 2.0; 2.5 per cent dextran in heparinized plasma. 0.2 ml from each is taken for the determination.

Lower dextran concentrations than 0.25 % can be determined when bigger amounts of blood are used. Up to 2.0 ml of blood can be used, diluted with water to 5.0 ml. Thus, the lowest determinable concentration of dextran will be 0.025 %.

When whole blood is used, the concentration of dextran in plasma can be computed by means of the hematocrit correction since the amount of dextran taken up by the red blood cells is negligible.

ERRORS

The values of dextran to be determined depend on the concentration of copper sulphate as well as sodium hydroxide, trichloracetic acid and sodium citrate. Consequently, citrate or oxalate blood should not be used.

Cellulose combines with copper as does dextran. Alkaline solutions should therefore not come into contact with filter paper, cork or similar substances.

The blood samples should be freshly taken, since the use of old plasma may yield a dirty grey sediment which sticks to the walls of the tube, rendering the values obtained too high.

The applicability of this method for the determination of dextran in other body fluids has as yet not been fully established. It can, however, be adopted with fluids such as exsudates, transsudates, cerebrospinal fluid and different organ extracts, but not with urine.

Glycogen does not give sediment, nor does its presence affect the results.

As mentioned above copper carbamate may give disturbing opalescence. This compound is only slightly soluble in water and the concentration of copper in the reagent is chosen accordingly in order to keep the compound in solution.

It is evident from what has been said above that a strict copper »sterility» should be maintained. While working with the Pulfrich photometer, caution should be taken not to close the covers of the cuvette containers if they are made of brass.

EXAMPLE

Average error

For the construction of the curve as a rule 2—3 determinations of each concentration will suffice. In the following, however, 15 determinations were performed for calculation of the average error. The average extinction coefficients were plotted on the millimeter paper, the curve was constructed and the deviation of each extinction coefficient was expressed in the corresponding deviation of the dextran concentration. From the dispersion the average error was then calculated and expressed in per cents of dextran concentration. See Table and Figure.

The average (with standard error) and the standard deviation of the extinction coefficients have been calculated according to current statistical methods.

The standard deviations of the concentration percentage are obtained by multiplying the resp. standard deviations of the extinction coefficients by the slope of the curve. The slope of the curve has been determined graphically.

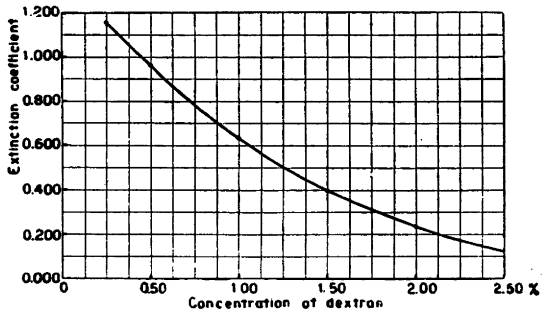

Fig. 1. Interpolation curve.

Table 1. Calculation of average error from 15 determinations.

Concentration of dextran	0.25 %	0.50 %	1.00 %	1.50 %	2.00 %	2.50 %
Average of obtained extinction coefficients in 9 cm cuvettes	1.155 ± 0.0053	0.963 ± 0.0036	0.634 ± 0.0026	0.395 ± 0.0016	0.234 ± 0.0015	0.123 ± 0.0011
Standard deviation of ext. coefficient	0.0206	0.0140	0.0102	0.0061	0.0057	0.0041
Slope of the (concentration) curve	1.29	1.39	1.76	2.55	3.55	4.80
Standard deviation of concentrations	0.027	0.019	0.018	0.016	0.020	0.020
Standard deviation in per cent of concentration	10.6	3.9	1.8	1.0	1.0	0.8

SUMMARY

A micromethod for determination of dextran — a plasmasubstitute — is described. Using 0.2—2.0 ml of fluid to be analyzed, concentrations from 0.025—2.5 % of dextran in body fluids, urine excluded, can be determined with sufficent accuracy for clinical purposes.

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Über den Oxydationsmechanismus der weissen Masse des Zentralnervensystems

Sauerstoffaktivierendes System der weissen Masse

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Bei der Erforschung des Oxydationsmechanismus des Zentralnervensystems (ZNS.) stehen wir infolge der verwickelten anatomischen und physiologischen Verhältnisse speziellen Problemen gegenüber. Wenn wir ein Stückchen Muskel oder Leber untersuchen, können wir unsere Ergebnisse ohne weiteres auf das ganze Gewebe beziehen. Dieses Verfahren ist aber beim Nervengewebe unbrauchbar, weil dieses kein einheitlich aufgebautes Gewebe, sondern eine Ansammlung verschiedener Zellen und Gewebselemente ist. Die Ganglienzellen sind die wichtigsten Träger der nervösen Funktionen. Sie bilden die sogenannte graue Masse des ZNS., in der sie zwischen anderen Gewebselementen eingebettet sind (Glia, Blutgefässe usw.). Die Fortsätze der Ganglienzellen, die sogenannten Achsenzylinder oder Neuriten, welche die leitenden Elemente der Nervenzellen darstellen, bilden im Zentralorgan die weisse Masse. In der weissen Substanz sind viel mehr Lipoide enthalten als in der grauen.

Von physiologischen Gesichtspunkte aus ist es wichtig zu bemerken, dass in den Ganglienzellen viel grössere Energieumwandlungen festzustellen sind als in dem leitenden Fortsatz derselben, in den Neuriten. Dementsprechend ist die Sauerstoffaufnahme der grauen Substanz viel grösser als die der weissen Masse.

Hieraus wird ersichtlich, dass in den verschiedenen Stellen des Neurons während der Funktion verschiedene primäre Reaktionen und Energieumwandlungen von wechselnder Grösse und Schnelligkeit vor sich gehen. Schon theoretisch wäre zu erwarten, dass auch die angeschlossenen letzten Energiequellen,

die Oxydationen, verschieden seien. Meine bisherigen Ergebnisse bestätigen diese Annahme. Ich konnte feststellen [1], dass der Oxydationsmechanismus der grauen und der weissen Substanz verschieden ist. Die weisse Masse des ZNS. vermag von den verschiedenen Kohlehydraten Glykogen und phosphorylierte Hexose oxydativ zu verwerten. Glukose und andere nicht phosphorylierte Zuckerarten werden nicht angegriffen. Demgegenüber wird die Glukose von den grauen Zentren des ZNS. sehr leicht oxydiert. Ich konnte ferner feststellen, dass diese Oxydation der Glukose nur nach ihrer Phosphorylierung stattfindet. Eine sogenannte »non phosphorylating glycolysis» nach der Annahme einiger englischen Forscher [2] gibt es im Gehirn nicht.

Über den Lipoid- und Eiweisstoffwechsel der weissen Masse wissen wir sozusagen garnichts.

Einen zweiten auffallenden Unterschied zwischen der weissen und grauen Masse des ZNS. konnte ich bezüglich ihres sauerstoffaktivierenden Systems feststellen [3]. Die Hirnrinde, die zentralen grauen Zentren, sowie die Ganglien der sensiblen Nerven enthalten das Warburg-Keilinsche eisenhaltige, katalytische System. Die zentrale weisse Substanz, die peripheren Nerven und die sympathischen Ganglien dagegen enthalten dieses System nicht.

In den letztgenannten Teilen des ZNS. konnte ich weder Cytochromoxydase noch Cytochrome finden.

DIE FRAGE DES SAUERSTOFFAKTIVIERENDEN SYSTEMS IN DER WEISSEN MASSE

Die Wirkung von Cyan und Kohlenmonoxyd auf die Atmung der weissen Masse:

KCN und CO hemmen die Atmung der weissen Masse fast eben so stark wie die der grauen Substanz. Auffallend ist aber, dass die CO-Hemmung nicht photosensibel ist (Abb. 1). Eine derartige Hemmung kann man bei den Cu-Proteinen beobachten. Unser Befund stellte uns also die Aufgabe, nach einem Cu-Protein zu suchen.

DER EISEN- UND KUPFERGEHALT DER WEISSEN MASSE

Ein Hund wurde durch die Carotis mit physiologischer NaCl-Lösung solange durchspült, bis die Spülflüssigkeit ganz klar und blutfrei blieb. Die sorgfältig abgetrennte weisse Masse wurde getrocknet und in der Trockensubstanz der Eisengehalt nach Reis und Chakmakjian [4], der Kupfergehalt mit der Methode von Eisler, Rosdahl und Theorell [5] bestimmt.

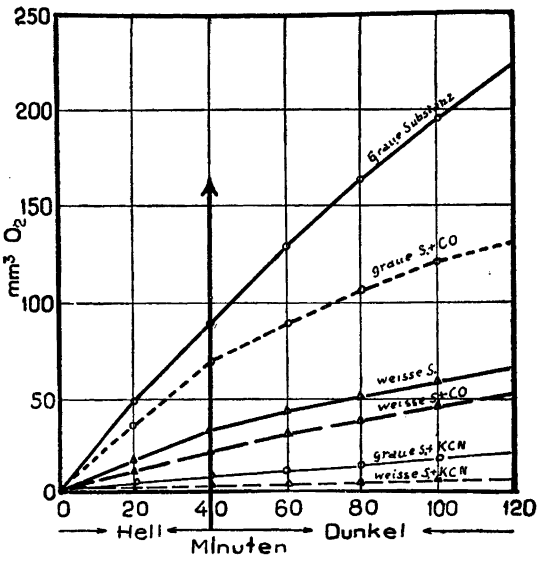

Abb. 1. Die Sauerstoffaufnahme der grauen und weissen Substanz in Gegenwart von CO und KCN.
Versuchsansatz: Weisse und graue Substanz eines Katzenhirnes (0,5 gr) wurde nach Latapimahlen in Phosphat-Ringerlösung von pH 7,4 suspendiert. Die Sauerstoffaufnahme wurde im Warburg-Apparat bei 37°C gemessen. KCN-Endkonzentration: M/1000.
Gasgemisch: Im Kontrollgefäss: 95 % N_2 + 5 % O_2.
Im Gefäss mit CO: 95 % CO + 5 % O_2.
KCN: in M/1000 Endkonzentration.

Wie aus Tabelle 1 ersichtlich, ist der Kupfergehalt der weissen Substanz ungefähr gleich ihrem Eisengehalt, dagegen ist in der grauen Substanz der Eisengehalt bedeutend grösser als der Kupfergehalt.

DIE WIRKUNG VON NA-DIAETHYLDITHIOCARBAMINAT AUF DIE ATMUNG ATMUNG DER WEISSEN UND GRAUEN MASSE DES ZNS

Abbildung 2 zeigt ,dass die Atmung der weissen Substanz durch Na-diaethyldithiocarbaminat viel stärker gehemmt wird als die der grauen Masse.

Nach dieser Feststellung ist anzunehmen, dass das sauerstoffaktivierende System der weissen Substanz ein Cu-Protein sein könnte.

Tabelle 1. Der Eisen- und Kupfergehalt der weissen und grauen Substanz.

Eisengehalt der		Kupfergehalt der	
grauen Subst. %	weissen Subst. %	grauen Subst. %	weissen Subst. %
0,032	0,008	0,012	0,024
0,043	0,011	0,007	0,018
0,038	0,007	0,011	0,014
0,029	0,006	0,021	0,009
0,041	0,012	0,006	0,011

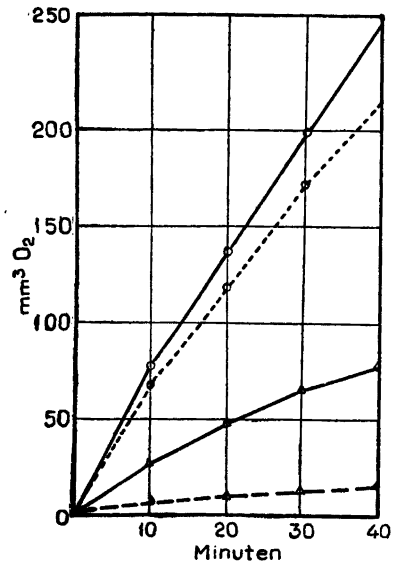

Abb. 2. Versuchsanordnung wie vorher
Na-diaethyldithiocarbaminat: M/100.
O———O *graue Substanz*
O- - - - - - O » » + *Na-diaethyldithiocarb.*
Δ———Δ *weisse Substanz*
Δ — — — Δ » » + *Na-diaethyldithiocarb.*

Die nächste Aufgabe wäre, dieses hypothetische Cu-Protein zu isolieren und zu untersuchen, ob das Mengenverhältnis dieses Proteins mit der Atmung der weissen Masse in Zusammenhang zu bringen ist. Im Falle der weissen Substanz wäre dieser Weg wegen der Labilität des kolloidalen Systems und der grossen Menge von Lipoiden, die bei der präparativen Darstellung von Fermenten störend wirken, ziehmlich schwer.

Die folgende Frage wäre, wie dieses Kupferprotein sich in das Oxydationssystem der weissen Masse einschaltet. Es ist nicht ausgeschlossen, dass auch hier ein Polyphenol als Vermittler zwischen Substrat und Cu-Protein mitspielt. Für die Richtigkeit dieser Hypothese spricht die Erscheinung, dass an der Schnittoberfläche des Gehirns die weisse Masse nach einiger Zeit rötlichbraun wird. Diese Frage zu klären wird die Aufgabe folgender Arbeiten sein.

ZUSAMMENFASSUNG

Die sauerstoffaktivierenden Systeme der weissen und grauen Substanz des Zentralnervensystems sind verschiedener Natur.

Die graue Masse des ZNS. enthält das Warburg-Keilinsche eisenhaltige, katalytische System, die zentrale weisse Substanz enthält dieses System nicht.

In der weissen Masse ist der Cu-Gehalt gleich dem Fe-Gehalt.

Die Atmung der weissen Masse wird von KCN und CO gehemmt, doch ist die CO-Hemmung nicht photosensibel.

Natriumdiaethyldithiocarbaminat hat eine starke hemmende Wirkung auf die Atmung der weissen Masse.

Diese Tatsache erlaubt den Schluss, dass in der Atmung der weissen Masse Cu-Proteine als sauerstoffübertragende Katalysatoren eine grosse Rolle spielen.

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Quantitative Colorimetrical Determination of Certain Sex Hormones belonging to the Steroid Group

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The biological methods for the determination of sex hormones are laborious and not very accurate. Of chemical methods, gravimetric ones are impossible, and colorimetrical ones are not very satisfactory, particulary in that they do not distinguish between substances closely related chemically. Therefore this work is presented.

The oldest colour reaction is that with conc. H_2SO_4, which is unspecific. It was first communicated by Wieland, Straub and Dorfmüller [1]. The blue fluorescence is given by a multitude of organic substances. Upon diluting with water a reddish, unstable colour is formed that is made more stable by addition of phenol or β-naphtol. The colorimetrical method of Kober [2] is built on this observation. Schmulowitz, Schwenk-Hildebrandt and Marx [3-5] have communicated experiments where they try to bring about a coupling between the hydroxyl group in the hormones of the oestrone family and the diazo group. Pincus, Wheeler, Young and Zahl [6] describe a colour reaction analogous to the cholesterol reaction with benzoyl chloride.

Scherrer [7] stated that sterols dissolved in conc. H_2SO_4 and covered with a layer of benzaldehyde will develop coloured rings in the interface. Oyama [8] described a microdetermination of cholesterol with salizylaldehyde and conc. H_2SO_4. The author, having had no knowledge of this work, made som preliminary tests of his own, and found that the steroid hormones dissolved in conc. H_2SO_4 will develop strong but unstable colours after treatment with benzaldehyde. The use of benzaldehyde in the presence of conc. H_2SO_4 is not to be recommended because of the lability of the benzaldehyde; but if *p*-dimethylaminobenzaldehyde is used instead, a distinct, stable and reproducible colour results. The fact that *p*-dimethylaminobenzaldehyde (reagent P) gives colours with a series of phenols was reported by Joachimowitz [9] and used in an investi-

gation on plant cells. The colours produced with the reagent P and these hormones can sometimes be considerably intensified by addition of water. The colour thus obtained is stable for hours, its intensity is proportional to the amount of hormone present (*e. g.* oestrone), and can easily be measured in the step-photometer (»stufo»). Instead of reagent P, *m*-Nitrobenzaldehyde (reagent M) can be used. *p*-Nitrobenzaldehyde gives weaker colours. *o*-Nitrobenzaldehyde gives no colour at all. Acetaldehyde as well as formaldehyde is usuable but these offer no advantage compared with the reagent P.

METHOD

A standard solution is made from the pure substance and the desired amount transferred to a test tube by a pipette. The solvent ($CHCl_3$) is evaporated on the boiling water-bath. After cooling, 0.2 ml of 1 % solution of reagent P or M in $CHCl_3$ is added and the solvent removed in vacuo at about 60°. When reagent P is used, the temperature must never be allowed to exceed 70° since melting of the reagent P (m. p. 73°) will lead to completely irregular blankvalues. Reagent P that has not been melted will always give reproducible blanks of low or neglegible value, depending on the age of the reagent P solution. By using reagent M all these precautionary measures are unnecessary since even after prolonged heating of reagent M (m. p. 58°) at 100° the blanks are always zero. — To the residue in the test tube 1.2 ml conc. H_2SO_4 (A. R.) is added and with oestrone, oestradiol, equilin and androsterone a cherry red colour will develop where the H_2SO_4 and the residue touch. This colour changes to red-orange after heating for 1 ½—2 min in the boiling water-bath. Oestradiol will then give a brown-orange tint. After completion of the heating the test tube is cooled under the tap, 0.8 ml water is added and the tube is vigorously and continously shaken in the stream of water. Thorough cooling is essential as overheating of the mixture will result in a variation in colour. The tube is now heated for 5 min in the boiling water-bath and after cooling under the tap ready for reading in the »stufo» with filter S 53. The compensation-cell is filled with the blank, or with water when reagent M has been used. The colour remains constant at least one hour and is permanganateviolet for the said hormones. The time given for the various steps is optimal and can easily be kept by aid of an alarm clock. The ratio H_2SO_4/H_2O is optimal. The colours given by the examined substances can be seen from Tables 1, 4 and 5. The sensitivity of reagent M is somewhat less than that of the reagent P. Beer's law is valid within wide ranges. For more than 250 μg oestrone the amount taken of reagent P or M must be increased. Insufficient quantities of the reagents can be recognized by change of colour compared with the colour when sufficient aldehyde has been used. Regarding the validity of Beer's law one is referred to Tables 2 and 3. Progesterone does not react with the reagent P or M. Pregnandiol should not be determined with the reagent P or M. The substance reacts but the results show poor conformity. Oestriol and equilinin were not available during the war. — The colour given by equilin is visually very similiar to that from oestrone, but maximal extinction is at S 57. — Phenols must be removed before determination as they also react with both reagent P and M.

The simultaneous determination of oestrone, oestradiol and equilin gives simple additive values. Instead of 0.2 ml of 1 % solution of reagent P or M, however 0.4 ml must be taken. For the determination of 50 μg oestrone together with 50 μg oestradiol

Table 1. Colours on reaction with reagent P and conc. H_2SO_4 + 50 µg hormone. a. undiluted b. diluted with 0.8 ml H_2O.

	a.	b.
1. Oestrone	red-orange	permanganate-violet
2. Oestradiol	brown-orange	red-brown
3. Equilin	red-orange	blue-violet
4. Androsterone	yellow-orange	brownish-permanganate
5. Testosterone	light-orange	yellow-brown
6. Corticosterone	light-yellow	brown-orange
7. Cholesterol	orange	red-orange
8. Pregnandiol	deep-orange	reddish-brown
9. Progesterone	colourless	faintly-orange

Table 2. Validity of Beer's law on reaction with reagent P. Final vol. 2 ml. 2.5 mm cell.

		Quantities in µg							
Substance	Filter	5	10	25	50	75	100	125	250
Oestrone	S 53	0.16	0.28	0.59	1.15	1.65	2.28*	2.87*	5.50*
Oestradiol	S 53		0.14**		0.68		1.36		
Equilin	S 57				0.78		1.58		
Testosterone	S 47				0.40		0.83		
Androsterone	S 53				0.36		0.80		
Corticosterone	S 47				0.30		0.60		
Cholesterol	S 47				0.16		0.36		

Validity of Beer's law on reaction with reagent P. Final vol. 1 ml. 10 mm microcell. Filter S 53. 0.6 ml H_2SO_4 diluted with 0.4 ml H_2O.

Substance	Quantities in µg					
	0.25	0.50	0.75	1.25	2.50	5.00
Oestrone	0.04	0.09	0.15	0.25	0.49	0.96

Table 3. Vality of Beer's law on reaction with reagent M. Final vol. 2 ml. 2.5 mm cell.

Substance	Filter	Quantities in µg		
		25	50	100
Oestrone	S 53	—	0.90	1.80
Oestradiol	S 53	0.27	0.53	1.03
Equilin	S 57	0.28	0.56	1.12
Testosterone	S 47	—	0.40	0.83
Androsterone	S 53	—	0.25	0.50
Corticosterone	S 47	—	0.30	0.55
Cholesterol	S 43	—	0.23	0.48

* Reduced from measurements in 1 mm cell.

** » » » » 5 » »

0.4 ml must be taken even if 0.2 ml is sufficient for 250 μg oestrone or oestradiol singly (Tables 6—8). The colours developed with the reagent P or M can all be destroyd by H_2O_2. With reagent P in this way were found yellow tints and the colour can be referred to the oxydation of reagent P. Reagent M will give colourless solutions. — In urine extracts the accompanying substances also react with conc. H_2SO_4 forming dark shades that disappear with H_2O_2 too.

Preparation of the solution of the reagent P and M

In all experiments solutions of 1 g reagent P or M in $CHCl_3$ were used. At room-temperature and even when kept in the dark the $CHCl_3$-solution of the reagent P will quickly turn rose-coloured and show an acid reaction. Storing at 2° will nearly suppress this process and solutions so stored will give neglegible values for the blanks for a considerable time. Ether can be substituted for $CHCl_3$; but before making up the solution it must be distilled over $SnCl_2$ because the smallest amount of peroxide will interfere with the reaction (colour as well as intensity will change). Carbontetrachloride is unsuitable

Table 4. Extinction on determination of 50 μg hormone with reagent P and conc. H_2SO_4. Final vol. 2 ml. Reading with 2.5 mm cell.

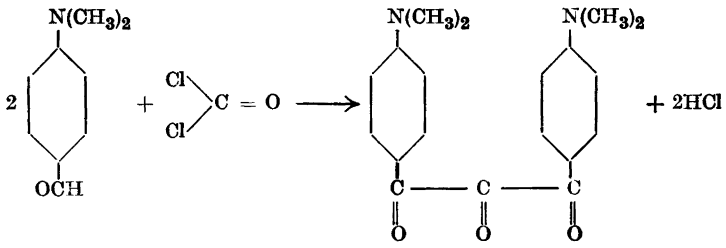
	Filters									
Substance	S 43	S 47	S 50	S 53	S 57	S 61	S 66.6	S 72	S 75	L II
Oestrone	0.22	0.37	0.73	1.15	0.33	0.22	0.11	0.00	0.00	1.00
Oestradiol	0.28	0.31	0.45	0.68	0.28	0.16	0.08	0.00	0.00	0.61
Equilin	0.29	0.40	0.44	0.68	0.78	0.58	0.15	0.03	0.00	0.62
Progesterone*	0.05	0.05	0.01	0.03	0.00	0.00	0.00	0.00	0.00	0.05
Testosterone	0.37	0.40	0.35	0.15	0.10	0.04	0.01	0.00	0.00	0.14
Androsterone ..	0.19	0.26	0.31	0.36	0.23	0.13	0.04	0.00	0.00	0.35
Pregnandiol	0.34	0.39	0.38	0.37	0.19	0.06	0.00	0.00	0.00	0.34
Corticosterone ..	0.23	0.30	0.26	0.16	0.12	0.08	0.03	0.00	0.00	0.17
Cholesterol	0.16	0.16	0.15	0.13	0.08	0.00	0.00	0.00	0.00	0.14

Table 5. Extinction on determination of 50 μg hormone with reagent M and conc. H_2SO_4. Final vol. 2 ml. Reading with 2.5 mm cell.

	Filters									
Substance	S 43	S 47	S 50	S 53	S 57	S 61	S 66.6	S 72	S 75	L II
Oestrone	0.23	0.32	0.52	0.90	0.42	0.31	0.19	0.07	0.06	0.86
Oestradiol	0.26	0.28	0.39	0.52	0.27	0.19	0.11	0.05	0.03	0.48
Equilin	0.23	0.31	0.36	0.53	0.56	0.40	0.13	0.03	0.03	0.50
Progesterone*	0.13	0.14	0.14	0.08	0.06	0.04	0.03	0.00	0.00	0.08
Testosterone	0.35	0.42	0.36	0.23	0.14	0.05	0.03	0.00	0.00	0.20
Androsterone ..	0.24	0.26	0.26	0.25	0.18	0.08	0.05	0.03	0.03	0.24
Pregnandiol	0.26	0.25	0.26	0.22	0.15	0.07	0.03	0.02	0.01	0.18
Corticosterone ..	0.25	0.32	0.24	0.10	0.09	0.04	0.00	0.00	0.00	0.13
Cholesterol	0.27	0.27	0.25	0.22	0.15	0.10	0.08	0.04	0.04	0.20

* For 100 μg progesterone the determination gives the same values.

as a solvent; a solution of reagent P quickly becomes cloudy and strongly acid. After some time a deep yellow precipitate is formed. When the solution in CCl_4 is exposed to the light from a mercury lamp, a yellow precipitate of very fine crystals is formed at once. This substance is propably 4-methyldiaminodiphenyltriketone following the reaction



Fosgen seems to be formed extremely rapidly under the influence of mercury-light in the presence of *p*-dimethylaminobenzaldehyd (acceptor-effect). There was used a »Philora» »HPW 125 W» *with* a reflector S.K.215/02 — Philiray. Without the reflector the reaction goes quite slowly. — The solution of P in $CHCl_3$ does not behave in this way; it becomes at once rose-coloured and shows an acid reaction.

Contrary to the reagent P, there is no difficulty in storing the solution of reagent M in $CHCl_3$ for months at 20°—25°. It remains stable and gives zero »blanks». Rapid, roughly quantitative tests can be carried out by adding a few crystals of reagent M and the conc. H_3SO_4 to the substance.

Table 6. Simultaneous determination of oestrone, oestradiol and equilin with reagent P. 0.4 ml of 1 % solution of reagent P. Final vol. 2 ml. Reading with 2.5 mm cell. 50 µg of each substance.

	Filters					
Substances	S 53	S 50	S 47	S 43	L II	
Oestrone + oestradiol ..	1.80	0.98	0.66	0.45	1.60	found
	1.79	1.08	0.68	0.50		calculated
Oestrone + equilin	1.66	1.04	0.72	0.45	1.10	found
	1.78	1.17	0.77	0.51	1.11	calculated
Oestradiol + equilin	1.25	0.86	0.68	0.54		found
	1.35	0.89	1.71	0.57		calculated
Oestron + oestradiol + equilin * ..	1.20	0.78	0.58	0.42		found
..	1.25	0.81	0.53	0.40		calculated

The reading with S 53 and S 50 is difficult as equality of shades and equal brightness rarely will be found. The reading is taken for equal brightness.

* 25 µg of each of the 3 substances.

Table 7. Determination of 250 µg oestrone with different quantities of reagent P. Final vol. 2 ml. Reading with 1 mm cell.

	Filters				
	S 53	S 50	S 47	S 43	
Extinction	1.44	1.13	1.08	0.54	0.1 ml of 1 % solution of reagent P
»	2.20	1.18	0.84	0.44	0.2 » » 1 » » » »
»	2.20	1.14	0.80	0.44	0.3 » » 1 » » » »
»	2.20	1.35	1.04	0.53	0.4 » » 1 » » » »

Table 8. Simultaneous determination of oestrone, oestradiol and equilin with reagent M. 0.4 ml of 1 % solution of reagent M. Final vol. 2 ml. Reading with 2.5 mm cell. 50 µg of each substance.

	Filters					
Substance	S 53	S 50	S 47	S 43	L II	
Oestron + oestradiol	1.47	0.87	0.60	0.45	1.34	found
	1.42	0.93	0.60	0.49	1.35	calculated
Oestrone + equilin	1.43	0.88	0.65	0.46	1.36	found
	1.43	0.90	0.63	0.46	1.36	calculated
Oestrone + equilin	1.09	0.73	0.61	0.53	1.00	found
	1.05	0.75	0.59	0.49	0.97	calculated
Oestrone + oestradiol + equilin *	0.98	0.68	0.48	0.38	0.87	found
	0.98	0.63	0.46	0.36	0.90	calculated

THE REACTION WITH SALIZYLALDEHYDE

Salizylaldehyde gives a colour with conc. H_2SO_4 and higher ketones. The reaction was first recommended by Täufel and Thaler [10] for the determination of the »ketone-rancidity». Under certain circumstances various sex hormones can be quantitatively determined with salizylaldehyde, conc. H_2SO_4 and glacial acetic acid. The order of mixing the reagents must strictly be adhered to.

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* 25 µg of each of the 3 substances. There are the same difficulties for reading with filters S 53 and S 50 as mentioned in Table 6.

Method

The substance to be examined is dissolved in 0.05 ml $CHCl_3$, and 2.4 ml glacial acetic acid (gl. a. a.) containing 2 % reagent S (salizylaldehyde) is added. It is first necessary to dissolve it in $CHCl_3$ because it is uncertain if the substance is completely soluble in gl. a. a. Then follows the addition of 1.2 ml conc. H_2SO_4 and vigorous shaking, immersing for 5 min in boiling water, cooling in water and dilution with 3.6 ml gl. a. a. Hereby the intensity of the colours is increased and Beer's law is valid. If the dilution with gl. a. a. is not made, other shades of colours will appear and Beer's law is *not* valid. — The compensation-cell must always be filled with the blank.

Table 9. View of the colours formed on reaction with the examined substances and gl. a. a. containing reagent S + conc. H_2SO_4. Dissolving the substance in 0.05 ml $CHCl_3$ + 2.4 ml gl. a. a. containing reagent S. Adding of conc. H_2SO_4. Diluting with 3.6 ml gl. a. a.

Substance (250 µg)	Colour
Oestrone	permanganate
Oestradiol	»
Pregnandiol	brownish permanganate
Equilin	brown
Progesterone	permanganate
Testosterone	blue-green, thick red dust *
Androsterone	brownish
Cholesterol	red violet, a tinge of brown
Corticosterone	olive coloured, thick red dust *

Table 10. View of the influence of alternating the order of mixing.

a. Dissolving the substance in conc. H_2SO_4 and adding gl. a. a. containing reagent S, no further dilution with gl. a. a.

b. Dissolving the substance in conc. H_2SO_4 and adding gl. a. a. containing reagent S. Dilution later on with more gl. a. a.

Substance (250 µg)	a.	b.
Oestrone	dirty brown	red-violet
Oestradiol	deep red	dirty orange
Pregnandiol	dirty brown	» brown
Equilin	» »	blue with a tinge of red, thick red dust *
Progesterone	red violet	dirty orange
Testosterone	deep red violet	» red
Androsterone	red, changes quickly into blue	smoky violet
Cholesterol	dirty brown	» »
Corticosterone	deep red	chestnut

* Looking at the tube against a dark background gives the impression that the glass is covered with a thin layer of red dust. The same impression is given by the walls of the »stufo»-cell when the cell is placed in the instrument.

Alternation of the order of mixing: If the substance is first dissolved in 1.2 ml conc. H_2SO_4, heated for 2 min at 100° and then 2.4 ml gl. a. a. containing reagent S is added, a shortlived vivacious play of colours is seen (Table 10). On subsequent dilution with gl. a. a. colours will appear that are considerably different from those seen by the first way of mixing. These colours do not follow Beer's law. — The sensitivity of the reaction with the reagent S is considerably less than of the corresponding reactions with the reagent P and M.

The results of the investigations on the reagent S with the various substances examined will be found in Tables 9—12.

Table 11. Extinction on determination of 500 µg substance with gl. a. a. containing reagent S and conc. H_2SO_4. Final vol. 7.2 ml. Reading in 2.5 mm cell. Compensated against the blank.

Substance	Filters									
	S 43	S 47	S 50	S 53	S 57	S 61	S 66.6	S 72	S 75	L II
Oestrone	0.06	0.18	0.38	0.62	0.25	0.20	0.08	0.00	0.00	0.66
Oestradiol	0.41	0.47	0.71	1.23	0.73	0.88	0.49	0.06	0.01	1.17
Equilin	0.36	0.63	0.70	0.60	0.29	0.27	0.13	0.00	0.00	0.57
Progesterone	0.03	0.04	0.10	0.05	0.00	0.00	0.00	0.00	0.00	0.12
Testosterone	0.32	0.52	0.47	0.56	0.57	0.54	0.42	0.16	0.08	0.60
Androsterone ..	0.35	0.33	0.36	0.40	0.31	0.20	0.08	0.02	0.00	0.44
Pregnandiol	0.22	0.27	0.35	0.43	0.49	0.30	0.12	0.02	0.00	0.46
Corticosterone ..	0.35	0.54	0.48	0.51	0.55	0.42	0.33	0.13	0.07	0.52
Cholesterol	0.31	0.40	0.43	0.48	0.34	0.28	0.19	0.03	0.00	0.54

Table 12. Validity of Beer's law on reaction with gl. a. a. containing reagent S. Final vol. 7.2 ml. Reading in 2.5 mm cell.

Substance	Filter	Quantities in µg	
		250	500
Oestrone	S 53	0.31	0.62
Oestradiol	S 53	0.59	1.23
Equilin	S 50	0.36	0.70
Progesterone	S 50	0.04	0.10
Testosterone	S 53	0.27	0.56
Androsterone	S 53	0.21	0.40
Pregnandiol	S 57	0.24	0.49
Corticosterone	S 57	0.27	0.55
Cholesterol	S 53	0.25	0.48

THE REACTION WITH ANTIMONY TRICHLORIDE

A whole series of substances, some of them strongly dehydrating, upon being heated with oestrone will produce an eosin red colour. This happens when oestrone is heated together with $AlCl_3$, P_2O_5, $SnCl_2$, $ZnCl_2$ as well as in a melt of boric acid, oxalic acid or sulphosalizylic acid. Oestradiol and equilin behave in the same way. The melts of benzoic, succinic, cinnamic, salizylic and phthalic acid give negative results; $CaCl_2$ is without effect. The colours are delicate and can not be satisfactory extracted. Contrary to this, $SbCl_3$ gives strong colours and the coloured substances dissolve easily in nitrobenzene. The solutions are deep red with green fluorescence. They can be discoloured by $CHCl_3$, glacial acetic acid and especially by alcohols. This process is accelerated by heating. Methanol, ethanol, buthylalcohol, amylalcohol (furfurolfree) and octylalcohol were examined. The red nitrobenzene solution gives the red dye to Al_2O_3 in the chromatographic column; but the red ring fades quickly. — The dark colours formed between $SbCl_3$ and the accompanying impurities of urine extracts (both human and equine urines were used) are also discoloured by alcohols. These dark colours can be adsorbed by Al_2O_3 but their chromatographical separation from the red dye in the nitrobenzene solution was only partly successfull.

Method

After evaporation of the solvent there is added 1 ml 23 % solution of $SbCl_3$ in $CHCl_3$ and the tube heated for 2 min to 100°; the substance then forms a melt with $SbCl_3$; it is heated for 5 min to 160° in a glycerin-bath. 2 ml nitrobenzene are added, the tube is chilled to room temperature and the extinction read through filter S 53 or L II. The compensation-cell must be filled with nitrobenzene. The reading through L II is a little easier than through S 53 where absolute equality of the colour is difficult to achieve. Heating for more than 5 min at 160° is unnecessary; after 15 min the same values are obtained as after 5 min. When *oestradiol* (25 μg, 50 μg) is heated *10 min* at 160° the nitrobenzene solution shows a strong turbidity and the reading in the »stufo» becomes impossible. If oestrone or equilin are heated to 140° the time must be extended to 15 min. Heating to 100° does not give reproducible values. Phenols must be removed before determination as they also react with $SbCl_3$. The reaction is typical for oestrone, oestradiol and equilin, which all give a deep red coloured lake, with strong green fluorescence.

The simultaneous determination of oestrone, oestradiol and equilin gives simple additive values.

The reaction can also serve as a qualitative test to show the presence of androsterone or testosterone.

The other examined substances gave either no colour or only a very faint one and therefore could not be determined with $SbCl_3$ colorimetrically.

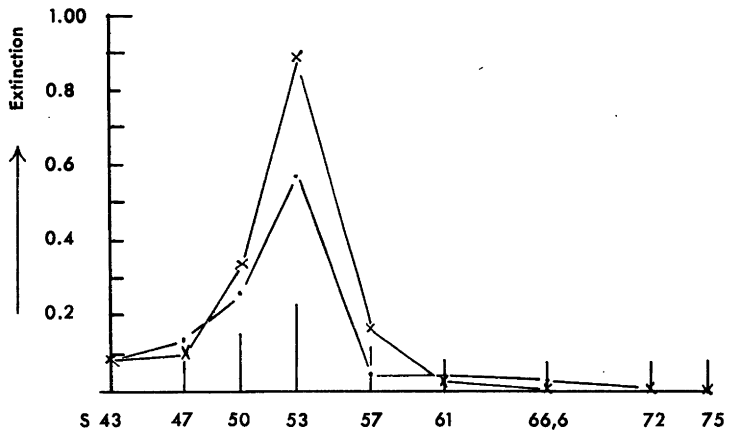

Fig. 1. Extinction on determination of 50 µg oestrone with $SbCl_3$ and $SnCl_4$. Volume 2 ml. Reading in 2.5 mm cell.

× ——— × *with* $SbCl_3$
· ——— · *with* $SnCl_4$.

The results of the experiments will be found in Tables 13—17 and curve 1.

In connection with the reaction with $SbCl_3$ will be mentioned a reaction with $SnCl_4$ communicated by Israel Kleiner [11]. As no scientific periodicals from the allied nations were available in Norway during the German occupation, the author did not know this publication. The author's investigations on the reaction with $SbCl_3$ were finished in autumn 1944.

On heating oestrone with phthalic anhydrid and $SnCl_4$ to 116°—120° a deep red substance is formed that is soluble in $CHCl_3$ with bright eosin-like colour that gradually fades away. The dye is also soluble in nitrobenzene. The author has made some experiments with the reaction of Kleiner after having read his publication after the end of the German occupation. The extinction of the nitrobenzene solution in the different wave ranges closely resembles the extinction of the coloured substance formed with $SbCl_3$ in nitrobenzene solution. The colour can also be got with $SnCl_4$ and oestrone without phthalic anhydrid; it is however less intense. The dye is very sensitive to alcohols and water. — It has not been possible to work out the reaction for quantitative purposes. Kleiner considers this dye as a phthalid; this is scarcely right as the reaction can take place without phthalic anhydrid. The great similiarity of the two extinction curves point to one and the same condensation of the substance in question under the influence of $SbCl_3$ or $SnCl_4$. The phthalic anhydrid serves only to prevent the $SnCl_4$ from escaping during the reaction, as the reaction temperature is considerably higher than the b. p. of $SnCl_4$,

Table 13. Colours on reaction with $SbCl_3$ and 50 µg substance, 5 min. at 160°.
a. Colour of the lake. b. Colour of the nitrobenzenesolution.

Substance	a.	b.
Oestrone:	purple	strong eosin-red, with strong green fluoresc.
Oestradiol:	»	» » » » » » »
Equilin:	»	» » » » » » »
Progresterone:	light brown	light yellow to orange
Testosterone:	in the beginning: deep lilac. Red dust*. Bluish at the end	faintly yellow
Androsterone:	in the beginning: yellow-brown. No dust	yellow
Pregnandiol:	faintly yellow	faintly yellow
Corticosterone:	in the beginning deep blue, light blue at the end	colourless-faintly yellow
Cholesterol:	yellow-brown	faintly yellow

Table 14. Extinction on determination of 50 µg substance with $SbCl_3$. Final vol. 2 ml. Reading in 2.5 mm cell.

	Filters									
Substance	S 43	S 47	S 50	S 53	S 57	S 61	S 66.6	S 72	S 75	L II
Oestrone	0.09	0.10	0.33	0.88	0.16	0.02	0.00	0.00	0.00	0.82
Oestradiol	0.17	0.15	0.28	0.54	0.00	0.00	0.00	0.00	0.00	0.50
Equilin	0.15	0.21	0.38	0.82	0.15	0.07	0.03	0.03	0.03	0.80
Progesterone	0.05	0.06	0.07	0.03	0.01	0.00	0.00	0.00	0.00	0.00
Testosterone	0.14	0.19	0.18	0.12	0.12	0.12	0.05	0.01	0.02	0.12
Androsterone ..	0.19	0.10	0.08	0.03	0.00	0.00	0.00	0.00	0.00	0.00
Pregnandiol**....	0.07	0.06	0.04	0.02	0.00	0.00	0.00	0.00	0.00	0.05
Corticosterone ..	0.16	0.17	0.22	0.16	0.16	0.23	0.04	0.03	0.00	0.16
Cholesterol	0.09	0.09	0.11	0.06	0.06	0.02	0.02	0.02	0.00	0.02

Table 15. Validity of Beer's law on reaction with $SbCl_3$. Final vol. 2 ml. 2.5 mm cell.

		Quantities in ug			
Substance	filter	25	50	100	
Oestrone	S 53	0.45	0.88	1.78	(reduced from 1 mm)
Oestradiol	S 53	0.28	0.54	1.08	
Equilin	S 53	0.42	0.82	1.65	(reduced from 1 mm)

Contrary to oestrone, oestradiol and equilin all other examined substances show bad agreement with Beer's law for nearly all examined filter ranges.

---

* »Red dust», look at Tables 9 and 10.

** 100 µg pregnandiol gives the same values!

Table 16. Simultaneous determination of oestrone, oestradiol and equilin with $SbCl_3$. Final vol. 2 ml. Reading with 2.5 mm cell. 25 µg each substance.

	Filters								
Substance	S 43	S 47	S 50	S 53	S 57	S 61	S 66.6	S 75	L II
Oestrone + oestradiol	0.09	0.14	0.32	0.78	0.19	0.07	0.02	0.04	0.77
Calculated:	0.10	0.12	0.31	0.75	0.19	0.08	0.03	0.03	0.76
Oestrone + equilin	0.14	0.20	0.39	0.84	0.18	0.07	0.04	0.02	0.82
Calculated:	0.14	0.20	0.39	0.87	0.15	0.07	0.04	0.01	0.86
Oestradiol + equilin	0.15	0.16	0.31	0.70	0.16	0.06	0.04	0.00	0.68
Calculated:	0.16	0.22	0.36	0.72	0.16	0.09	0.05	0.01	0.72
Oestrone + oestradiol + equilin	0.22	0.28	0.51	1.12	0.24	0.08	0.05	0.03	1.04
Calculated:	0.20	0.27	0.53	1.17	0.26	0.12	0.06	0.01	1.18

Table 17. Extinction of the lake produced with 100 µg oestrone and $SnCl_4$ (reaction of Israel Kleiner). The lake is dissolved in 5 ml $CHCl_3$. Reading with 5 mm cell.

	Filters									
Substance	S 43	S 47	S 50	S 53	S 57	S 61	S 66.6	S 72	S 75	L II
Oestrone	0.16	0.26	0.52	1.14	0.08	0.08	0.06	0.00	0.00	0.98

but under that of the m. p. of phthalic anhydrid. Moreover the result of the reaction is uncertain unless care is taken to get a homogenous melt through a short heating above the m. p. of the phthalic anhydrid, before dropping in the $SnCl_4$. The results will be found in Table 17 and Fig. 1.

THE REACTION WITH THE REAGENT OF FOLIN-WU

The reagent of Folin-Wu (phospho-molybdo-tungstic acid) can be used for the determination of absolutely pure oestrone. A blue colour is developed as the result of a reducing process.

Method

To the substance is added 0.5 ml conc. H_2SO_4 and the tube is heated for 2 min to 100°, cooled and 2 ml of the reagent of Folin-Wu are dropped in. The tube is now heated for 5 min to 100°, cooled and 5 ml saturated solution of sodium carbonate added. Then the tube is heated again to 100° for 3 min, cooled and read in the »stufo» through filter

S 72. The success of the reaction depends on working in an acid medium also after the addition of the carbonate solution. If not, the development of the blue colour will come to an early standstill and Beer's law is not valid. In a separate experiment the amount of saturated carbonate solution which is necessary must be determined for the exact neutralisation of the Folin-Wu solution. For the real experiment a little less must be taken. For the author's work 5 ml saturated carbonate solution were used while 5.8 ml were needed for the neutralisation.

The results are given in Table 18. Since even traces of impurities may cause trouble, only very pure samples of oestrone can be determined in this way.

Table 18. Validity of Beer's law on determination og oestrone with the reagent of Folin-Wu. Final vol. 7.5 ml. Reading with 10 mm cell.

		Quantities in μg oestrone		
Substance	Filter	250	500	1000
Oestrone	S 66.6	0.64	1.28	2.60
Oestrone	S 72	0.77	1.49	3.00*

DISCUSSION

Above some colour reactions have been described that can be used for quantitative determination of certain substances with hormonal properties from the steroid group. They are distinguished by a considerable sensitivity. They are typical for the examined substances in so far as the presence of troublesome impurities can be excluded either as a result of previous purification or from the nature of the substance in question. All the reactions described are sensitive to impurities. On filtering solutions containing lipoid solvents through qualitative filterpaper (Norwegian war-time manufacture) substances (resins) were dissolved that made the described reactions impossible in the filtrate. Owing to this glass filter crucibles were used for all filtrations. As conc. H_2SO_4 reacts even with traces of impurities experiments were carried out to find a substitute for the conc. H_2SO_4. Both phosphoric acid (85 %) and pyrophosphoric acid were tried, but without any success.

Experiments carried out in connection with this work — to purify extracts from mare's urines and from human urines, so far as their content of oestrogenic substance could be determined with one of the described reactions — were without success. The extensive purification of such extracts with the reagent »Girard P» could not be tried as the reagent was unobtainable during the occupation.

* Reduced from reading in 5 mm cell (found: 1.50).

SUMMARY

The author communicates some reactions that can be used for the quantitative determination of oestrone, oestradiol, equilin, androsterone, testosterone, corticosterone and cholesterol.

1. Reaction with *p*-dimethylaminobenzaldehyde (reagent P) that will allow the determination of 0.5 μg using 10 mm microcells.

2. Analogous reaction with *m*-nitrobenzaldehyde (reagent M) and *p*-nitrobenzaldehyde.

3. Reaction with salizylaldehyde (reagent S) that permits the seizure of the said substances qualitatively and quantitatively.

4. Reaction with $SbCl_3$ by the aid of which oestrone, oestradiol and equilin can be determined quantitatively, while the other examined substances do not give the deep red colour that is typical for the said 3 substances.

5. The working conditions are given for the determination of oestrone with the reagent of Folin-Wu.

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The Distribution of Sizes of Particles in Some Polymeric Methyl Methacrylates *

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By the sedimentation of polydisperse substances in the ultracentrifuge a concentration gradient will appear in the cell due to the various sedimentation velocities of particles with different molecular weights. The shape of the concentration gradient curve will depend upon the nature of the frequency function of the sedimentation constants or — as the sedimentation constant is some function of the molecular weight — the frequency function of the molecular weights. From a sedimentation diagram, therefore, it must be possible to deduce the distribution of sizes of particles in a polydisperse substance.

Since the development of the ultracentrifugal technique this method has been used by Rinde [1] to obtain the frequency curves for the particles in gold sols, by Nichols *et. al.*[2] on suspensions of ferric oxides, barium sulphates, on emulsions and rubber latex etc. More recently Jullander [3] has thoroughly discussed different ways of obtaining frequency curves for cellulose nitrates. In this connection the work of Signer and Gross [4] on polystyrene can also be mentioned. The earlier work has mainly been concerned with substances, the frequency curves of which show only one maximum. Further their sedimentation has been almost independent upon the concentration; an exception is of course the behaviour of cellulose nitrate and polystyrene.

When the frequency curve of the substance under investigation has more than one maximum and when the sedimentation velocity is dependent upon the concentration, the problem of obtaining the frequency curve from the sedimentation diagram is very complicated. In this paper some of the prob-

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Table 1. Sedimentation measurements in acetone solution.

Sample B			Sample E		
c	s	dB/dx	c	s	dB/dx
0.500	22.1		0.500	26.0	0.27
0.330	24.3		0.333	30.5	0.34
0.314	25.1	0.58	0.200	40.9	0.50
0.280	27.8		0.100	49.6	0.82
0.250	27.9		0	58.2	1.23
0.221	30.0				
0.214	28.7	0.89			
0.165	27.8				
0.125	28.1				
0.105	31.8	1.22			
0.054	30.4	1.49			
0	37.2	1.80			

lems involved are discussed in connection with an ultracentrifugal investigation of polymeric methyl methacrylate. The sedimentation diagrams at low concentrations are compared with frequency curves obtained from fractions of the samples used.

SEDIMENTATION AND DIFFUSION MEASUREMENTS ON UNFRACTIONATED SAMPLES

The samples used for this investigation were two commercial methyl methacrylates, here called sample B and E. These samples were characterized by means of sedimentation and diffusion measurements. The sedimentation measurements are given in Table 1 (for details about the ultracentrifugal technique, *cf.* reference 2). They were performed in acetone solution and the values obtained for the sedimentation constant, s, are given in S-units and refer to a temperature of 20° C. The s-values have been corrected for the influence of the increased hydrostatic pressure in the cell due to the high compressibility of acetone (Mosimann and Signer [5]). The concentrations, c, are given in g/100 ml. For concentrations lower than 0.1 g/100 ml it has been very difficult to calculate reliable s-values because the width of the curve in the sedimentation diagram is very large and it has been almost impossible to determine the true maximum of the curve. In Table 1 the values of dB/dx are also given. These have been calculated according to Gralén [6] and show how the width, B, of the sedimentation curve varies with the distance, x

Table 2. Sedimentation measurements in ethyl acetate solution.

Sample B		Sample E	
c	s	c	s
0.500	11.0	0.507	12.8
0.333	13.4	0.406	13.8
0.200	14.8	0.203	19.5
0	17.7	0.101	25.3
		0	32.2

from the centre of rotation (B is the ratio between the area of the curve and the maximum height). The values extrapolated to zero concentration indicate a very high polydispersity. In order to check the s-values obtained in acetone solution the samples were also measured in ethyl acetate solution. The results follow from Table 2. Using the formula given by Kraemer and Nichols [7]

$$[s]_0 = \frac{\eta V s_0}{1 - V\varrho} \tag{1}$$

we can calculate the intrinsic sedimentation constant, *i.e.* the sedimentation constant reduced to a common basis involving only the volume of the sedimenting particle and shape factors (η is the viscosity and ϱ the density of the solvent; V the partial specific volume of the solute). The following values are obtained:

	s_0	$[s]_0$*
Sample B, acetone	37.2	26.9
ethyl acetate	17.7	23.1
Sample E, acetone	58.2	42.0
ethyl acetate	32.2	42.1

The agreement is satisfactory and hence the results from the sedimentation measurements quite reliable.

To get the molecular weights of the samples the diffusion constants were determined in acetone solution at 20° C by means of Lamm's [8] scale method. Both of the samples gave skew diffusion curves and a reduction to zero concentration was made according to Gralén (*l. c.*). The results are given in Table 3, where the area value, D_A, the moment value, D_m, and the reduced

* $[s]_0$ is expressed in units of 10^{-15} cgs.

Table 3. Diffusion measurements in acetone solution.

Sample	c	D_A	D_m	D_0
B	0.494	4.36	4.78	4.31
E	0.382	3.23	3.87	3.32
	0.255	3.14	3.29	3.29
				3.31

value, D_0, of the diffusion constants are given in units of 10^{-7} cgs. The centrations are given in g/100 ml.

From Svedberg's formula

$$M = \frac{RTs_0}{(1 - V\varrho)D_0} \tag{2}$$

with $V = 0.80$ the following molecular weights have been calculated: sample B, $M = 570000$; sample E, $M = 1170000$. It is to be emphasized that these values are not well defined averages. According to Jullander [9] the values obtained from equ. (2) in the way used above are close to a $M_{w,w}$-value obtained by using a weight average value of s instead of s_0.

The sedimentation diagrams of the two samples are shown in Figs. 1 and 2. Here the scale line displacement, Z, divided by the scale distance, b, and the concentration, c, is given as a function of the distance from the centre of rotation at different times, t, of sedimentation. The diagrams show that the polydispersity of the samples is high and that the gradient curves tend to distribute themselves over almost the whole cell.

Furthermore the irregularities to the left and right of the main peak (sample B: $c = 0.105$ g/100 ml, $t = 40$ min; sample E: $c = 0.100$ g/100 ml, $t = 15$ and 25 min) indicate that the frequency curves of the substances have at least three maxima. Attempts to measure the substances at still lower concentrations indicate a possibility of a better resolution of the peaks.

FRACTIONATION OF THE SAMPLES

In order to get the frequency curves of the samples in another way they were divided into fractions by means of fractional precipitation. The precipitation was made with cyclohexane from benzene solution according to Schulz and Dinglinger [10] and was performed in a thermostat at 20° C. The cyclohexane

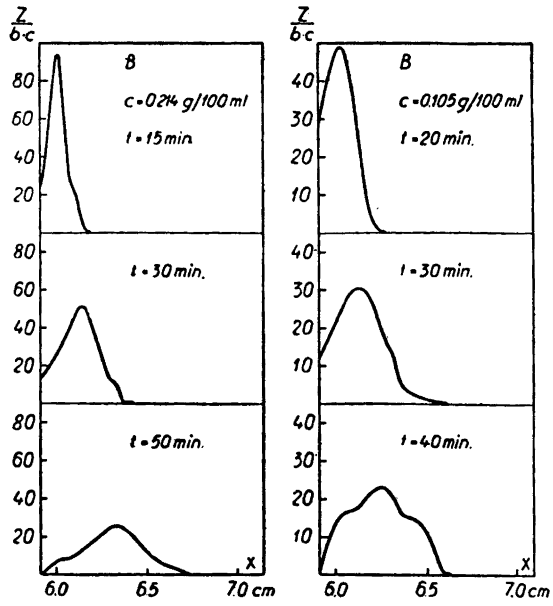

Fig. 1. Sedimentation diagram of sample B at different concentrations and different times.

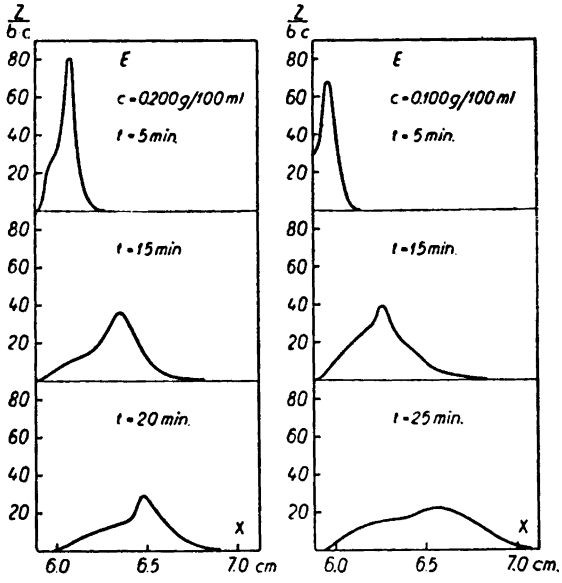

Fig. 2. Sedimentation diagram of sample E at different concentrations and different times.

was added to the benzene solution very slowly (about 1 ml/min) and under intensive stirring until the critical composition of the solvent/non solvent system was almost reached. Then the cyclohexane was added drop by drop until the solution became turbid. The temperature of the thermostat was slowly raised to about 30° C and in this way the precipitate was dissolved. By lowering the temperature slowly it precipitated again and the precipitate formed a gel which could easily be removed. From the supernatant liquid a new fraction could be obtained. In this way, it was possible to get about 10 fractions. The concentration of the original solution was about 2 %. This is a little high and certainly it has had an effect upon the sharpness of the fractions (*cf.* Scott and Magat [11] and Scott [12]). The sample B was not completely soluble in benzene. After 10 days an insoluble residue of about 25 % remained; hence only one fractionation of this sample was performed. On sample E, five independent fractionations were made in all. The sum of the weights of the fractions corresponded within 5—10 % to the original amount of substance. The loss is due to the difficulties involved in working quantitatively with the small original amounts of substance (about 5 g). The fractions were characterized by means of viscosity measurements in benzene and/or acetone solution. In Table 4 the data for the fractions are given. The amount of a fraction in per cent is based on the total weight of all the fractions in one experiment. The intrinsic viscosity has been calculated with the concentration expressed in g/ml for B (1) and E (1)—E (2) and in g/g for E (3)—E (5). In the table are also given for fractionations E (3) and E (4), the k'-values defined by the equation

$$\frac{\eta_{sp}}{c} = [\eta]\,(1 + k'\,[\eta]c) \tag{3}$$

As to the notations of the fractions E (4) III means the third fraction of sample E in the fourth fractionation experiment.

In Fig. 3 the distribution and frequency curves are drawn according to Schulz [13]. The former have been obtained by plotting the quantity $(\sum_{1}^{n-1} p_\nu + \frac{1}{2}\,p_n)$, were p_ν denotes the percentage amount of the ν:th fraction, against the intrinsic viscosity $[\eta]_n$. The latter have been constructed by taking the derivatives of the distribution curves at a number of points.

All the frequency curves have distinct maxima. Sample B has three maxima at the intrinsic viscosities 60, 120 and 180 respectively. Sample E in acetone has four maxima at the intrinsic viscosities 60, 120, 180 and 280 respectively. The third maximum is the highest one. In benzene we have four maxima for E (3) and E (4); E (5) does not show the first small maximum

Table 4. Fractionation experiments. (The intrinsic viscosities for B (1), E (1)—(2) measured in acetone and for E (3)—(5) in benzene).

Fraction		Amount %	$[\eta]$	Fraction		Amount %	$[\eta]$	k'
B (1)	I *	24.6	120	E (3)	I	16.3	446	0.38
	II	14.2	228		II	23.6	369	0.32
	III	16.3	174		III	9.6	352	0.31
	IV	14.0	149		IV	9.9	303	0.34
	V	5.6	129		V	3.6	283	0.32
	VI	10.1	122		VI	16.6	253	0.36
	VII	5.4	106		VII	7.3	200	0.37
	VIII	4.9	80		VIII	5.1	170	0.40
	IX	5.1	56		IX	4.0	142	0.34
					X	4.0	86	0.27
E (1)	I	58.3	202					
	II	13.6	130	E (4)	I	27.9	425	0.36
	III	2.5	94		II	17.0	388	0.34
	IV	19.9	71		III	17.7	321	0.33
	V	5.7	43		IV	19.0	264	0.34
					V	6.6	202	0.32
E (2)	I	23.4	282		IV	4.2	171	0.29
	II	21.2	240		VII	3.1	82	0.27
	III	12.7	191		VIII	4.6	79	0.28
	IV	14.5	175					
	V	9.5	136	E (5)	I	20.5	358	
	VI	6.0	120		II	26.8	352	
	VII	7.1	105		III	14.0	303	
	VIII	4.7	74		IV	11.5	222	
	IX	3.4	58		V	11.9	202	
					VI	5.2	183	
					VII	4.5	151	
					VIII	2.4	134	
					IX	1.8	110	
					X	1.4	87	

* In benzene insoluble part.

In this case the fourth (third for E (5)) is the highest one. As regards the quantity of substance represented by the maxima, however, it seems to correspond to the fourth maximum for E (2). The discrepancy between the heights of the maxima in the two solvents certainly depends upon the relation between intrinsic viscosity and molecular weight.

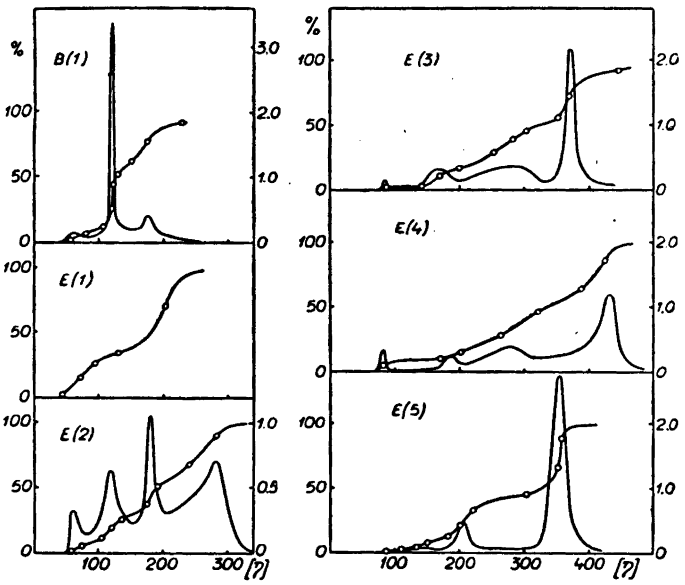

Fig. 3. Distribution and frequency curves from viscosity data.

SEDIMENTATION MEASUREMENTS ON FRACTIONS

In order to give further evidence for the frequency curves shown in the preceding section, the sedimentation constants for the fractions B (1) I—IX and E (2) I—IX have been determined in acetone solution. The results obtained are given in Table 5. There is also given for E (2) the k_s-values

Table 5. Sedimentation measurements on fractions.

Fraction	s_0	Fraction	s_0	k_s
B (1) I	42	E (2) I	94	7.3
II	90	II	97	9.4
III	59	III	80	4.7
IV	55	IV	70	—
V	51	V	60	—
VI	44	VI	53	—
VII	27	VII	39	1.7
VIII	38	VIII	34	1.6
IX	30	IX	26	1.1

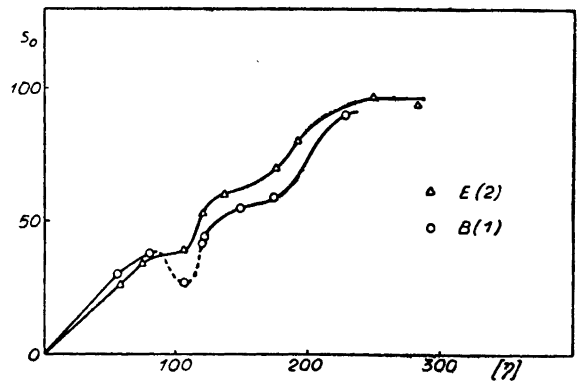

Fig. 4. The relation between sedimentation constant and intrinsic viscosity.

(c expressed in g/100 ml) of those fractions whose concentration dependence of s follows the relation.

$$s = \frac{s_0}{1 + k_s \cdot c}$$

(*cf.* Gralén, *l. c.*). The k_s-values show that the concentration dependence for the highest fractions is very pronounced.

Before we discuss the frequency curves, it is interesting to study the sedimentation constants as functions of the intrinsic viscosity. This last quantity is a simple algebraic function of the molecular weight and hence in this way we get an idea of the general behaviour of the molecules during the sedimentation. In Fig. 4 the s_0-values are plotted against the corresponding $[\eta]$-values. In their treatment of different hydrodynamical models Kuhn and Kuhn [14] have discussed two limiting cases of chain-like molecules: free draining coils and matted coils. The frictional resistance of the first type is proportional to the molecular weight M and that of the second proportional to $M^{1/2}$. As the sedimentation constant according to Svedberg [15] is defined by the relation

$$s = \frac{M(1 - V\varrho)}{f} \tag{5}$$

where f is the molar frictional coefficient, s for the free draining type is independent of M and for the matted coil proportional to $M^{1/2}$. If the molecules were built up in the same way the curves in Fig. 4 should indicate that the folding of the molecule depends very much on the molecular weight. In the regions $[\eta] = 80$—110, 130—170 and > 230 the molecules should behave

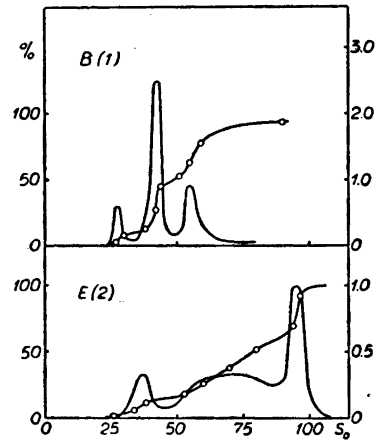

Fig. 5. Distribution and frequency curves from sedimentation data.

as free draining coils and between these regions more as matted coils. In this case, however, where we have synthetic polymers, the behaviour of the molecules may depend upon the internal structure and not only on the folding of the chain. Especially, branching of the chains may have a large effect on the frictional resistance. The k'-values in Table 4 give some evidence that structural differences really exist. According to Ewart [16], linear molecules in good solvents should give k'-values of about 0.38. Since we can assume benzene to be a good solvent for linear molecules of polymethyl methacrylates independently of their molecular weights, the only reason for the change in the k' values must be changes in structure. At high intrinsic viscosities we have values near to 0.38; then they decrease with decreasing viscosity but have for $[\eta]<300$ a tendency to increase; finally they decrease to values below 0.30. Alfrey, Bartovics and Mark [17] have shown that polystyrene polymerized at the temperatures 60, 120 and 180° C gives decreasing k'-values: 0.42, 0.33 and 0.21. Probably a higher temperature gives molecules which are more branched or more cross-linked.

From the s_0-values in Table 5 and the percentage amount of fractions B (1) I—IX and E (2) I—IX in Table 4, the distribution and frequency curves for the sedimentation constants have been drawn (Fig. 5). The frequency curve for B (1) shows three maxima. These occur for the s_0-values 27, 42 and 55 S. The frequency curve for E (2) also shows three maxima; the corresponding s_0-values are 36, 70 and 95 S.

It is possible to get the frequency curve for an unfractionated substance by adding the frequency curves for each fraction. This method has been tried for sample E assuming that the frequency curves of the sedimentation con-

stants for the fractions can be approximated with a logarithmic frequency function (given by Lansing and Kraemer [18] for the molecular weights):

$$\frac{dc}{ds_0} = K_s \cdot e^{-\frac{1}{\gamma_s^2}\log^2 s_0/s_1} \tag{6}$$

where γ_s is a distribution coefficient, K_s, the maximum height, and s_1, the s_0-value at the maximum. By knowing the dB/dx-values, it is possible to calculate γ_s according to the relation

$$\frac{dB}{dx} = \gamma_s \cdot e^{\frac{1}{4}\gamma_s^2} \sqrt{\pi} \tag{7}$$

and K_s from

$$K_s = \frac{\text{Area}}{s_0 \cdot (dB/dx)} \tag{8}$$

If the areas are taken equal to the fractional amount of each fraction, we get the values in Table 6 for the fractions E (2) I—IX. The frequency curve of sample E obtained in this way is drawn in Fig. 6. It shows only one maximum, but there are two irregularities on the left side of the curve. These indicate the existence of at least another two maxima (dotted lines).

Still another method has been used to get the frequency curve of sample E. In this the fractions have been assumed to have a symmetrical triangular frequency curve, the height of which is equal to K_s and the base equal to

Table 6. Data corresponding to frequency curves given in Figs. 6 and 7.

Fraction	s_1	dB/dx	Area	γ_s	$K_s \cdot 10^2$
E (2) I	94	0.58	0.234	0.319	0.430
II	97	0.56	0.212	0.309	0.394
III	80	0.60	0.127	0.330	0.265
IV	70	0.53	0.145	0.293	0.391
V	60	0.43	0.095	0.239	0.368
VI	53	0.42	0.060	0.234	0.269
VII	39	0.44	0.071	0.245	0.413
VIII	34	0.51	0.047	0.282	0.272
IX	26	0.40	0.034	0.223	0.327

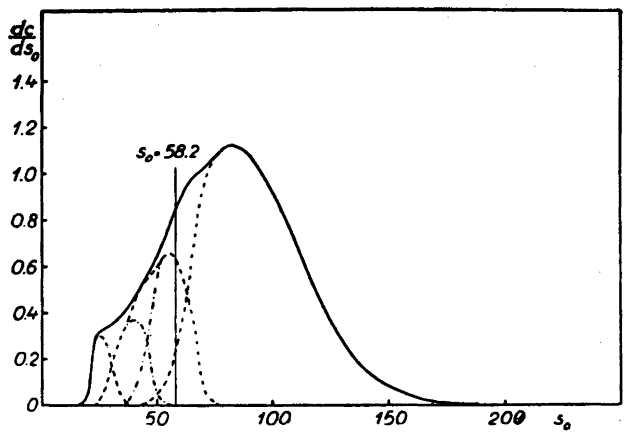

Fig. 6. Frequency curve obtained by adding the frequency curves of the fractions (logarithmic frequency curve).

$2s_0 \cdot (dB/dx)$. The frequency curve obtained (Fig. 7) shows two maxima, for $s_0 = 27$ S and $s_0 = 70$ S.

DISCUSSION

The results presented in the previous sections give evidence that the two samples of polymethyl methacrylate should have frequency curves with at least three maxima. This follows from the sedimentation diagrams and the curves in Figs. 3 and 5. The attempt to add the logarithmic frequency curves for the fractions does not give any clear cut decision. One of the reasons for

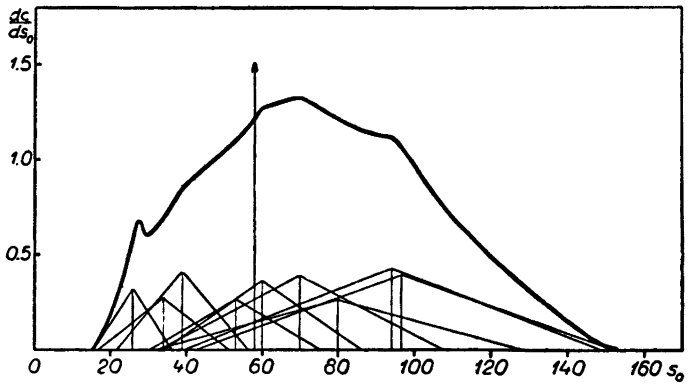

Fig. 7. Frequency curve obtained by adding the frequency curves of the fractions (symmetrical triangular curve).

this is certainly connected with the nature of the logarithmic frequency function. It will distribute too much substance towards higher s_0-values. From a theoretical point of view the fractions should have a tail towards smaller s_0-values instead (*cf.* Scott *l. c.*). A better result is obtained by adding the triangular frequency curves (Fig. 7). The two maxima correspond very well to the first and the third maxima of the viscosity frequency curve E (2) in Fig. 3 (the s_0-values for the peaks are according to the relation in Fig. 4, $s_0 =$ 27, 53, 72 and 96 S). Between 90 and 100 S the curve shows a tendency to form a third maximum corresponding to the fourth maximum in Fig. 3. The large maximum of the curve probably contains the two maxima shown in this region in Fig. 3. The agreement with the second and the third maxima in Fig. 5 is also good. As regards the curve in Fig. 7 its shape can depend upon the conditions under which the fractionation is performed. In some work on cellulose nitrate Rånby [19] has shown that the sharpness of the fractions depends upon the solvent/non solvent system used in the fractionation and that the frequency curve obtained is the more rich in details the sharper the fractions.

Examining Figs. 6 and 7 we see that the determined s_0-value of 58 S for the unfractionated sample E does not correspond to the highest maximum. From Fig. 5 it is seen that $s_0 = 58$ S corresponds to the left side of the middle maximum. This discrepancy may have two reasons. It has been shown by Kinell [20] that for polydisperse substances, the sedimentation of which is not dependent upon the concentration of the solute, the determined s_0-values does not correspond to the maximum of the original frequency curve except for the time $t = 0$. The higher the polydispersity the larger the difference between the observed value and the original maximum value for a given time. On the other hand the dilution with the sector shape of the cell causes an increase in the s-values. The combination of these two effects for a polydisperse, concentration dependent substance may cause the measurements to give an s_0-value which is too low. The other explanation of this may be found in the discussion given by Johnston and Ogston [21]. If two components with different sedimentation constants sediment in the ultracentrifuge, the slower moving component will show an increase in amount if the sedimentation velocity changes when the substance passes the boundary. (This effect was first observed by McFarlane [22] and Pedersen [23] in mixtures of proteins; it has also been studied on mixtures of fractions of cellulose nitrate by Sihtola [24]). For a substance which has a frequency curve with several maxima, it is very likely that in the sedimentation the heights of the maxima may change considerably according to this boundary effect. If this is the case here the maximum of the sedimentation curve should not correspond to the highest maximum of the

frequency curve. With decreasing concentration the effect should be less pronounced and disappear completely at zero concentration. It is not possible to run concentrations lower than 0.1 g/100 ml, when the concentration dependence is still quite considerable, and hence it is not excluded that, according to the boundary effect, the extrapolation gives an s_0-value which is too low. (An experimental error in the s_0-value must be excluded in this case where the agreement between the $[s]_0$-values is very good). A final decision as regards the reason for the observed discrepancy is not possible.

In this paper mainly the frequency curve of the sedimentation constants has been discussed. It is to be emphasized that conclusions as to the frequency curve of the molecular weights can be drawn from the sedimentation diagram only if we are dealing with molecules having certain sedimentation properties. If the molecules are of the free-draining type only a very bad or no resolution at all will be obtained in the ultracentrifuge, because the sedimentation constant is the same for all molecular weights. In this case nothing can be said about the distribution of the molecular weights without other methods being applied.

Finally, it can be emphasized that a synthetic high polymer giving a frequency curve of the shape shown in this paper must have been formed by a rather complicated mechanism of reaction. At least sample B is known to have been prepared by bulk polymerisation with a peroxide catalyst. This process should, therefore, be much more complicated than, for instances, the polymerisation in a reduction-oxidation system used by Baxendale, Bywater and Evans [25]. They get a frequency curve with only one maximum and in good agreement with the curve corresponding to the assumed simple reaction mechanism. A preliminary result from the investigations of Eriksson and Kinell [26] is that in bulk polymerisation a frequency curve with at least two maxima appears after quite a short time of polymerisation. The positions and the heights of the maxima is then changed as the polymerisation proceeds.

SUMMARY

Two samples of polymeric methyl methacrylate have been characterized by means of sedimentation and diffusion measurements. The samples have been divided into a series of fractions by fractional precipitation. The fractions have been characterized by viscosity and sedimentation measurements. From the data obtained, frequency curves have been constructed and it follows that each of the samples probably has three maxima. This is also in agreement with the conclusions which can be drawn from the sedimentation diagrams. The relation between sedimentation constant and intrinsic viscosity suggests

that the molecules have different structures (branching) or that the folding of the molecules depends on the molecular weight. This fact makes it difficult to draw definite conclusions about the distribution of molecular weights from the sedimentation diagram alone.

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On the Splitting-off of the Amide Group from Proteins

The Amides of Zein

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The amide group belongs as an essential part to most of the proteins In connection with the breaking of the peptide bonds by pepsin and trypsin ammonia is also formed. According to Damodaran and Ananta-Narayanan [1] the formation of ammonia is a non-enzymatic reaction whereby the amide groups which are present in peptides formed by proteolytic enzymes are split off at the acid or alkaline reaction. True, Melville [2] has noted that the peptides of glutamine, where the amino group of glutamine is free, are very unstable. The amide group is quantitatively split off from such peptides without any enzymatic effect at pH 1.8 at 37°, *i. e.* in the conditions of peptic hydrolysis.

In examining zein and casein we have noted that at the acid reaction (pH 1.5—1.8) at 37° C or at room's temperature amide nitrogen splits off from these proteins without any preceding pepsin effect. In the following we shall present our experiments pertaining to this as well as our observations on the amide nitrogen in zein.

EXPERIMENTAL

The first experiments were made in Erlenmeyer flasks. In the parallel experiments each flask contained 1 g zein suspended in 50 ml of 0.175 *N* HCl and 2 ml toluene. To one of the flasks was added besides crystalline pepsin (0.6 mg N), to its parallel none. At definite intervals samples were taken from the flasks and ammonia was determined in them. A continuous formation of ammonia was observed even without pepsin. Since, however, in these conditions certain errors are involved in the experimental results owing to the fact that the sample taken from the suspension does not always exactly represent the whole suspension, even be it thoroughly shaken, another method was adopted.

Samples of 100 mg zein, suspended in 5 ml of 0.175 *N* HCl and 0.2 ml toluene,were measured to ampuls. In addition, to the parallel ampuls crystalline pepsin (0.06 mg N) was added. The ampuls were sealed and kept at 37° C shaking occasionally. For each

interruption 1 or 2 ampuls were kept thus the contents of the whole ampul could always be used to determinations of ammonia and amino nitrogen. Frequently 2 parallel analyses were made using 2 ampuls. The results were then regularly in good agreement. The zein used in the experiments (fraction II) was isolated from yellow maize according to the method of Mason and Palmer [3] and fractionated according to the principle of Williams and Watson [4] in the manner modified by Laine [5]. The nitrogen content of the moisture-free zein preparation was 16.2 %. Similar experiments as with zein were made correspondingly with casein (Hammarsten). Ammonia was determined according to Pucher *et al.*[6] using in each receiver 0.01 *N* H_2SO_4 and titrating the excess of acid with 0.01 *N* NaOH. As indicator Tashiro's mixed indicator was used. A quantity of 0.01 *N* acid was equivalent to 0.14 mg ammonia nitrogen. The determination was made as follows.

The contents of the whole ampul were washed to a distilling flask and made alkaline with the Folin's solution. Distillation time was 15 min. Ammonia was determined from the distillate in the above manner. After distillation the residue was made up to 25 ml and amino nitrogen was determined according to Pope and Stevens [7] by the Cu-method.

Glutamic acid was determined according to the principle of Olcott [8] which is based upon a measurement of the loss in amino nitrogen occasioned by the transformation of glutamic acid to pyrrolidonecarboxylic acid at 125° and pH 3.3. pH was adjusted in our experiments to 3.3 by means of acetic acid. The accuracy of the method was tested both with pure glutamic acid and by adding a known amount of glutamic acid to the amino dicarboxylic acid fraction which was obtained by Foreman precipitation from the acid hydrolysate of zein. In the latter case glutamic acid was determined from the Foreman precipitate both before and after addition of glutamic acid. The results of these control experiments are given in the following

No. of expt.	Pure glutamic acid	Glutamic acid added to the hydrolysate of zein
	Found glutamic acid N, % of added	Found glutamic acid N, % of added
1	94.0	94.0
2	95.2	95.0
3	95.3	—
4	93.5	—

According to the above determinations the method in the form employed by us gives on the average 94.5 % of the theoretical value. The values found by us for the glutamic acid in the acid hydrolysate of zein are therefore correspondingly corrected.

Aspartic acid was determined according to Arhimo [9] using the method developed by Pucher *et al.*[6] for determination of malic acid.

RESULTS

Glutamic acid N was found in zein 15.2 % of the total N. Chibnall [10] recently reported an unpublished value of Rees 15.8 %. Taking into account the possible errors in the method used by us the agreement between our values

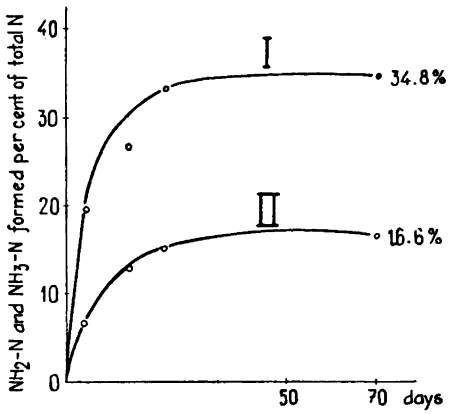

Fig. 1. Formation of amino-N (curve I) and ammonia-N (curve II) in hydrolysis of zein by crystalline pepsin at pH 1.5—1.8 at 37° C.

and those of Rees is fairly good. Laine [5] found in zein glutamic acid N 18.4 %. The method used by him has, however, evidently given too high values.

Aspartic acid N was found by us in zein 2.9 % of total N (2.87—2.99 %). The unpublished value of Rees reported by Chibnall [10] is 3.0 %, the value found by Laine [5] 2.2 %, and that of Dakin [11] 1.18 %. There is a good agreement between Rees's and our values which were obtained by different methods of analysis. The total amount of glutamic acid N and aspartic acid N of zein is according to our findings 18.1 % of total N, according to the values of Rees 18.8 %.

The amount of amide nitrogen found by us in zein was 18.4 % of total N, by Laine [5] 18.6 % and by Chibnall [12] 18.3 %. In regard to amide nitrogen the agreement is thus fairly good. Amide nitrogen corresponds rather accurately the sum total of glutamic and aspartic acid-N, accordingly, the total amide nitrogen in zein can belong to asparagine and glutamine.*

Amino nitrogen was found in zein by the Cu-method 73.2 %, the value of Laine [5] is 74.2 %.

The splitting-off of amide and amino nitrogens from zein *by pepsin* appears from the curves in Fig. 1. The values were obtained by the Cu-method and are too high since the bound NH-groups of lower peptides partly react in this method. The value of 34.8 % found by the Cu-method for amino nitrogen corresponds to about 25 % by the van Slyke method. The average molecular weight in the hydrolysate of zein is of the magnitude of tri-tetrapeptide.

* If a part of the ammonia resulting from acid hydrolysis is split from amino acids, the share of real amide-N is less, being calculated on ammonia. On the basis of the ammonia liberating in peptic hydrolysis it can be concluded that glutamic acid at least is completely amidated.

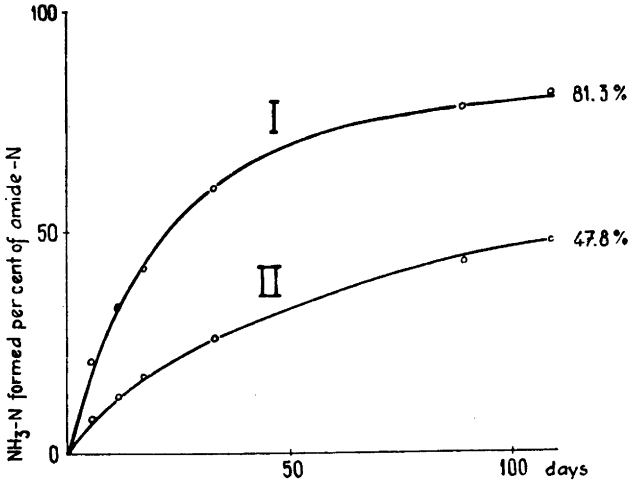

Fig. 2. Splitting-off of ammonia-N from casein (curve I) and from zein (curve II) at pH 1.5—1.8 at 37° C without pepsin.

The ammonia formed in peptic hydrolysis, 16.6 % of total nitrogen, is equivalent to 90.2 % of total amide nitrogen. The entire amide nitrogen is thus not liberated as ammonia in peptic hydrolysis during the experiment (70 days).

Splitting-off of amide nitrogen from proteins as ammonia *without pepsin* is shown by Fig. 2 (zein and casein).

As can be seen from the results large amounts of ammonia have been liberated both from zein and casein at pH 1.5—1.8 without pepsin. From the amide nitrogen contained in zein there have been liberated in 33 days 26 % ammonia-N and in 109 days 48 %. The graph illustrating the velocity of decomposition indicates that the reaction probably ceases at about 50 % unless new decomposition is started by possible breakdowns of the molecule. We have so far followed the ammonia formation in 109 days. In the course of 90 days only 1.8 % amino-N was liberated without pepsin from zein which shows that the peptide bonds are not to any greater extent broken in the zein molecule.

In order to ascertain that the enzymes probably present in the preparation of zein had not caused decomposition of ammonia we heated a water suspension of zein for ½ h at 110° C and made a decomposition experiment with this preparation at pH 1.5—1.8 without pepsin. Ammonia-N was split off in 8 days 10.7 % of the amide nitrogen of zein, hence practically the same quantity of ammonia was set free from heated zein as from unheated.

The amide-N of casein was liberated as ammonia without pepsin in a considerably greater measure than that of zein. Ammonia N amounted in 33 days to 60 % of the amide nitrogen, in 109 days to 81 %. Also the peptide bindings in the casein molecule seem, however, to break down appreciably at pH 1.5—1.8 without pepsin as indicated by the formation of amino nitrogen. After 13 days amino-N was formed 2.2 % and after 109 days 13.6 % of total nitrogen.

These results are in agreement with the results of Carpenter[13] who estimated the soluble and amino nitrogens formed from casein at different temperatures in dilute solutions of casein. Ammonia-N then split off was not determined by him. Osborne and Nolan[14] and Vickery[15] have earlier shown that boiling of gliadin with dilute hydrochloric acid when a relatively small proportion of the peptide bindings are broken yields nearly the same amount of ammonia as is formed during total hydrolysis with concentrated acid.

DISCUSSION

The result obtained show that amide nitrogen is split off at an acid reaction (pH 1.5—1.8) at 37° C from proteins to a very great extent *without enzymatic effect.* Approximately a half of the amide nitrogen of zein seems to be split off in our experimental conditions. The simultaneous increase of amino nitrogen has been very slight, and consequently, no corresponding breaking of peptide bonds has occurred. A small increase in the amino nitrogen was, however, noted.

In the light of the analyses the amide nitrogen in zein corresponds quantitatively to the sum total of glutamic and aspartic acid-N, accordingly, all the amide-N may belong to glutamine and asparagine. Since the share of the amide group of glutamine is about 85 % of the total amide nitrogen and only about a half of the amide nitrogen is split off at pH 1.5—1.8 *without pepsin*, the entire glutamine residue does not give up its amide group in the form of ammonia. Liberation of ammonia is continuously retarded in the course of the experiment. This seems to imply that a part of the amide groups of glutamine is in such a position that its splitting is easily affected at an acid reaction, whereas a part again is located so that the splitting is difficult or impossible. It could be postulated that a part of the amide groups of the glutamine residues is protected in the zein molecule, for instance, by being located between the submolecules united by hydrophobic groups, and consequently, the acid solution has no chance of attacking them, at least not during an experimental period of nearly 4 months.

On decomposition of zein *by pepsin* amide nitrogen is simultaneously split off. The question concerns then, however, the unstability of the glutamine

peptides formed and not the splitting off of amide groups from the zein molecule itself. The entire amide nitrogen is not split off in the peptic hydrolysis. The ammonia formed in about 30 days is approximately equal to the glutamine in zein. This indicates that ammonia is apparently not split off from asparagine peptides, which is in agreement with the findings of Damodaran *et al.*[1] Strange enough, in a period of long duration (70 days) somewhat more ammonia is split off than corresponds to the glutamine residues in the zein molecule, accordingly, ammonia is also formed from some other N-compound besides glutamine. If asparagine peptides were able to give up a little ammonia during prolonged experiment the origin of the excess of ammonia would be explicable. More experiments are needed in this respect. Addition to the proof.

In a later experiment (Virtanen and Kerkkonen), in which after a hydrolysis of 49 days pepsin solution was added to the hydrolysate, total amide-N was split off in 64 days as ammonia (found NH_3-N 18.2 % of total N).

From casein amide nitrogen is split off at pH 1.5—1.8 *without pepsin* even to a much greater extent than from zein. Over a period of nearly 4 months well over 80 % of the amide nitrogen of casein were split off. Since there is no information about whether amide nitrogen in casein belongs entirely to glutamine — the quantity of amide nitrogen (9.1 % of total N) does not by far require the presence of the entire glutamic acid (13.4 % N of total N) in the form of glutamine — it is difficult to say whether ammonia is split off more easily from the glutamine in casein than from that in zein. It must, however, be duly considered that at an acid reaction there is formed appreciably amino nitrogen from casein, and accordingly, the casein molecule seems to break down considerably without pepsin during a longer period. More investigations are needed to explain the breakdown of casein at an acid reaction.

Laine [5] arrived in his studies at the conclusion that pepsin breaks the peptide bonds at the point of glutamine residues in zein, hence, in the peptides formed the amino group of glutamic acid is free and can be oxidized to a hydroxy-group (α-hydroxy-glutaric acid) by means of nitrous acid. The quantity of glutamic acid in zein was according to his determinations so high that it would have recurred (as glutamine residues) as every fourth amino acid in the zein molecule. Since, however, according to the recent determinations the amount of glutamine in zein is lower, this concept does not hold good at least not as such. The sum total of glutamine and asparagine residues, again, is so high that these amino dicarboxylic acids together can replace every fourth amino acid in the peptide chains. The elucidation of this question requires more research.

SUMMARY

It has been shown, that both from zein and casein high percentages of amide nitrogen are liberated as ammonia at an acid reaction (pH 1.5—1.8) at 37° C wthout pepsin. The reaction is non-enzymatic. Over an experimental period of about 4 months about 50 % of the amide nitrogen have been split off from zein, and over 80 % from casein. Since only a few amino groups are liberated from zein during the experiment the amide nitrogen is split off at least to a great extent from undecomposed zein molecule. On the contrary, casein seems to split considerably in these circumstances as indicated by the great increase in the amino nitrogen.

The amide nitrogen of zein (18.4 % of the total nitrogen) well corresponds to the sum of aspartic and glutamic acid N (18.1 % of total N according to our determinations, 18.8 % according to Rees). It is plausible that the amide nitrogen belongs to the asparagine and glutamine residues of zein. In a peptic hydrolysis 90 % of the total amide nitrogen is split off as ammonia (cf. addition on page 852). The glutamine peptides formed in the hydrolysis are first split off.

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Determination of α-Alanine by Ninhydrin Oxidation

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Virtanen and collaborators [1-3] have in this laboratory worked out a method for determination of certain amino acids by ninhydrin oxidation. In this method the amino acids are converted to aldehydes with one less carbon atom ($R \cdot CH(NH_2) \cdot COOH \rightarrow R \cdot CHO$); the volatile aldehydes formed are distilled into a bisulfite solution, and their total amount is determined by means of iodometric titration. The group of amino acids which can be determined in this way, the so-called »volatile aldehyde amino acids», is comprised by α-alanine, valine, leucine, isoleucine and possible other isomers, phenylalanine, and methionine.

It has been shown in recent years that α-alanine has some special importance in the metabolism of amino acids and proteins. It is one of the amino acids active in the transamination reaction, and investigations concerning the protein synthesis in yeast (Roine [4]) showed that alanine together with the dicarboxylic amino acids is abundantly formed in the very beginning of the nitrogen uptake by yeast. Alanine, thus, occupies a special position in the group of »volatile aldehyde amino acids», and a separate determination of it seems to be important in many cases.

As reported earlier by one of us [4] alanine can be determined conveniently in connection with the determination of »volatile aldehyde amino acids» by means of some colour reaction, specific for acetaldehyde. The blue colour which acetaldehyde gives with sodium nitroprusside and piperazine is well suited for this purpose. The solution, the alanine content of which is to be analyzed, is first extracted with ether to remove acetaldehyde, pyruvic acid, and other possibly disturbing substances. After the oxidation with ninhydrin, distillation and titration, acetaldehyde is determined photometrically in a way similar to that used by Fromageot and Heitz [5].

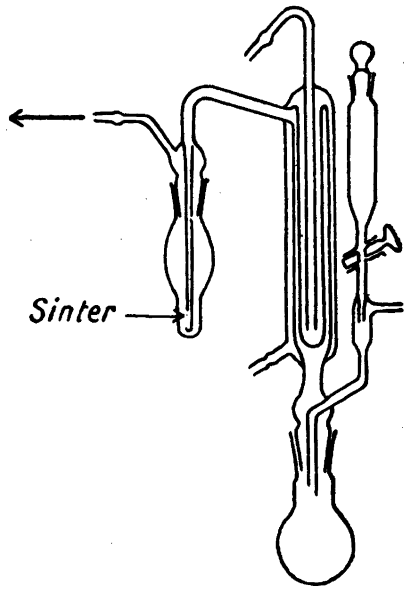

Fig. 1. Lieb-Zacherl apparatus.

PROCEDURE

A sample of the solution to be analyzed, the alanine content of which corresponds to 0.5—20 mg, is made acid with sulphuric acid and extracted over night with aldehyde-free ether in a Partheil-Rose extractor [6]. The excess of sulphuric acid is neutralized with sodium hydroxide, the remaining ether evaporated on a water bath at 60—70° C, and the volume of the residue made up to 20 ml. 2—10 ml of this solution are placed in the reaction flask of a Lieb-Zacherl apparatus (Fig. 1), made up to 10 ml and 1 g of potassium dihydrogen phosphate, and 2.1 g of sodium chloride are added. The receiver is charged with 5 ml of 1 % sodium bisulphite solution. The solution in the reaction flask is brought to boil, whereupon 3 ml of an 1 % ninhydrin solution are introduced through the side tube. The distillation is continued for 60 min after which the receiver is disconnected, the tube with sintered end washed twice with small amounts of water, and the excess of bisulphite destroyed with 0.1 *N* iodine solution. After the addition of starch the solution is titrated colourless with 0.01 *N* sodium tiosulphate. 10 ml of a saturated sodium bicarbonate solution are added and the bisulphite thus liberated, titrated with 0.01 *N* iodine. After the addition of some drops of 0.01 *N* sodium tiosulphate, the titrated solution is placed in a 50 ml volumetric flask and filled to the mark.

A 6 ml aliquot of the solution is placed in a 10 ml measuring cylinder, 0.5 ml of 4 % sodium nitroprusside solution and 1.5 ml of 25 % (saturated)

piperazine solution are added, mixed thoroughly and placed in a 2 cm cell of Pulfrich photometer (of the two piperazine preparations tried by us, only that of Schering A. G. (Berlin) gave reliable results). The colour reaches its maximum intensity in some 2 or 3 min and remains there for 1—2 min during which time the readings are to be made, using colour filter S 57. The amount of alanine equivalent to the reading is read from a calibration curve constructed with pure acetaldehyde.

DISCUSSION

In addition to acetaldehyde only acrolein and propionaldehyde give the same colour reaction with sodium nitroprusside and piperazine, the intensity of the colour, however, in the latter cases being very weak. The aldehydes which are formed of valine, leucine, isoleucine and possible other isomers, phenylalanine, and methionine, do not give this reaction. Under the conditions described above, the method is thus specific for alanine. The smallest amount of alanine which can be reliably determined with the given procedure, is about 0.5 mg.

The procedure is rapid and simple and is especially well suited to be performed in connection with the determination of »volatile aldehyde amino acids» (*cf.* Virtanen and Rautanen [3]). In this laboratory the method has been used for the determination of alanine in yeast [4] and plant [7] extracts as well as in protein hydrolysates.

SUMMARY

A micro method for determination of α-alanine is described. Alanine is oxidized with ninhydrin and the acetaldehyde formed is determined photometrically using the specific colour reaction which it gives with sodium nitroprusside and piperazine.

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Some Remarks on Schwédoffs Experiment with a Gelatin Sol

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In 1889 Schwédoff made a wellknown rheological experiment with a 0.5 % gelatin sol [1] in an apparatus (similar to a Couette viscometer) consisting of two co-axial cylinders; the solution was poured into the cylindrical shell. The inner cylinder could be turned by a torsion wire, and the shearing angle, Ω, as well as the torsion angle, Δ, could be determined.

After having made a certain initial twist of the wire Schwédoff read the angle, Ω, that the inner cylinder had rotated. Then he tried to keep Ω constant by means of continuously diminishing Δ, *i. e.* back twisting of the torsion head. He took up Δ as a function of the time t. When he tried to obtain a formula to fit his results he came to the following equation.

$$\Delta = \Delta_\infty \left[1 + \left(\frac{\Delta_0}{\Delta_\infty} - 1\right) e^{-\frac{t}{\tau}}\right] \qquad (1)$$

τ is a »relaxation» constant. The indices of Δ relate to time. This equation with three constants was well satisfied by Schwédoff's experimental values. Later Hatschek and Jane [2] made investigations on similar sols but were unable to fit their experimental values into equation 1.

In spite of that Reiner [3] has proposed a system of rheological classes, one of which has been named after Schwédoff. Thus that class is based on one single experimental series.

It is now reasonable to suppose that the gelatin sol of Schwédoff's experiment obeyed de Waele—Ostwald's law [5,6] as well as a comparatively concentrated gelatin solution usually does so; compare Auerbach [7], Ostwald [6] and Ostwald and Stuart [8].

I have made experiments with gelatin sols in three types of apparatus *viz.*, the Tsuda viscometer [9], the Bungenberg de Jong, Kruyt and Lens pressure

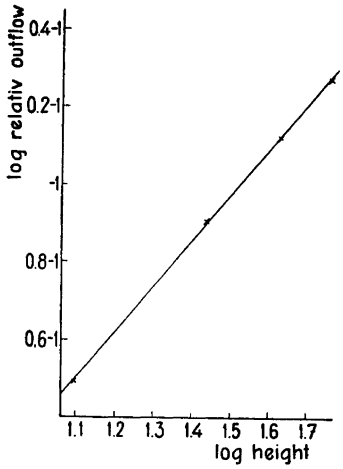

Fig. 1. A gelatin sol in a Tsuda viscometer. log relativ outflow (in cm/s) as a function of the pressure (in cm solution).

viscometer [10] and the Auerbach apparatus [7]. In all these types of instruments gelatin sols behaved like de Waele—Ostwald materials. An example of the experiments is shown in Figure 1.

As is known de Waele—Ostwald's law can be written

$$\psi \cdot \sigma = p^{\beta} \cdot t \tag{2}$$

where β is a constant depending on the material, and ψ is a constant that is identical with viscosity when $\beta = 1$. p is shear stress and σ shear strain.

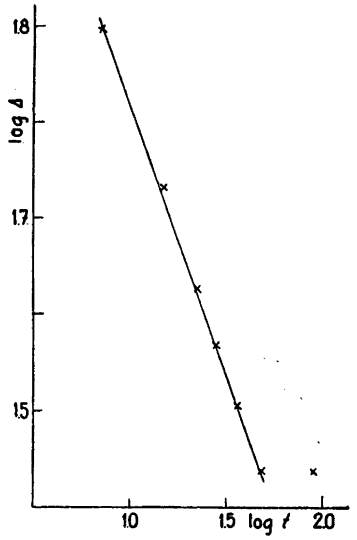

Fig 2. Schwédoffs experiment. log Δ as a function of log time.

In the Schwédoff case Ω was kept constant. That means that there was no streaming after the initial torsion, *i. e.* σ was constant. As p is proportional to Δ, the experimental values ought to follow a straight line in a logaritmic diagram over Δ as a function of t, if de Waele-Ostwald's law is valid. As can be seen from Figure 2 this is also the case. The first value has the greatest experimental uncertainty. As regards the last value it may be that the inner cylinder had got stuck. The other values lie close to a straight line.

SUMMARY

It has been shown that the gelatin sol of Schwédoff obeyed de Waele—Ostwald's law. The so-called Schwédoff class in Reiner's rheological system has therefore no justification.

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Preliminary Communication

On the Melting Point of Hydratropic Acid

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Hydratropic (α-phenyl-propionic) acid was first prepared 80 years ago by Kraut[1], who described it as »nicht krystallisierbar». Later it was investigated more thoroughly by Fittig and Wurster[2], who purified it through the calcium salt and found that it did not solidify even at — 20°. Later investigators invariably describe the acid as a viscous liquid, and in modern handbooks and tables we often find the statement of Fittig and Wurster, sometimes in the erroneous form m. p. $< -20°$. The optically active acid, first prepared by Raper[3], also failed to crystallise.

On comparison with related acids it seemed unlikely that the melting point of hydratropic acid would be extremely low, and for stereochemical reasons it was desirable to study the melting point diagrams given by this acid with certain other compounds. One of us has for other purposes worked out a convenient method to prepare the ethyl ester of α-cyan-hydratropic acid in a very pure state[4]. On boiling with alkali this ester is hydrolysed and decarboxylised, yielding hydratropic acid. After standing for some time in an open beaker at — 6°, this acid solidified to a crystal cake. It was dissolved in about twice its volume of ligroin (b. p. 60—70°) at room temperature, cooled to — 6°, and left to crystallise after seeding. The acid could also be crystallised from petrol ether (b. p. 30—50°). It is obtained as large, short-prismatic crystals; m. p. after two recrystallisations 16—16.5°.

0.2081 g: 13.07 ml 0.1060 *N* NaOH. — 25.21 mg: 66.36 mg CO_2 and 15.30 mg H_2O.

$C_9H_{10}O_2$ (150.2)

Calc.	Equiv. wt. 150.2	C 71.98	H 6.71
Found	» » 150.2	» 71.79	» 6.79

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On the Relation between Nitrogen Fixation and Leghaemoglobin Content of Leguminous Root Nodules. II

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In the previous paper under the same title Virtanen *et al.* [1] reported on some results which suggest that the rate of nitrogen fixation depends on the haemoglobin content of the root nodules. In order to obtain more elucidation on this correlation we made parallel determinations of the nitrogen content of peas and horse beans, inoculated with bacterial strains of different effectiveness, and of the haematin * content of the nodules. In addition, in the experiments on soya bean, the nitrogen content of the plants and the haematin content of the nodules was determined at different stages of growth. In the following we shall describe our experiments. The results of the pea experiments were already briefly discussed at the IV. International Congress for Microbiology in Copenhagen [2].

EXPERIMENTAL

Methods

In the experiments 1 to 3 we examined the haematin content of nodules formed by bacterial strains with different activity. Pea and horse bean were grown in unglazed pots. Culture medium was quartz sand, pH about 6.5. Watering solution was free of combined N, pH 6.5. Its composition was: 3.6 g $MgSO_4$, 4.5 g KCl, 4.2 g KH_2PO_4, 4.0 g $CaSO_4$ in 60 litres of tap water. Each pot contained 3 plants. Seeds in 3 pots were inoculated with the same bacterial strain. When the experiment was interrupted the 3 parallel pots, 9 plants altogether, were taken for each determination. — In fine weather the plants were kept out in the open air, in the night-time and on rainy days in the greenhouse.

The plants were cut just above the sand and their dry matter and nitrogen contents were determined by drying the plants in an air-oven after which they were weighed

* Haematin has been used to designate the prosthetic group of haemoglobin independent of the valency of iron.

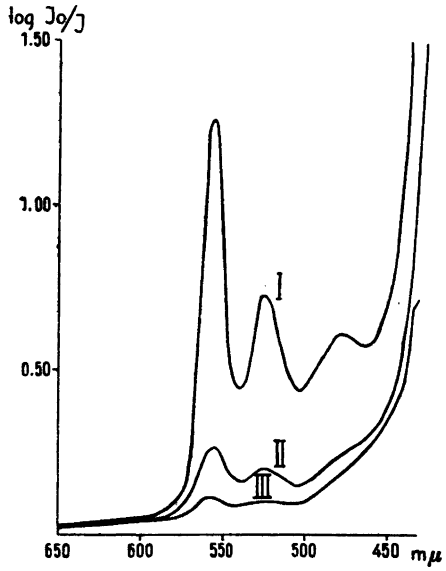

Fig. 1. Absorption spectrum of the pyridine extraction from different root nodules of pea.
I. H 7 nodules, red (experiment 28. VI—6. VIII. 1947).
II. H 7 nodules, partly green (experiment 29. VIII—17. X. 1947).
III. H 5 nodules, green (experiment 29. VIII—17. X. 1947). Plants were kept in the dark for 66 hours after the end of the experiment.

and ground in a mill to fine powder. The Kjeldahl-determinations were made from the powder. The results of the analysis are expressed per plant.

The roots were carefully separated from the sand by pouring the contents of the pot upon a large sheet of paper. As far as possible all nodules were removed. Haematin was determined as pyridine hemochromogen as follows.

A sample of 500 mg was taken from the nodule mass, washed and quickly dried between sheets of blotting-paper, pyridine and some sodium hydrosulphite were added, the mixture was crushed in a mortar, and centrifuged. Extraction with pyridine was repeated twice, after which red colour was no longer detected in the solution. The extinction of the clear extract was determined in the experiment 1 (pea) by Pulfrich photometer using the filter S 53 and the cell 0.25 cm. In the experiment 2 (pea) extinction was determined in the same way. In addition, the absorption between 650 and 440 mμ was measured by the Beckman spectrophotometer. Determination of haematin was even then made on the basis of the absorption at 530 mμ and the haematin content was expressed as chlorhaematin. As standard solution we used the pyridine solution prepared from haematin-HCl-compound isolated from blood.

The curves in Fig. 1 indicate the absorption between 440 and 650 mμ of the pyridine solutions obtained from red and green nodules. As shown by curve I the pyridine extraction of the red nodules has a very distinct maximum at about 555 mμ and another pronounced maximum at about 525 mμ. When the plants come to full bloom and the pods are partly formed the red pigment begins to turn green. The absorption maxima are then rapidly lowered as shown by curve II. The velocity of the change in the colour depends on the intensity of light and, on the whole, upon the factors affecting the growth. In unfavourable conditions the nodules may start to turn green already at the early part of flowering or even before flowering, whereas in more favourable conditions the change does not take place until pods begin to form. Great differences are also noted between the nodules of different bacterial strains in respect to the rapidness of the change.

When the plants are kept in the dark for a couple of days the nodules turn green, as previously reported by Virtanen [3] and the absorption maxima almost entirely disappear. This is clearly seen from the curve III.

The curves in Fig. 1 seem to suggest that for determination of haematin the absorption range from 550 to 560 mμ is the most suitable. But as we at first had available only the filter S 53 of Stufen photometer we had to determine the extinction by that and therefore, when we later obtained the Beckman spectrophotometer we chose the same wavelength (530 mμ) for the basis of the measurements to facilitate comparison.

The curves reveal that the method employed by us for determination of haematin gives the less reliable values the more abundant the formation of green pigment since the green pigment has an even, though weak, absorption between 500 and 560 mμ, and this raises the total absorption. Therefore in comparing the haematin content of the nodules of different strains the determination has to be made at a rather early stage when the red pigment has not yet turned green to any greater extent. Even then there may be in certain cases so much green pigment as to slightly affect results.

According to the spectrophotometric determinations the nodules formed by H VIII strain do not contain leghaemoglobin and therefore the very weak absorption of the pyridine solution from the H VIII nodules at 530 mμ has been subtracted from the extinction value of the nodules of other strains. The haematin content of H VIII nodules is thus marked as 0 in the figures. In this way the haematin values can be considered to have nearest correspondence to the haematin groups found in leghaemoglobin.

In experiment 3 (horse bean) the absorption was determined by Beckman spectrophotometer at 530 mμ.

In experiment 4 (soya bean) the haematin content of the nodules was examined at different stages of growth. Inoculated soya beans were grown in large boxes of sand out-of-doors and five times during the growth period (73 days) 20 plants were taken for analysis. After separation of the nodules from the plants dry matter and nitrogen were determined in them. From the nodule mass haematin was determined by the Beckman spectrophotometer in the manner described above in connection with the pea experiments. The remark on the effect of the green pigment on the haematin values refers also to this experiment.

Experiments

The 1st set of experiments on Torsdag pea was carried out 23. IV—17. VI. 1947. The experiment comprised 39 pots, each 3 of them were inoculated with the same strain. Thus the whole set came to include 13 parallel experiments with 13 different strains. The strain H VIII forms completely ineffective nodules, H 130 is slightly effective, H 9, too, is weak. The other strains are effective or very effective. The experiments were interrupted at the same time at the early part of flowering. The nodules were, on the whole, red, but some green pigment could be seen in the nodules of certain strains.

The nitrogen content of the plants (3 in each pot) and the haematin content of the nodules are given in Table 1 and Figs. 2 and 3.

The 2nd set of experiments on pea was conducted 28. VI—5. VIII. 1947. The experiment was otherwise similar to the previous one, except that it was interrupted before flowering. The nodules were still red, hardly any green nodules were perceptible. Table 1 and Fig. 4 show the results of this experiment.

The 3rd set of experiments was made on horse bean. For inoculation 7 different strains were used, 2 of them were very weak. Period of growth was 11. VII—27. VIII. 1947.

Table 1. 1st experiment on different bacterial strains. Test plant: Torsdag pea in full bloom. Period of growth: 23. IV—17. VI. 1947, 55 days.

Strain	Number of plants	Plants (green parts)		Nodules							
							Haematin			N	Fe
		Dry matter g/plant	N mg/plant	Fresh weight mg/plant	Dry matter %	Dry weight mg/plant	γ/g fresh weight**	γ/g dry weight**	γ/plant	mg/g dry weight	γ/g dry weight
H 1	9	1.006	32.4	693			136 (116)		78		
H 2	9	1.031	42.3	380	10.6	40.3	172 (152)	(1210)	39	71.1	592
H 3	9	1.162	49.3	400	9.5	38.0	176 (156)	(1540)	59	78.8	662
H 4	9	0.651	33.1	503	10.0	50.3	104 (84)	(730)	37	86.5	542
H 5	9	1.201	49.0	351	9.7	34.0	160 (140)	(1330)	45	89.5	628
H 6	9	0.054	42.4	345	9.2	31.8	144 (124)	(1260)	40	98.6	592
H 7	9	0.929	36.7	294	10.5	30.8	127 (107)	(900)	28	74.3	662
H VIII	9	0.267	5.5*	430	6.3	27.1	20 (0)	(0)	0		
H 9	9	0.492	20.6	456			120 (100)		41		
H 10	9	0.802	34.6	606	10.4	63.0	116 (96)	(800)	50	102.6	482
H 43	9	1.023	38.1	332	7.7	25.6	147 (127)	(1600)	41	77.1	654
H 47	9	0.913	33.1	199	9.7	19.4	129 (109)	(1020)	20	94.6	798
H 130	6	0.349	8.9	100	7.5	7.5	44 (24)	(280)	2	47.1	370
2nd experiment. Torsdag pea in bud. Period of growth: 28. VI—5. VIII. 1947, 39 days.											
H 1	9	0.588	21.4	506	8.3	41.4	108 (83)	(1040)	43		510
H 2	9	0.579	24.6	316	8.5	26.8	136 (111)	(1340)	36		568
H 3	9	0.600	25.6	309	9.8	30.3	177 (152)	(1550)	47		565
H 4	9	0.457	19.9	453	10.6	48.0	142 (117)	(1080)	52		411
H 5	9	0.557	24.7	346	10.6	36.7	156 (131)	(1200)	44		512
H 6	9	0.647	29.2	338			151 (126)		39		
H 7	9	0.676	30.6	359	10.1	36.2	172 (147)	(1450)	53		551
H VIII	9	0.214	4.8*	318	9.6	30.6	25 (0)	(0)	0		384
H 9	9	0.261	7.3	557	8.9	49.6	73 (48)	(550)	27		396
H 43	9	0.891	37.8	445	9.8	43.6	170 (145)	(1480)	65		602
H 47	9	0.740	29.8	370	9.9	36.6	177 (152)	(1550)	57		541
H 130	6	0.246	6.1	251	8.3	20.8	38 (13)	(200)	4		373

* No N-fixation. N originates from seed. N-content of a seed of Torsdag pea is about 7—8 mg.

** The figures in brackets are after subtraction of »haematin» in H VIII nodules.

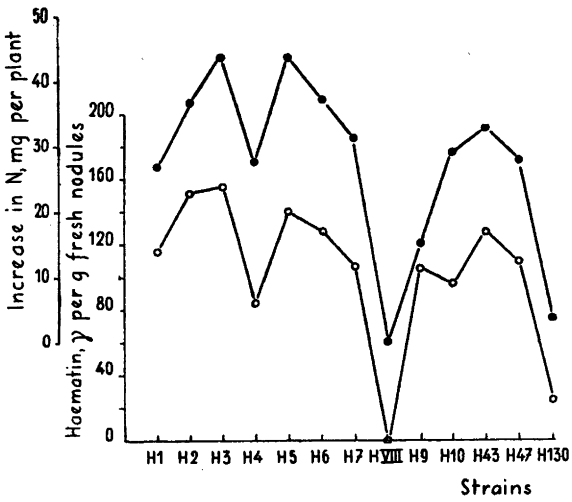

Fig. 2. Haematin content of the nodules of Torsdag pea inoculated with different bacterial strains. Period of growth: 23. IV—17. VI. 1947. Plants in full bloom at the end of the period, nodules chiefly red, a few green nodules in some experiments. The low absorption of inactive H VIII nodules is subtracted from each experiment.

●——● *Increase in N, mg/plant.*
○——○ *Haematin, γ/g fresh nodules.*

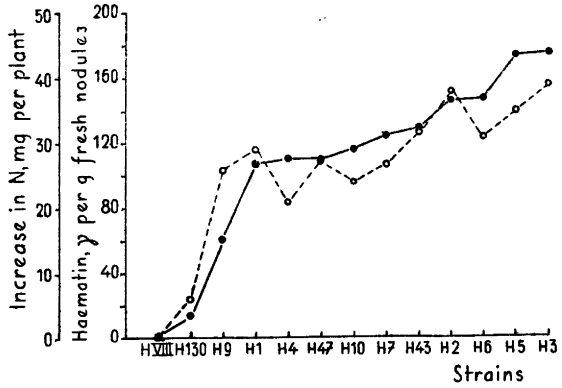

Fig. 3. Similar experiment as in Fig. 2 but interrupted before flowering.

●——● *Increase in N, mg/plant.*
○— — —○ *Haematin, γ/g fresh nodules.*

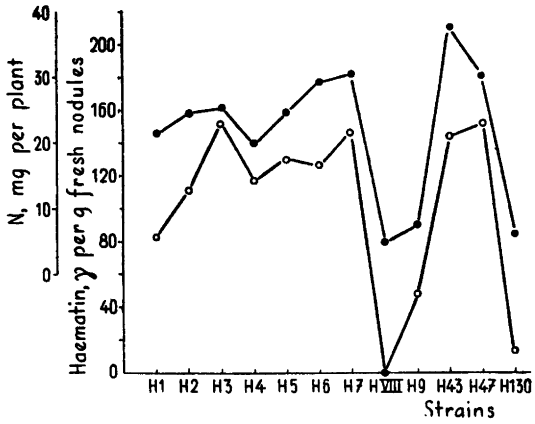

Fig. 4. Haematin content of the nodules of Torsdag pea inoculated with different bacterial strains. Period of growth: 28. VI—5. VIII. 1947. Harvested before flowering, nodules red. The low absorption of H VIII nodules is subtracted from each experiment.

●——● *N, mg/plant.*
○——○ *Haematin, γ/g fresh nodules.*

Table 2. 3rd experiment on different bacterial strains. Test plant: horse bean. Period of growth: 11. VII—27. VIII. 1947, 47 days.

Strain	Number of plants	Plants (green parts)		Nodules					
				Fresh weight mg/ plant	Dry matter %	Dry weight mg/ plant	Haematin		
		Dry matter g/plant	N mg/ plant				γ/g fresh weight	γ/g dry weight	γ/plant
F 1	6	2.472	90.0	796	12.3	98.1	182	1480	145
F 2	6	2.705	99.0	933	15.5	140.0	195	1260	176
F 3	6	2.405	85.4	784	13.0	102.0	152	1170	119
F 4	6	2.420	87.6	927	14.3	132.6	195	1350	180
F 5	6	3.040	112.8	776	12.6	97.8	216	1710	167
F 6	6	0.526	14.3	100	13.8	13.8	68	495	68
H 5*	6	0.718	20.9	192	11.8	22.6	32	272	62

* Strain of pea bacterium.

We are indebted to Mr. P. I. Forsius for determinations of haematin in this experiment.

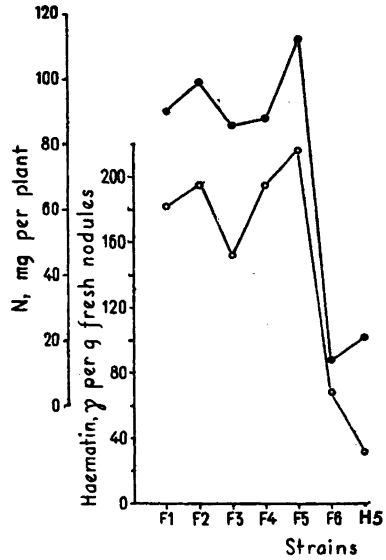

Fig. 5. Haematin content of the nodules of horse bean inoculated with different bacterial strains. Period of growth: 11. VII—27. VIII. 1947. Plants in the beginning of flowering, nodules red.

●——● *N, mg/plant.*
○——○ *Haematin, γ/g fresh nodules.*

Table 3. 4th experiment. Test plant: soya bean. Period of growth: 30. VI—12. IX. 1947. Haematin content of nodules and nitrogen content of plants determined 5 times in the course of growth.

Date	Age days	Stage of growth	Plants				
			Number	Average length cm	Fresh weight g/plant	Dry weight g/plant	N mg/plant
17. VII	18	Before flowering	5	15	6.94	1.30	42.2
26. VII	27	Flowering begins	5	25	20.8	3.92	134.2
11. VIII	43	Flowers and pods	5	38	44.2	8.81	261.0
22. VIII	54	Pods, some flowers	5	45	49.3	9.70	322.0
12. IX	73	Pods, no growth	5	45	55.4	13.38	412.4

Date	Nodules								
	Number	Colour	Fresh weight mg/plant	Dry matter %	Dry weight mg/plant	Haematin γ/g fresh weight	Haematin γ/g dry weight	N mg/plant	Fe γ/g dry weight
17. VII	20	red	309	21.1	65	155	730	3.15	340
26. VII	30	»	755	24.1	182	242	1000	9.40	300
11. VIII	46	»	1487	22.9	340	358	1560	17.0	410
22. VIII	75	»	1574	25.2	397	382	1560	19.2	360
12. IX	71	green	2084	19.0	397	176*		18.4	355

* Haematin value of no significance, since the nodules were green.

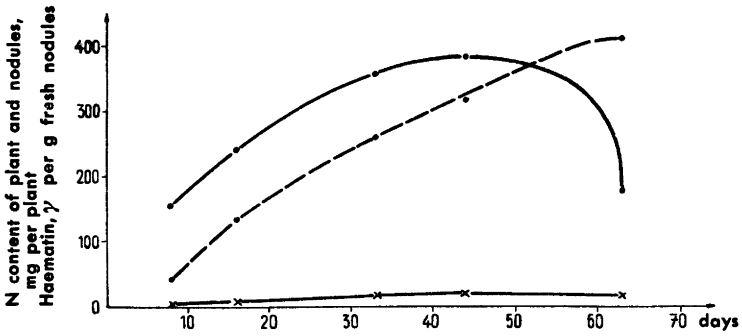

Fig. 6. Nitrogen content of plant and haematin content of nodules at different stages of growth of soya bean.

●———● *Haematin, γ/g fresh nodules.*
●— — —● *N, mg/plant without nodules.*
×———× *N in nodules, mg/plant.*

When the experiment was interrupted the plants had just started to bloom and the nodules had not yet even partly turned green. Table 2 and Fig. 5 illustrate the results.

The 4th set of experiments on soya bean was carried out 30. VI—12. IX. 1947. Samples were taken 5 times at different stages of growth. At the last time after 73 days of growth the soya beans had already ceased to grow and were producing seeds. The nodules had turned green and the haematin determinations had no longer significance in quantitative sense. On the former samples taken at the age of 18, 27, 43, and 54 days nodules were red. The results are given in Table 3 and Fig. 6.

DISCUSSION

All the experiments give the general impression that the ability of the nodules to fix nitrogen is correlated with the haemoglobin content of the nodules. Although haematin only was directly determined and not leghaemoglobin the values, nevertheless, chiefly refer to leghaemoglobin. In young peas the haematin of nodules originates for the most part from leghaemoglobin.

In examining the relation of the haematin content of the root nodules and the nitrogen fixation a distinctly positive correlation is observed between the nodules formed by weak and effective strains. If the differences in the effectiveness of strains are but slight the variations in the haematin content of the nodules do not always parallel the differences in the nitrogen fixed in the plants, as illustrated by Fig. 3. It is evident that a complete correlation between the nitrogen fixation and the haematin content of the nodules can hardly be expected since many various factors affect the growth and the

nitrogen fixation. Such a parallelism as was found in our experiments is almost surprising in these conditions.

While a parallelism exists between the nitrogen fixation and the haematin concentration of the nodules (haematin γ per g nodules), a corresponding relation is not found, at least by far not to the same extent, between the nitrogen contained in the plant and the quantity of haematin in the total number of nodules. If the weight of the root nodules is great but the concentration of haemoglobin small (haematin γ per g nodules), the nitrogen fixation is weaker than in a reverse case (compare for instance, the nitrogen amount per plant and the weight and haematin of the nodules in experiment 1 on strains H 1 and H 10, on the one hand, and on strains H 3 and H 5, on the other). This can be possibly interpreted so that with the rise of the concentration of haemoglobin in the nodules its effect on the occurrences controling the nitrogen fixation increases more than is directly implied by the rise. This may be accounted for by the fact that because of the small concentration of haemoglobin and its great dilution in the nodule tissue the linkage of oxygen to the iron of leghaemoglobin is incomplete. The higher the haemoglobin concentration in the nodule the greater the chance of every haemoglobin molecule to participate in the transfer of oxygen.

The variations in the total iron content of the nodules do by no means regularly correspond to the variations in the nitrogen fixation although the iron content in the ineffective and slightly effective nodules is, as a rule, lower than in very effective nodules. Since the major part of the nodule iron does not belong to leghaemoglobin (Virtanen *et al.*[1]) a distinct positive correlation can hardly be expected between the nitrogen fixation and the total iron content, even when such a relation exists between the nitrogen fixation and the leghaemoglobin content.

In an experiment where the haematin content of the nodules of soya bean were examined at different stages of growth (Fig. 6) a pronounced parallelism was observed between the nitrogen fixation and the haematin content of the nodules almost until the end of the vegetative growth. After that the nodules turned green and the haematin disappeared. Simultaneously nitrogen fixation ceased.

SUMMARY

Root nodules formed by bacterial strains of different effectiveness were crushed with pyridine. Spectrophotometric examination of the extracts showed that the strong absorption maxima of the red nodules at 555 and 525 mμ disappear after nodules have turned green and leghaemoglobin is no

longer found. The strong absorption of the extracts from the red nodules is thus at least chiefly attributed to the prosthetic group of leghaemoglobin.

Comparative experiments with 13 different strains of pea bacterium and 6 different strains of horse bean bacterium indicated that a positive correlation exists between the haematin concentration of nodules (haematin γ per g nodules) and nitrogen fixation. The absolute quantity of pigment in the total number of root nodules (haematin γ per plant) is not by far so decisive as is the concentration of pigment in the nodules.

In experiments on soya bean where the haematin content of the nodules was determined at different stages of growth, a clear parallelism was noted between the nitrogen fixed in the plant and the haematin content of the nodules.

Correlation is not distinct between the total iron of the nodules and the nitrogen fixation although the nodules formed by very effective strains, as a rule, contain more iron than do the nodules of ineffective or poor strains.

Grateful thanks are expressed to the Rockefeller Foundation for the grant which made possible the acquisition of the Beckman spectrophotometer.

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The Action of Anesthetics upon Interfaces

On the Mechanism of Anesthesia

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The different theories which try to explain the anesthetic action can be divided into two groups, those which in some way try to find a correlation between surface activity and anesthetic action and those which do not. Theories of the first type are those of Traube [1] and Warburg [2]. Many authors [3–7] have discussed the relation between the surface activity at the interface air/water and the anesthetic action. None of these authors have found a general correlation between the surface and the anesthetic activity.

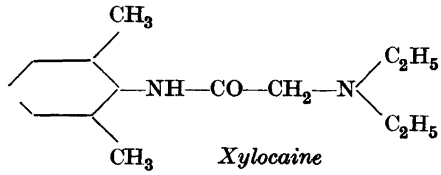
A theory of another type is that of Meyer [8] and Overton [9] who have found a good correlation between the physiological action and the distribution coefficient at oil/water. »Narkose tritt ein wenn ein beliebiger chemisch indifferenter Stoff in einer bestimmten molaren Konzentration in die Zellipoide (richtiger: die lipoiden Alkohole der Zellsubstans) eingedrungen ist. Diese Konzentration ist von der Tier- oder Zellart abhängig» (Meyer and Hemmi [10]). *The permeability theory* advocated originally by Höber and Lillie states that stimulation causes an increase in the permeability of the plasma membrane and this is the primary cause of exitation, and that anesthetics would inhibit exitation by preventing an increase in permeability. *A calcium release theory* is put forward by Heilbrunn [11] (further information about this theory is given in the discussion). Finally, Seelich [12] used an interesting model to which we will return later. He studied the action of anesthetics upon an adsorbed layer of ergosterol at the interface water/paraffin oil.

Since very little is known about the chemical structure of cell membranes it is impossible to make an exact interpretation of the phenomena connected with the action of anesthetics at these membranes. This is also the reason why every model is necessarily incomplete. Norris [13] has made a summary of what we know about the cell membrane. It has a low tension about 0.1 dyn and its

probable constitution (Danielli [14]) is that of two monomolecular layers of lipids at which proteins are adsorbed. Warburg suggests that some of the molecules with enzymatic effect, adsorbed at cell interfaces, are necessary for the transport of impulses.

The problems connected with the action of anesthetics after injection or inhalation in the body where the distribution to the vital parts of the nerve is of importance, are very complicated. In this paper the problems are limited to those which are connected with the single cell.

We had a great many local anesthetics and related compounds synthesized by Löfgren and co-workers at our disposal. It was therefore an attractive task to test if measurements in conformity with those described in the papers mentioned above could give any positive results. Many of the compounds studied are closely related to a new, available, clinically tested, local anesthetic [16], *i. e.* ω-diethylamino-2,6-dimethylacetanilide.



Xylocaine

SURFACE ACTIVITY AT VARIOUS INTERFACES

The only conditions which produce correlation between surface and physiological activity are those in homologous series. Any physical property in one homologous series is likely to give such a correlation and positive results received in this way tell nothing about the mechanism of anesthesia. Miescher [15] found a maximum of surface activity in the percaine series. The same phenomenon was found by us in homologous series of the type ω-alkylamino-acet-*o*-toluidide * and ω-alkylamino-acet-*vic.*-*m*-xylidide ** (Fig. 1). In the diagram the surface tension of 1.47 mC solutions in Sørensen buffer at pH = 7.4 is put against the number of C-atoms in the alkylamino groups; γ_0 is the surface tension of the pure 0.25 C buffer and γ is the surface tension of the solutions.

The uncharged form of organic protolytes are more strongly adsorbed than the corresponding ion (Adam [18], Gardner and Semb [19]). Anomalies like maxima or minima in surface activity for protolytes in homologous series could therefore depend on a variation in pK_a. The pK_a values for the ω-alkylamino-acet-

* Substances made by Löfgren [16].

** Substances made by Löfgren and Widmark [17].

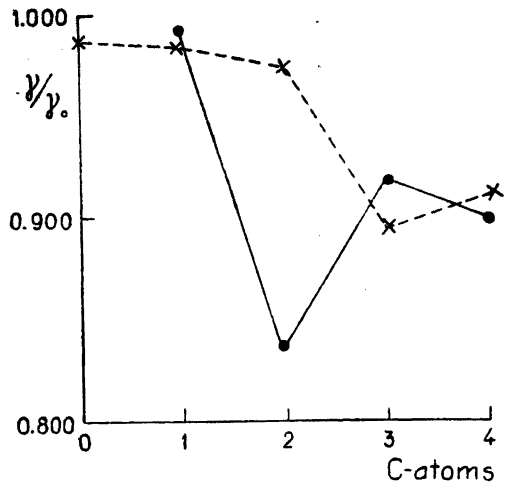


Fig. 1. Surface tensions of equimolar solutions of homologues in the series ω-alkylamino-acet-orto-toluidides (×— — —×) and ω-alkylamino-acet-vic.-m-xylidides (●———●).

vic.-m-xylidides are in order from the methylamino to the butylamino derivative 7.992, 8.075, 8.012 and 8.036 (Löfgren and Östlund, unpublished). If the base is the most surface active these values do not explain the maximum value in surface activity for the ethyl derivative because here the base concentration must be the lowest in the series. The explanation to this maximum value must be that the molecules are orientated at the surface and that a new methylene group added to one derivative is not equally situated in relation to the surface as the preceding one.

Since it is impossible to predict anything about the way these molecules orientate at the cell interface, it is impossible to infer anything about the mechanism of anesthesia through comparisons with the anesthetic effect in the same series. Moreover, the activities in the alkylamino-acet-*vic.-m*-xylidide series measured as minimal effective concentrations (action on frog sciatic nerve at pH = 7.4) show neither maxima nor minima. The activities increase with increasing molecular weight.*

The importance of selecting the right model for testing the correlation between surface and physiological activity appears from the following results. The surface activity at the interface air/water for some common local anesthetics is compared to the interfacial activity at the interface benzene water. Column I in Table 1 gives the surface tension in dynes/cm of 1.46 mC solutions of the anesthetics in 0.25 C Sørensen buffer at pH = 7.4. Column II gives the surface tension in dynes/cm in the interface benzene/water if 0.5 mmoles

* L. Ehrenberg at this laboratory, unpublished.

of anesthetic are distributed to equilibrium between 50 ml benzene and 25 ml 0.25 C Sørensen buffer at pH = 7.4.

Table 1. Surface tensions of equimolar solutions at I air/water and II benzene/water.

	I	II
Novocaine	70.0	25.7
Tutocaine	67.0	24.4
Benzocaine	63.5	28.4
Pantocaine	56.5	25.7
Cocaine	54.7	25.7
Percaine	49.6	26.3
Pure buffer solution	74.4	30.5

The two interfaces give different orders of the values if the anesthetics are ordered after increasing surface activity. It is obvious that it is impossible to estimate the anesthetic effect through measurements of the surface tension at an *arbitrary* interface, as for instance air/water.

ACTION OF ANESTHETICS UPON ADSORBED LAYERS

The Warburg theory points out that anesthetics, because of their surface activity, would *displace*, other molecules necessary for the transport of impulses, — a theory which is criticized by Seelich (*l. c.*). As far as that a displacement is very unlikely, we agree. Furthermore, it is not probable that inhibition of some enzymatic effect directly causes anesthesia. Seelich believes that a dehydration of the cell membrane is responsible for the anesthesia. His reasoning about variations of permeability is not in agreement with modern conceptions (Heilbrunn [20]). Seelich mentions that it is possible that besides the dehydration, even a variation in the adsorption equilibrium for some enzymes adsorbed at the cell membrane might be responsible for the anesthesia.

Seelichs model contains a liquid/liquid interface which has a low interfacial tension. A 0.05 % solution of ergosterol in paraffin oil is one of the phases and water the other. Ergosterol is strongly adsorbed at the interface, and (a) in Fig. 2 shows the variation of the interfacial tension with time until equilibrium is reached. The equalities between this interface at equilibrium and the cell interface are naturally only of physical character.

If the anesthesia is correlated to an alteration in adsorption equilibriums for molecules at the cell membrane, Seelichs's model is useful. Thus we decided to test what effect some of our local anesthetics would have on the adsorbed ergosterol layer. Seelich tested chloroform and the five first alcohols in the aliphatic series.

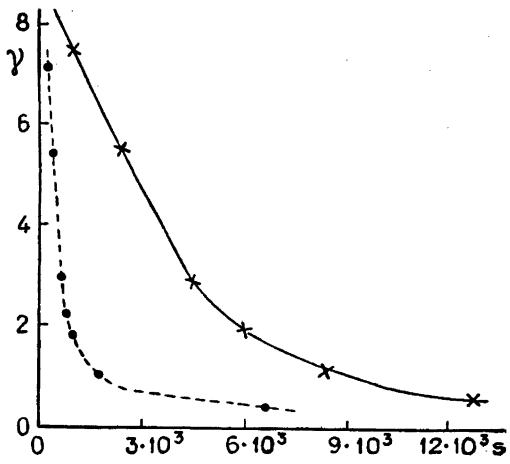


Fig. 2. a) ×———× *The interfacial tension — time curve caused by the adsorbtion of ergosterol at the interface paraffin oil/buffer solution.*
b) ●– – –● *The similar curve if the oil phase contains propanol.*

PRELIMINARY EXPERIMENTS

1. Adsorption of ergosterol at the interface oil/water

a) 20 ml of paraffin oil with 0.05 % ergosterol is poured over 20 ml Sørensen buffer (0.25 C, pH = 7.4). The interfacial tension is put against time and thus we get (a) shown in Fig. 2.

b) 20 ml of paraffin oil with 0.05 % ergosterol and 0.3 ml *n*-propanol is poured over 20 ml Sørensen buffer (0.25 C, pH = 7.4). Time and interfacial tension, — see (b) in Fig. 2.

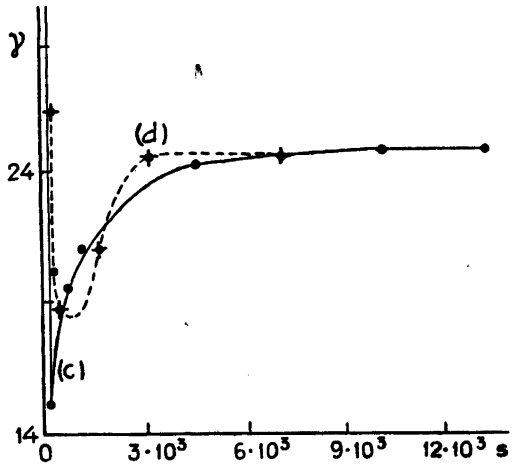


Fig. 3. c) The interfacial tension — time curve showing the effect when propanol is distributed from the water phase to the oil phase.
d) The similar curve for the opposite direction of the distribution.

The interfacial tension is expressed in dynes/cm and the time in seconds. Time is zero at the moment of overpouring.

It is important for the following that we are able to establish that (a) and (b) are going against the same value (about 0.5 dynes/cm) when time increases.

2. Propanol at the interface pure paraffin oil/ Sørensen buffer (0.25 C, pH = 7.4)

c) 20 ml pure paraffin oil is poured over 20 ml Sørensen buffer (0.25 C, pH = 7.4) and then 0.3 ml propanol, dissolved in 3 ml of water, is injected into the water phase. Time and interfacial tension are put against each other in (c) in Fig. 3.

d) 20 ml pure paraffin oil containing 0.3 ml propanol is poured over 23 ml Sørensen buffer (0.218 C, pH = 7.4). Time and interfacial tension, — see (d) in Fig. 3.

The interfacial tension is expressed in dynes/cm and time in seconds. Time is zero at the moment of overpouring in (d).

The initial interfacial tension ((c) and (d)) between pure paraffin oil and buffer solution was 33 dynes cm; (c) and (d) reach the same (equilibrium) value (24.6 dynes/cm) when time increases, which was to be expected. (c) shows that the propanol concentration in the layers next to the interface is abnormally high immediately after the injection, and that equilibrium is reached through diffusion *from* the interface. The minimum value of the interfacial tension in (c) is unknown because the tension was already increasing between the two first points which we were able to determine.

If the propanol comes from the oil phase as in (d), the course is in point of principle the same, but the minimum value is not so low as in (c). The propanol concentration in the layers next to the interface does not reach such high values as during the course (c).

3. Action of propanol upon ergosterol adsorbed at the interface paraffin oil/buffer solution

After ergosterol is adsorbed to equilibrium (see Fig. 2 (a)) at the interface paraffin oil + 0.05 % ergosterol/Sørensen buffer (0.25 C, pH = 7.4), 0.3 ml propanol is injected into the waterphase (before the injection the propanol was dissolved in a few ml of the water phase).

The interfacial tension after the injection is put against time in Fig. 4. Time is zero at the moment of injection and γ is expressed in dynes/cm.

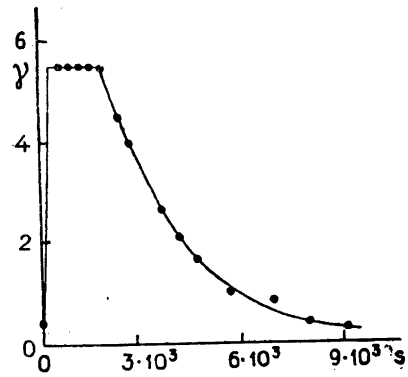


Fig. 4. Interfacial tension — time curve showing the action of propanol on an interface paraffin oil/buffer solution at which ergosterol is adsorbed, — the propanol is injected into the water phase.

The increase of the surface tension is caused by the *first* high propanol concentrations in the layers next to the interface. The ergosterol film at this stage is partially dissolved by these layers. That the propanol concentrations in these layers (immediately after the injection) are high, is clearly indicated by (c) of Fig. 3. When the high propanol concentrations are levelled, the interfacial tension in Fig. 4 decreases to a value very close to that of the injection point (0.5 dynes/cm). Fig. 2 also shows that the equilibrium values with and without propanol in the oil phase are almost the same. Injection of propanol in the oil phase (before the injection the propanol was dissolved in a few ml of the oil phase) gave no alteration in the interfacial tension. It is obvious that the concentrations of propanol in the layers next to the interface, corresponding to the lowest values in (d) of Fig. 3 are not high enough to effect any decrease in the (two dimensional) ergosterol concentration at the interface. Our interpretation of the decrease of the interfacial tension, *e. g.* in Fig. 4 is not in coincidence with Seelich's. He believes that evaporation of the anesthetic causes the decrease which apparently is impossible.

EXPERIMENTS WITH LOCAL ANESTHETICS AND RELATED COMPOUNDS

The same effect as in Fig. 4 was received if 0.176 mmoles LL 31 * was injected in the same way as the propanol (Fig. 6). In an experiment with the same model the distribution of LL 31 between paraffin oil + 0.05 % ergosterol and Sørensen buffer was studied; the quotient C_{oil}/C_{water} was determined at various times after the injection of 0.176 mmoles in the water phase. The result is illustrated in Fig. 5.

* Trivial name for ω-diethylamino-2,4,6-trimethylacetanilide, an available, new local anesthetic (N. Löfgren *l. c.*).

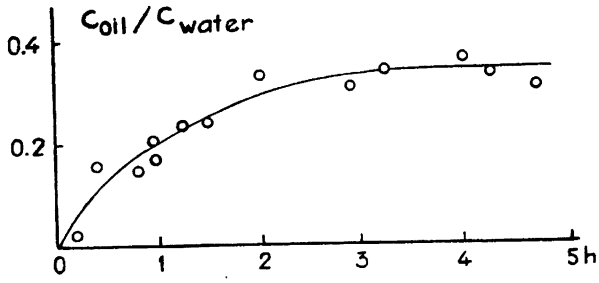


Fig. 5. Distribution of a local anesthetic (LL 31) between paraffin oil with ergosterol and buffer solution.

A comparison between Figs. 5 and 6 indicates that the effect in Fig. 6 is maintained as long as the value of C_{oil}/C_{water} perceptibly differs from the distribution coefficient (*i. e.* the quotient at equilibrium).

Fig. 7 shows the effect of 0.176 mmoles of a) ω-diethylaminoacetocyclohexylamide, b) LL 31, c) Xylocaine and d) ω-diethylaminoacetanilide injected in the same way as in Fig. 4.

Xylocaine produced a complication because after twenty minutes we could observe a precipitation in the interface. The interfacial tension once raised, remained unchanged even after 24 hours.

APPARATUS AND FORMULAS

For the measurements of surface and interfacial tension the ring method with du Nöuy tensiometer was used. Temperature was kept constant in the solutions with a water thermostat at 25° C. The vessel with the solutions was attached to a metal stand which could be raised or lowered by screws. In this way the vessel was in contact with the thermostat during measurements.

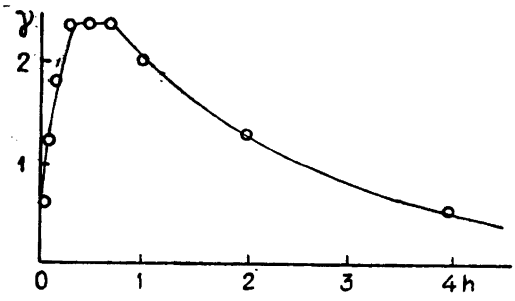


Fig. 6. Interfacial tension — time curve showing the action of a local anesthetic (LL 31) on an interface paraffin oil/-buffer solution at which ergosterol is adsorbed, — the anesthetic is injected into the water phase.

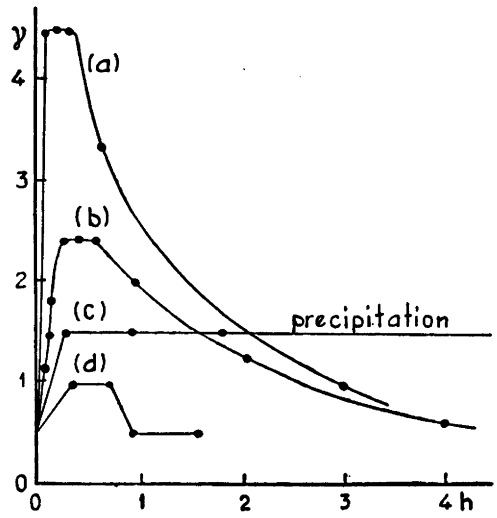


Fig. 7. Interfacial tension — time curves showing the action of various local anesthetics on an interface paraffin oil/buffer solution at which ergosterol is adsorbed, — the anesthetics are injected into the water phase.
a) ω-diethylamino-acetocyclohexy amid.
b) LL 31.
c) Xylocaine.
d) ω-diethylamino-acetanilide.

The injections were performed with a common syringe for medical use. After the injection, buffer solution from the water phase was sucked up and injected again three times. In that way we got a homogeneous solution of the anesthetic in the waterphase before the measurements began.

The surface and interfacial tensions were obtained according to the equation

$$\gamma = \frac{P'}{4\pi R} \cdot F$$

given by Harkins and Jordan [21]. In this equation γ is the surface or interfacial tension in dynes/cm, P' is the maximum pull on the ring in dynes, R is the radius of the ring in cm. F is a correction factor and is determined by the relation:

$$(F-a)^2 = \frac{4b}{\pi^2 R^2} \cdot \frac{P}{D-d} + C$$

given by Zuidema and Waters [22].

P = maximum pull on the ring in dynes/cm.
D and d = densites of the lower and upper phases respectively.
R = radius of the ring.
C = a constant which depends upon the ratio r/R (where r = radius of the wire of the ring) in the following manner.

$$C = 0.04534 - 1.679 \frac{R}{r}$$

$a = 0.7250$ and $b = 0.0009075$ are universal constants for all rings.

DISCUSSION

Traube's and Warburg's theories about anesthetics as compounds acting because of their surface activity (surface phenomenons governed by the Gibb's adsorption law) are criticized by Meyer and Hemmi (*l. c.*).

They point out that if an anesthetic should be surface active at the interface cell membrane/water the interfacial tension anesthetic/water must be lower than the interfacial tension at the membrane *in vivo* (lower than about 0.1 dynes/cm). For hydrophobic general anesthetics (methane for instance) this is impossible. Local anesthetics always have polar (hydrophilic)-nonpolar structures. But in spite of this fact, it is highly improbable that any interface local anesthetic/water would have an interfacial tension lower than that of the membrane in vivo.

The lipid-solubility law given by Meyer and Overton is the only one that gives a mathematical formulation that is applicable. If oleyl alcohol is used as a model compound (Meyer and Hemmi, *l. c.*), the law has validity for general narcotics. Plotting the logarithms of the minimal effective concentrations against the logarithms of the distribution coefficients inverse proportionality is obtained —. However, this law gives no explanation of the *mechanism* of anesthesia.

The permeability theory is criticized by Heilbrunn [23] who emphasizes that the magnesium ion which has anesthetic effect certainly lowers permeability, but the calcium ion that lowers permeability still more, is able to break off a magnesium anesthesia. Moreover, recent studies of numerous anesthetics have shown that organic anesthetics do not always lower permeability. These facts show that Seelichs' (*l. c.*) theory is improbable because it is founded on the hypothesis that a lowering of permeability would be followed by anesthesia.

The Heilbrunn [24] calcium-release theory says that stimulation causes a release of calcium from the cell membrane and this calcium then causes a clotting or gelation of the interior protoplasm. The peculiar inhibiting action of anesthetics is due to a preventing effect on the clotting reaction.

After having described some results from measurements of salt potentials of resting muscles and nerves Höber [25] says: »Taking into account these various observations on the salt potentials and further referring to the aforementioned effects on excitability of muscle and nerve, *it is suggestive to interpret the bioelectric phenomena as surface effects taking place in the plasma membrane.* — The ionic effects on the potential suggest the presence of a colloidal membrane which shows both hydrophilic and hydrophobic attributes.» — Furthermore, by studying the birefringense of the cell membrane, it has been shown that there must be a strong organisation of the molecules in the plasma membrane (see Heilbrunn [26]).

From experiments by Kato [27] we are able to draw some important conclusions about the site of action of anesthetics on nerve tissue. Tasaki and Kato found that when nodes of Ranvier (of a single nerve fibre) are exposed to relatively dilute (though over the minimal effective concentration) narcotising solutions (such as urethane and cocaine) *anesthesia is induced after less than*

a quarter of a second. It seems to us that *the immediate effect shows that the mechanism of anesthesia is a surface course.* Moreover, when *Ranvier's* nodes are exposed to Ringer solution containing an anesthetic (urethane), the conc. of which is below the minimal effective conc. necessary for complete anesthesia, the alteration of the threshold of the stimulating current occurs abruptly. The effect of anesthetics on adsorbed lipids measured by us was also almost immediate (see Fig. 4).

If we assume, which is reasonable, that *the transport of impulses is dependent on a special organisation of molecules and metal ions at the cell membrane,* we are able to propose that *a disturbing of the equilibrium sites of these molecules and ions may cause the phenomena of exitation and anesthesia.* The physical difference between these is likely to be a difference in the nature and degree of the disorder caused.

In our experiments with ergosterol adsorbed at the interface paraffin oil/water, we found a marked alteration in the adsorbtion equilibrium of the film, when a narcotic was injected in the water face. *The effect was marked only before equilibrium distribution for the anesthetic was reached* (Figs. 5 and 6). The equilibrium value for the adsorbtion of the lipid molecules returns to a value very close to the original value when distribution equilibrium is reached for the anesthetic. A similar course is likely to take place at the cell membrane, *the equilibrium state of the impulse conducting organised layer* (built by lipo-proteins and metal ions) *is likely to be altered when an anesthetic is distributed to the lipid bulk phase* (about this phase, — see *e. g.* Höber [28]).

For general anesthetics the disturbing effect must be ruled by the value of the distribution coefficient lipid/water. Immediately after the injection, layers with high concentration of the anesthetic in the oil phase, develop near the interface (see Fig. 3) *because of a stopping effect of the viscous oil.* The dissolving power of these layers on the adsorbed interfacial layer must be stronger, the stronger the attracting forces is between the anesthetic and the adsorbed interfacial lipid molecules. The distribution coefficient lipid/water for the anesthetic is a measure of the attracting forces lipid-anesthetic in relation to the attracting forces water-anesthetic. Thus it is very *probable* that the distribution coefficient is a measure of the attracting forces between the adsorbed lipid layer and the anesthetic. The former reasoning is probably applicable to the cell in vivo and thus seems to be the reason for the known parallelism (see *e. g.* Meyer and Hemmi, *l. c.*) between the anesthetic effect and the distribution factor oil/water for general anesthetics.

For the local anesthesia it is impossible to get a mathematical relation based on the Meyer-Overton rule. (Löfgren-Ehrenberg, — unpublished). The distribution coefficient seems to have some significance but is not dominating.

Local anesthetics probably have a more specific way of action. These compounds always have one lipophilic and one hydrophilic part (aromatic residue — intermediate chain—amino group).

Rideal *et al.*, have made fundamental experiments with »penetration» of surface films. It is especially a lipophilic-hydrophilic construction that constitutes a »penetrant», Rideal [29] considers that such compounds have a strong physiological action. According to Schulman and Rideal [30] two types of complexes can be identified »those formed by association of a submerged polar group in the material injected, and those in which a subsequent penetration of the hydrophobic portion of the film by the hydrophobic portion of the material injected takes place. This subsequent stage is termed film penetration, and the stability of the resulting mixed films are shown to be due to molecular association.»

In our experiments the effect of local anesthetics was similar to that of general anesthetics. Even here we must propose a development of layers with high concentrations of the anesthetic in the oil phase next to the interface, and a dissolving effect of these layers on the adsorbed lipid layer at the beginning of the distribution of the anesthetic. Even here it seems reasonable to presume that a similar course in vivo causes anesthesia. The reason why it is impossible to get a mathematical relation based on the parallelism between distribution coefficient and anesthesia is that the distribution coefficient, because of the peculiar nature of a local anesthetic, is much less probable to be a measure of its power to attract lipid molecules. We must assume that local anesthetics act in a more specific way than general anesthetics, perhaps with forces like those described by Rideal *et al.* (*l. c.*) which are established at »penetration».

Metal ion anesthesia can be understood if we make one assumption, namely that a calcium ion-lipo-protein complex is responsible for the transport of impulses. If magnesium ions are injected, calcium is displaced at the lipids by magnesium (as at a permutite filter) and anesthesia is established. The inverted course represents the inhibition of magnesium anesthesia by calcium. Organic anesthetics affects the lipoproteins as is described above and thus disturb the metal ion-lipoprotein complex and cause anesthesia.

SUMMARY

Immediately after injection, the anesthetic dissolves in the layers of the lipid bulk phase of high viscosity next to the water phase and these layers are now at the highest concentration of anesthetic. The lipids adsorbed at the cell membrane which are organised and together with metal ions responsible for the transport of impulses, dissolves in the aforementioned bulk layers, and the organisation of the adsorbed lipid layer is altered. This alteration is respon-

sible for anesthesia. For general anesthetics this theory is in accordance with the known parallelism between distribution coefficients and minimal effective concentrations.

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A Modification of the Microhydrogenation Apparatus of Breitschneider and Burger

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For the accurate determination of the hydrogen absorption of catalytically hydrogenated organic compounds, the best methods so far developed are based on the principle shown in Fig. 1. Hydrogenation takes place in the vessel I, which communicates with a compensation vessel II by a manometer. During hydrogenation, the drop of pressure in I is compensated by the addition of mercury from a burette, and the manometer is in this way kept at zero. The amount of hydrogen h (in ml at 0°/760 mm Hg) consumed by the substance is given by equation (1)

$$h = \frac{273}{260}(B - e)\frac{a}{T} \qquad (1)$$

a is the volume of added mercury in milliliter; T the absolute temperature; B the atmospheric pressure in mm Hg and e the vapour pressure of the solvent in millimeter Hg at $T°$.

The above principle was introduced by Smith [1], and later employed by Slotta and Blanke [2], Jackson and Jones [3], Breitschneider and Burger [4] and Prater and Haagen-Smit [5]. The advantage of the method as compared to other methods has been reviewed by Breitschneider and Burger (*cf.* also Pfeil [6]).

In this communication we report some modifications of the apparatus of Breitschneider and Burger. Magnetic stirring has been employed to avoid the use of the fragile glass coil which connects the hydrogenation vessel with the manometer; the air is washed out by a stream of hydrogen without evacuating the entire apparatus; this makes it possible to dispense with an arrangement to remove the manometer liquid. The new apparatus is simpler, and easier to handle, while the obtainable accuracy remains the same.

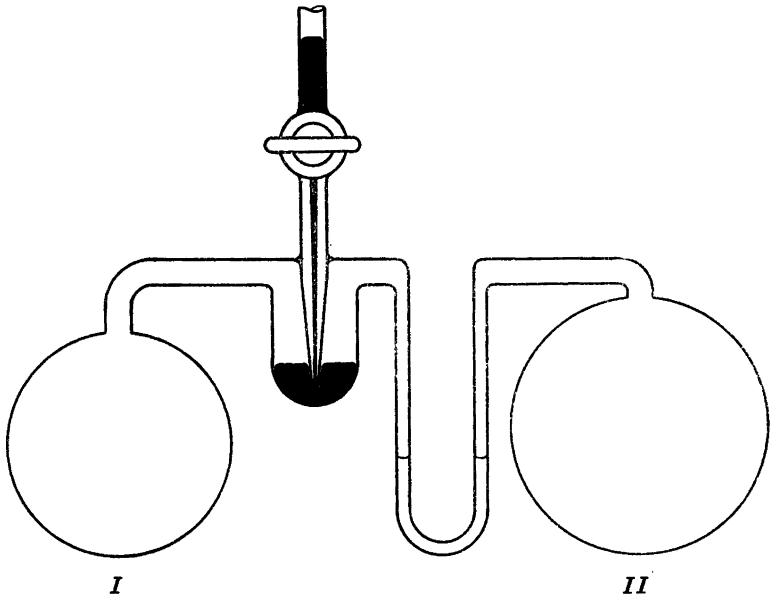

Fig. 1. Principle of the method. I hydrogenation vessel, II compensation vessel.

APPARATUS

Figure 2 gives a detailed design of the apparatus [7]. The total volume of the parts on either side of the manometer is about 70 ml and the two volumes should be of the same size within 2—3 ml.

The graduation of the manometer must not necessarily be very accurate inasmuch as it is only used to adjust the pressure difference to zero. A 2 centimeter long graduation in millimeters is very convenient.

The burette is about 40 cm long and contains 5 ml. Every milliliter is graduated in 50 parts; of course a longer and more accurate burette may equally well be employed. The tip of the burette is so fine, that one milliliter of mercury flows through it in about one minute. It is therefore not necessary to adjust the flow of mercury with the cock *b*.

p is a platinum beaker to which a 25 mm long iron rod is attached, so that the beaker may be hooked off and dropped into the solvent by means of a magnet. The rod is wrapped in a platinum sheet, soldered with gold, to avoid corrosion of the iron by the solvent.

The whole apparatus is supported by a clamp just beneath the cock *b*, and immersed into a glass disk filled with water. The water is stirred efficiently to assure equality of temperature throughout the bath. The liquid in the

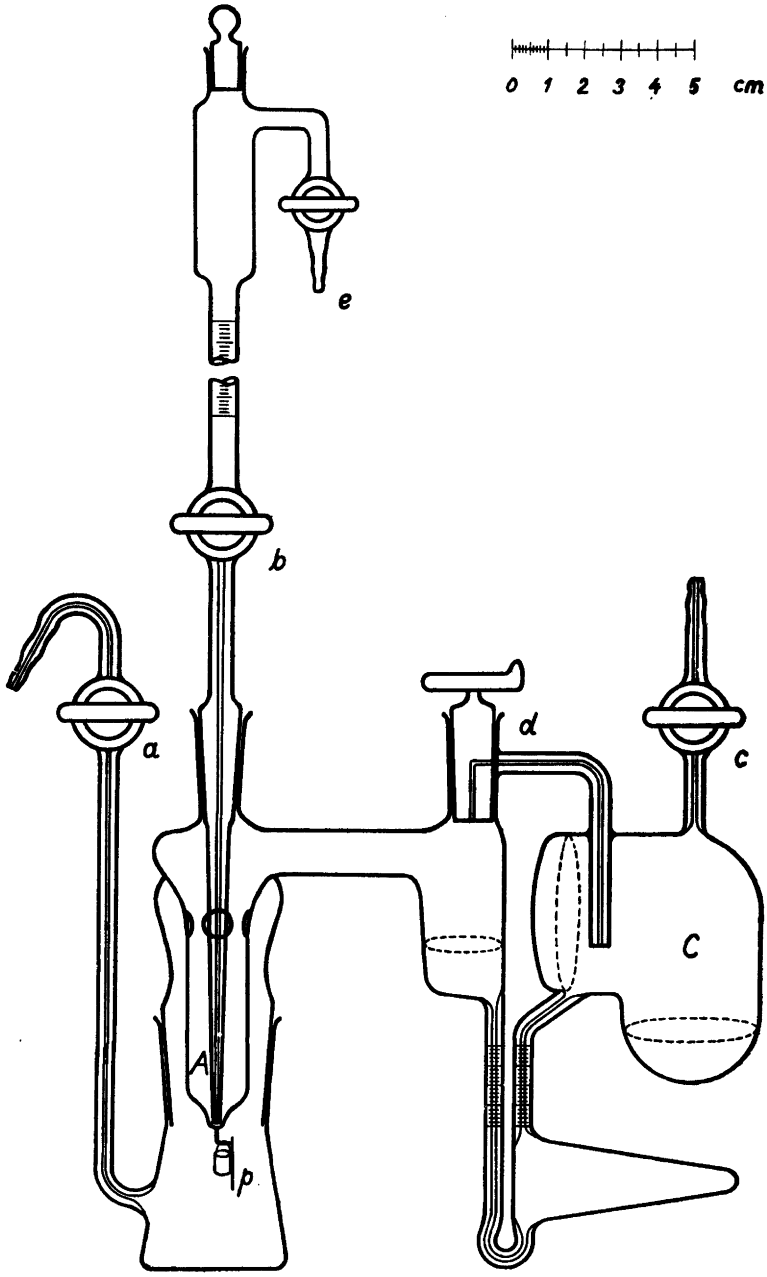

Fig. 2. Apparatus. A reservoir for mercury; C compensation vessel; a, b, c, d and e cocks; p platinum beaker.

hydrogenation vessel is agitated by a glass-jacketed rod, which is rotated by a revolving magnet placed under the glass disk.

Before use the apparatus is cleaned, and the cocks carefully greased. Mercury is filled into *A*, and sucked up into the burette by applying suction to *e*. When the mercury has reached the upper mark of the burette, *b* is closed, and suction turned off. There should now only be a small amount of mercury left in *A*, just enough to cover the tip of the end of the burette.

PROCEDURE

The solvent is filled into *C* (about 2 ml) and into the manometer. These operations are performed by disconnecting the cock *d*. Now the catalyst and 2 ml of the solvent are placed in the hydrogenation vessel. The substance to be analyzed is weighed into the platinum beaker, which eventually is suspended on the glass hook. Then the hydrogenation vessel is connected with the main part of the apparatus and the air in the whole apparatus removed by leading a stream of hydrogen through *c* with *d* and *a* open (note 1).

When all air has been removed, first *a* and, shortly after, *c* are closed. In this way the pressure inside the apparatus will become slightly higher than that of the atmosphere. Stirring is started in order to hydrogenate the catalyst. At the same time the hydrogen becomes saturated with the vapours of the solvent. When the catalyst is perfectly hydrogenated, the manometer will remain on level if *d* is closed. Now the excess pressure is relieved by opening *a* for a moment (*d* must be open). The temperature and the atmospheric pressure are registered. *d* is closed and hydrogenation commenced by dropping the beaker with the substance into the solvent.

Absorption of hydrogen causes the left part of the manometer to be filled with the manometer liquid (note 2). When a reading of the consumption of hydrogen is desired, stirring is stopped, and mercury let into *A* until the manometer again stands on level (note 3). Hydrogenation is continued till no decrease of pressure is observed and the manometer remains on level.

Note 1. For the usual precautions concerning the employed reagents etc. see the monograph of Pregl on quantitative organic microanalysis. (5th ed., Vienna 1947.)

Note 2. The volumes of the different parts of the apparatus are chosen so that 5 ml of hydrogen, that is the total content of the burette, may be consumed without causing the solvent to flow into *A*. The apparatus must thus not be attended to during hydrogenation, if only the equivalent weight of the substance is to be determined.

Note 3. Temperature fluctuations of the order of one degree may cause a small pressure difference due to volumetric changes of the solvent and the glass. If a very accurate determination is desired, the temperature of the water bath should therefore be kept constant within about 0.5°.

EXPERIMENTS

Sorbic acid ($M = 112.06$) was employed to test the apparatus. The sample was alternately sublimated in vacuum (twice) and recrystallized from water

(three times). The results of five hydrogenations in 2 ml of alcohol with Adam's catalyst are cited below.

Sample mg	PtO_2 mg	B mm	e mm	$T°$	a after 90 minutes ml	a after 5, 10, 15, 20 and 40 hours, respectively	a calc.	Error %
8,931	13	771.7	38.0	273 + 17.7	3,952	3,952	3,955	0.1
8,726	10	771.2	39.6	273 + 18.4	3,882	3,883	3,885	0.1
8,583	8	773.8	42.3	273 + 19.5	3,835	3,835	3,836	0.0
8,845	5	764.4	39.0	273 + 18.1	3,961	3,961	3,967	0.1
3,451	4	764.8	38.2	273 + 17.8	1,541	1,542	1,544	0.1

The experiments demonstrate, that the error is less than 0.2 % even after hydrogenation for 40 hours.

During the investigation we have received financial aid from *Det teknisk-videnskabelige Forskningsraad* (N. Clauson-Kaas) and from *Kemisk Værk Køge A/S, Copenhagen* (F. Limborg). We are indebted to the Director of the Chemical Laboratory of the University of Copenhagen, Prof. Dr. A. Langseth, for permission to carry out this work in his laboratory.

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An Examination of the Sulphur-binding Capacity of Charcoal by Means of X-Ray and Electron Ray Diffraction

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In an earlier investigation Wetterholm and Davidson [1] discussed briefly the properties of charcoal (degree of carbonisation, specific surface etc.) and its influence on the capacity for binding sulphur so as to make it impossible to extract it with solvents such as carbon disulphide and hot aniline. Juza and Blanke [2] and Wibaut *et al.*[3] have given some evidence that surface compounds can be formed in the charcoal-sulphur system. Wetterholm and Davidson [1] could show by extraction with hot aniline that the content of sulphur in the system decreased slowly with the extraction time. Thus it seemed probable that the system could not be regarded as a chemical compound. Instead, the sulphur may, during the preparation of charcoal-sulphur, be distilled into the capillaries of the charcoal. (It must be difficult to detect any sulphur in these capillaries). This is also confirmed by Wibaut,[3] that at a temperature of 1000° C in vacuum the sulphur can be distilled off except for a very small portion.

In order to elucidate the structure of the charcoal-sulphur system, electro-optical and X-ray investigations have been made on pure charcoal, mixtures of charcoal and sulphur, and the charcoal-sulphur »compound» (extracted with either hot aniline or carbon disulphide). The last two samples contained 13 % of sulphur.

In the *electro-optical investigation* the ordinary technique was used (V. K. Zworykin *et al.* Electron Optics and the Electron Microscope, New York 1945). The samples were suspended both in water and in octyl alcohol. The magnification was 1,200 and 20,000 times. No difference was observed between the charcoal-sulphur »compound» and the charcoal. Thus, the particles retain their form and size after the absorption of sulphur.

The electron microscope was then used to give diffraction diagrams of the samples. These are shown in Figs. 1 and 2. Charcoal gives three diffuse rings

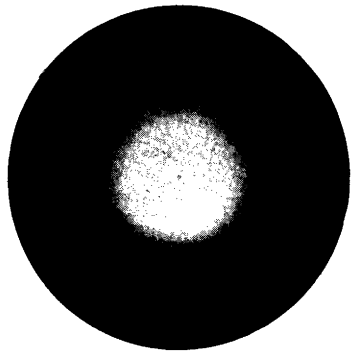

Fig. 1. Electron ray diffraction diagram of charcoal.

Fig. 2. Electron ray diffraction diagram of charcoal-sulphur compound (approx. 13 % S). The preparation has been subjected to extraction with aniline.

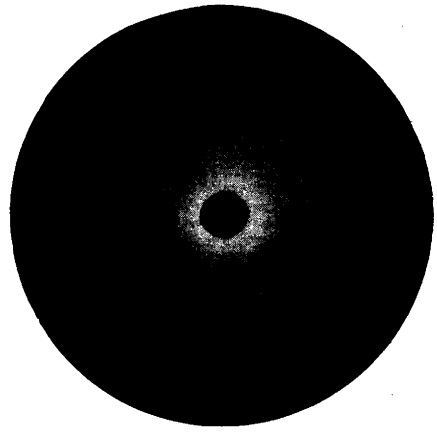

Fig. 3. X-ray diffraction diagram of charcoal.

(Fig. 1). The charcoal-sulphur samples, however, can show either diffuse rings only or both these and a number of sharp rings (Fig. 2). The sharp rings of the various preparations did not always correspond, and they also varied in number. The existence of the sharp rings show that a crystalline structure

Fig. 4. X-ray diffraction diagram of sulphur.

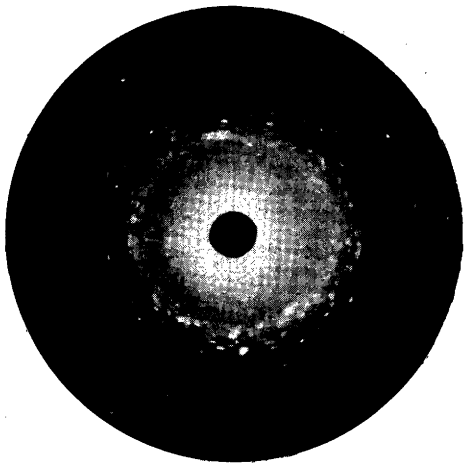

Fig. 5. X-ray diffraction diagram of a mixture of charcoal and sulphur (13.0 % S). The preparation has not been heated.

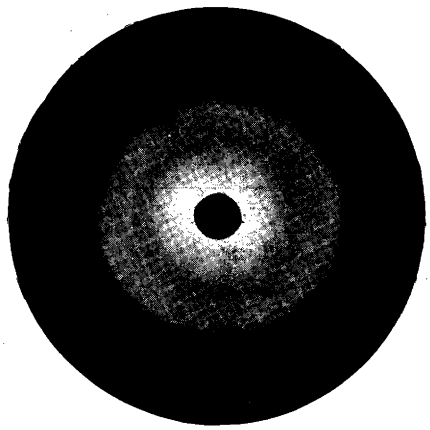

Fig. 6. X-ray diffraction diagram of charcoal-sulphur compound (approx. 13 % S). The preparation has been heated and then extracted with hot aniline.

Fig. 7. X-ray diffraction diagram of charcoal-sulphur compound (approx. 13 % S). The preparation has been heated and then extracted with carbon disulphide.

must be present in the charcoal-sulphur system. In the sample consisting of the charcoal-sulphur mixture, the sulphur evaporated in the microscope and a pure charcoal picture was obtained. A comparison could therefore not be made.

For the *X-ray investigation* CuKα-radiation was used. The samples were compressed into a small rod with 0.8 mm. diameter. With this powder diagrams were obtained (Figs. 3—7). Charcoal gives a very diffuse ring (Fig. 3) whereas sulphur produces a diagram with interference points (Fig. 4). The sulphur is thus distinctly crystalline. The mixture of charcoal and sulphur shows a diffraction diagram which contains the diffraction pictures of both charcoal and sulphur (Fig. 5). The charcoal-sulphur »compound» gives diffraction diagrams containing only one diffuse ring. This ring has, however, the same radius as that of the charcoal but is a little more distinct (Figs. 6 and 7). This is certainly due to some ordered structure in this compound. Crystalline sulphur alone gives quite a different diagram (Fig. 4).

From the investigations performed it seems very probable that on heating charcoal and sulphur some sort of a chemical compound with crystalline structure is formed. It was not possible to detect any change in the diffraction diagrams due to the method of extraction used, in spite of small differences in sulphur content after extraction (Wetterholm and Davidson).[1]

SUMMARY

A compound obtained by heating charcoal and sulphur has been investigated by means of electron diffraction and X-rays. There is evidence that a definite chemical compound with crystalline structure is formed.

This investigation has been made in connection with research work carried out at the request of *Nitroglycerin AB.*, Gyttorp. We are very indebted to the Head of the Institute, Professor The Svedberg, for his kind interest in this work and for the facilities placed at our disposal.

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The Crystal Structures of Molybdenum and Tungsten Borides

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For the past two years binary systems, composed of a transition element and boron, have been studied at this institute. This report is concerned specifically with the borides of molybdenum and tungsten for which structure determinations have been carried out.

Chemical litterature is rather scanty concerning these compounds. In earlier experiments by Binet du Jassoneix [1], Tucker and Moody [2], and Wedekind [3] some intermediate phases, obtained at high temperatures by several methods, are reported. Varying compositions of these phases were recognized, which seem to depend on difficulties attending the isolation of the pure phases. Halla and Thury [4] isolated a compound of the composition WB_2 with a complicated X-ray pattern, prepared by aluminothermic methods. The purity of this compound, however, is questionable.

During the course of this study, Weiss [5] reported the existence of the phases Mo_2B,MoB, and WB, produced by electrolysing melts of borax together with the anhydrides of molybdenum and tungsten acids in the presence of sodium fluoride. The phases are well defined and some chemical and physical properties are described. These compounds are discussed below.

GENERAL METHODS

The borides were prepared in two ways. The general method was to heat weighed mixtures of boron and metal in evacuated quartz tubes for 48 hours at about 1200° C. After this time complete equilibrium seems to be established. The starting materials were molybdenum powder (Kahlbaum) which had been heated *in vacuo* at about 400° C, tungsten powder (Merck, puriss.) and boron. The initial studies were carried out with boron (amorph, Kahlbaum), purified

by chemical methods and with a boron content of 88 %. The results were later affirmed by using 98—99 % boron, prepared by thermic dissociation of BBr_3 in a heated quartz tube at about 700° C in the presence of hydrogen (Kiessling, unpublished). For control purposes mixtures of boron and metal were compressed to pastilles, placed in magnesia crucibles and heated for some minutes at a temperature of 1500—1600° C in a vacuum furnace.

In general, samples prepared by the first method were not analysed as there were no losses during the course of preparation. Some control analyses were made by the methods, given by Weiss [5], and samples prepared by the second method were also analysed in this way.

The preparations were examined under the microscope. The structure determinations were made by X-ray powder methods, since single crystals large enough for X-ray investigation by means of single crystal methods could not be prepared. The powder photographs were obtained with a set of focussing cameras (»A», »B», and »C»-cameras) which provided different glancing angles. The intensities of the reflections in the photographs were estimated visually, starting from 4 : 2 : 1 as relative values of the intensities of Cr-$K\alpha_1$, Cr-$K\alpha_2$, and Cr-$K\beta$ radiation. The lines in the three cameras were then coordinated to the same scale of intensity by means of lines common to the A—B and B—C cameras respectively. From the estimated intensities relative values of $p|F|^2$ were calculated by means of the expression, given by Hägg and Regnström.[6]

$$I = C \cdot \frac{\lambda^3}{\mu} \cdot \frac{1 + \cos^2 2\,\Theta}{\sin^2 \Theta \cdot \cos \Theta} \cdot \frac{1}{\sin\,(2\,\Theta - \alpha) + \sin \alpha} \cdot A_a \cdot A_p \cdot A_f \cdot p|F|^2$$

where the absorption factors of the cameras and the film have been taken into consideration. For a given wave length, camera, and preparation all factors preceeding p will be functions of Θ only.

Observed and calculated values of $p|F|^2$ are given in the tables. In these tables all intensity calculations are based on the metal atoms only; the scattering power of the boron atoms being too low compared with that of the molybdenum and tungsten atoms to give any variations great enough to be observed, even in the most boron rich phases. The positions of the boron atoms were determined using space considerations.

GENERAL SURVEY OF THE SYSTEMS

The phases of the two systems have been found to be isomorphous to a large extent and therefore will be described together.

The solubility of boron in the metallic lattices is apparently very low since the interferences in the powder photographs within the limits of error always occurred at fixed angles.

Three intermediary phases (γ,δ, and ε) were found in each system. The γ phases are isomorphous and have the composition Me_2B.

The δ phases are also isomorphous and exist in a slightly extended homogeneity range of about 48—51 atomic % boron.

The ε phases are not isomorphous but the two structures are very similar. Their »ideal» boron content is 71.4 atomic % but the real boron content is slightly lower.

THE γ PHASES

These phases were found to be homogeneous in samples, prepared at 1200° C, consisting of 33.3 atomic % boron. In each instance the lines in the powder photographs occurred at fixed angles, indicating that the homogeneity range of these phases is very narrow. Both phases were hard enough to scratch an agate mortar. Weiss [5] prepared Mo_2B and this phase seems to be identical with the γ phase in the Mo-B system. He gives a hardness of 8—9 according to Mohs, and describes some chemical properties all of which were found to be possessed by the γ phase.

The interpretation of the powder photographs (Cr-K and Cu-K radiation) showed the phases to be isomorphous and gave tetragonal cells with the dimensions:

Mo_2B: $a = 5.543$ Å, $c = 4.735$ Å, $c/a = 0.854$, $V = 145.5$ Å^3,
W_2B: $a = 5.564$ Å, $c = 4.740$ Å, $c/a = 0.852$, $V = 146.7$ Å^3.

With a cell content of 4 Me_2B the calculated densities are 9.31 for Mo_2B and 16.72 for W_2B, in good agreement with the observed values 9.1 and 16.0 respectively.

Fe_2B [7, 8], Co_2B, and Ni_2B [9], which are tetragonal have the following dimensions: *

Fe_2B: $a = 5.109$ Å, $c = 4.249$ Å, $c/a = 0.842$,
Co_2B: $a = 5.016$ Å, $c = 4.220$ Å, $c/a = 0.843$,
Ni_2B: $a = 4.993$ Å, $c = 4.249$ Å, $c/a = 0.851$,

with 4 molecules of Me_2B in the cell. These borides are of the $CuAl_2$-type

* All values, given in the litterature in Ångström units have been converted to true Ångström units = 10^{-8} cm by multiplying by the factor 1,00202.

Table 1. Mo_2B, Cr—K radiation.

h k l	R	$\sin^2\Theta$ obs.	$\sin^2\Theta$ calc.	I obs.	$p\|F\|^2$ obs.*	$p\|F\|^2$ calc. $x = 1/6$
1 1 0	α	0.0857	0.0853	sv	0.4	1.5
2 0 0	α	1709	1706	m	5	6
0 0 2	α	2342	2338	st	9	12
2 1 1	α	2720	2717	st +	48	57
1 1 2	α	3191	3191	sv	1.6	3.7
2 2 0	α	3409	3412	sv	1.4	1.8
2 0 2	α	4049	4044	m	11	12
3 1 0	α	4268	4265	m	12	12
2 2 2	α_1	5742	5750	sv —	1.6	1.6
3 2 1	—	—	6129	—	0	0
3 1 2	α_1	6607	6603	m	18	14
4 0 0	α_1	6829	6824	sv —	2.0	2.6
2 1 3	α_1	7395	7392	st	38	35
3 3 0	α_1	7677	7677	sv	12	14
4 1 1	α_1	7832	7835	m	37	33
4 2 0	α_1	8533	8530	sv —	1.6	2.3
4 0 2	α_1	9160	9162	sv —	7	8
0 0 4	α_1	9351	9351	sv —	6	7

(C 16). The space group is D_{4h}^{18}—I $4/mcm$ with the metal atoms in an eightfold position.**

8 : (*h*) x, $1/2 + x$, 0; $\bar{x}$, $1/2-x$, 0; $1/2 + x$, $\bar{x}$, 0; $1/2-x$, x, 0; $1/2 + x$, x, $1/2$; $1/2-x$, $\bar{x}$, $1/2$; x, $1/2-x$, $1/2$; $\bar{x}$, $1/2 + x$, $1/2$.

The value of the parameter x is 1/6.
The boron atoms are in a fourfold position:

4 : (*a*) 0, 0, 1/4; 1/2, 1/2, 3/4; 0, 0, 3/4; 1/2, 1/2, 1/4***.

* Referred to α_1 for all lines.

** Notations according to »International Tables for the determination of Crystal Structures», Berlin 1935.

*** In the work of Bjurström[9] Fe_2B, Co_2B, and Ni_2B are said to belong to space group D_{4h}^{18}—I $4/mcm$ with the metal atoms in an eightfold position u, u, v; $\bar{u}$, $\bar{u}$, v; u, $\bar{u}$, $\bar{v}$; $\bar{u}$, u, $\bar{v}$; $u + 1/2$, $u + 1/2$, $v + 1/2$; $u + 1/2$, $1/2-u$, $1/2-v$; $1/2-u$, $1/2-u$, $v + 1/2$; $1/2-u$, $u + 1/2$, $1/2-v$; and the parameters $u = 1/6$ and $v = 1/4$. In space group D_{4h}^{18}, however, this eightfold position with two parameters does not exist. The position, given by B. is 8 : (*i*) in D_{2d}^{11}—$I\bar{4}2$ m. With a value of

Table 2. W_2B Cr-K radiation.

h k l	R	$\sin^2\Theta$ obs.	$\sin^2\Theta$ calc.	I obs.	$p\|F\|^2$ obs.*	$p\|F\|^2$ calc. $x = 1/6$
1 1 0	α	0.0847	0.0847	sv +	2	7
2 0 0	α	1694	1693	st	12	25
0 0 2	α	2334	2334	st	34	46
2 1 1	α	2698	2700	st +	121	194
1 1 2	α	3179	3180	sv +	8	10
2 2 0	α	3384	3386	sv	6	5
2 0 2	α	4025	4027	st	63	38
3 1 0	α	4229	4233	m	58	37
2 2 2	α_1	5724	5720	sv	9	8
3 2 1	—	—	6086	—	0	0
3 1 2	α_1	6563	6567	st	66	62
4 0 0	α_1	6773	6773	sv —	12	15
2 1 3	α_1	7362	7367	st	176	132
3 3 0	α_1	7609	7619	st	64	57
4 1 1	α_1	7772	7779	st	109	127
4 2 0	α_1	8456	8466	sv —	8	7
4 0 2	α_1	9101	9106	sv	19	26
0 0 4	α_1	9330	9334	sv +	25	23

For Mo_2B and W_2B reflections $h\ k\ l$ were observed only for $h + k + l = 2n$, $h\ k\ 0$ for $h + k = 2n$, $h\ h\ l$ for $l = 2n$ and $0\ k\ l$ for $k = 2n$ and $l = 2n$, showing that the space group D_{4h}^{18} is possible. The further structure analysis showed, that the metal lattices of these borides have the symmetry of 8:(h) m D_{4h}^{18}. The agreement between observed and calculated $p|F|^2$ values was good for both borides with a value of the parameter $x = 1/6$ (Table 1 and 2).

The arrangement of the metal atoms is described by Hägg [7]. The metal atoms lie in the corners of slightly deformed tetrahedrons, packed in a regular manner. In this network of tetrahedrons, there are channels running parallel

$v = 1/4$ the symmetry increases to D_{4h}^{18} and the correct position for the metal atoms in this group is 8 : (h), given above. The boron atoms are said to be in a fourfold position in D_{4h}^{18}; 1/2, 0, 0; 1/2, 0, 1/2; 0, 1/2, 0; 0, 1/2, 1/2; this is, however, the correct position for the boron atoms in D_{2d}^{11}. In D_{4h}^{18}, because of the transformation of the coordinate system, the correct position for the boron atoms is 4 : (a), given above. This note is only a formal one, introduced to avoid mistakes, and does not alter the structure described by Hägg [7]. Compare the note of Hägg [8].

* Referred to α_1 for all lines.

to the c-axis, and these channels cut the ab plane in the points 0,0,0 and 1/2, 1/2,0. Each metal atom is surrounded by three other metal atoms at about the same distance, which, for molybdenum is 2.61, 2.71, and 2.71 Å, for tungsten 2.62, 2.71, and 2.71 Å. Four other metal atoms are situated at distances of 2.92 Å for Mo and 2.93 Å for W and four others at distances of 3.00 Å for both Mo and W. With a slight variation of the parameter x from 1/6 (0.167) to 0.170 the distances to the three nearest neighbours become: for Mo 2.66, 2.67 and 2.67 Å, for W 2.67, 2.68 and 2.68 Å. The rather small number of interferences used for the determination of the parameter and the rough estimation of their intensities renders an accurate determination of the parameter difficult, but the latter value seems to be more correct. If the atoms are assumed to be spherical and in contact with each other, the radius will be 1.33 Å for molybdenum and 1.34 Å for tungsten.

The boron atoms then must be placed in holes of the metal lattice. The only holes large enough to be considered are in the channels. In D_{4h}^{18} there are two positions where four boron atoms per cell may be placed in the channels:

4 : (a) 0, 0, 1/4; 0, 0, 3/4; 1/2, 1/2, 3/4; 1/2, 1/2, 1/4,
or 4 : (c) 0, 0, 0; 0, 0, 1/2; 1/2, 1/2, 1/2; 1/2, 1/2, 0.

If the locations were those given in 4 : (c) the radius of the boron atom would be 0.72 Å. In all earlier determinations the radius has been found to have a value between that of carbon (0.77) and that of beryllium (1.12), and in no case has a value less than 0.86 Å been found. For this reason the position 4 : (c) is excluded. The remaining position, 4 : (a) is the same as has been found for boron in Fe_2B, Co_2B, and Ni_2B. It corresponds to the largest holes of the lattice, the distance molybdenum-boron beeing 2.37 Å and tungsten-boron 2.38 Å, which gives a radius for the boron atom of 1.03 Å. For Fe_2B, Co_2B and Ni_2B the values given for the boron radius are 0.97, 0.94, and 0.92 Å respectively, whereas from the other phases of the molybdenum and tungsten systems the value is about 0.87 Å. The reason for the varying values for Me_2B may depend on the fact that the lattice is established by the metal atoms, even if the holes have a slightly greater radius than the boron atom.

The γ phases, Mo_2B and W_2B, thus crystallize in space group D_{4h}^{18}, isomorphous with Fe_2B, Co_2B, and Ni_2B, with the metal atoms in:

8 : (h) x, 1/2 + x, 0; $\bar{x}$, 1/2—x, 0; 1/2 + x, $\bar{x}$, 0; 1/2—x, x, 0; 1/2 + x, x, 1/2; 1/2—x, $\bar{x}$, 1/2; x, 1/2—x, 1/2; $\bar{x}$, 1/2 + x, 1/2,

and the boron atoms in:

Table 3. Mo—B, δ phase, Cr-K radiation.

h k l	R	$\sin^2\Theta$ obs.	$\sin^2\Theta$ calc.	I obs.	$p\|F\|^2$ obs.*	$p\|F\|^2$ calc. $z = 0.197$
0 0 4	α	0.0732	0.0728	sv	0.4	1.1
1 0 1	α	1406	1405	sv	1.3	3
1 0 3	α	1771	1769	st	9	20
1 0 5	α	2502	2497	st	21	25
1 1 2 0 0 8	α	2910	2900 2912	st +	46	30 4
1 0 7	α	3595	3589	m	12	13
1 1 6	α	4361	4356	sv	8	7
1 0 9	α	5050	5045	sv —	1.2	0.5
2 0 0	α_1	5441	5436	m	19	18
2 0 2	—	—	5618	—	0	0
2 0 4	α_1	6165	6164	sv —	1.9	2.0
0 0 12	α_1	6561	6552	sv	4	4
2 1 1	α_1	6844	6841	sv	4	3
1 0 11	α_1	6874	6865	sv	4	4
2 0 6	—	—	7074	—	0	0
2 1 3	α_1	7209	7205	sv +	20	22
1 1 10	α_1	7276	7268	m	32	30
2 1 5	α_1	7934	7934	sv +	23	29
2 0 8	α_1	8352	8348	sv +	21	22
2 1 7	α_1	9026	9025	sv	11	15
1 0 13	α_1	9059	9049	sv	10	11

4 : ($\bar{a}$) 0, 0, 1/4; 0, 0, 3/4; 1/2, 1/2, 3/4; 1/2, 1/2, 1/4.

The value of the parameter $x = 0.170$.

THE δ PHASES

At compositions of about 50 atomic % boron a pure phase occurred in each system. These phases were isomorphous and possessed a slightly extended homogeneity range. They were hard, had metallic properties and appeared to be identical with MoB and WB described by Weiss [5].

The interpretation of the powder photographs (Cr-*K* and Cu-*K* radiation) gave tetragonal cells with the following dimensions at the ideal composition, 50.0 atomic % boron:

* Referred to α_1 for all lines.

Table 4. W—B, δ-phase, Cr-K radiation.

h k l	R	$\sin^2\Theta$		*I* obs.	$p\|F\|^2$	
		obs.	calc.		obs.*	calc. $z = 0.197$
0 0 4	α	0.0725	0.0732	sv	1.2	5
1 0 1	α	1394	1397	sv	5	11
1 0 3	α	1768	1763	st	24	69
1 0 5	α	2497	2494	st	52	88
1 1 2	α	2880	2885	st +	92	105
0 0 8	α	2926	2927	sv	11	16
1 0 7	α	3592	3592	m	48	42
1 1 6	α	4345	4348	sv +	37	24
1 0 9	α	5057	5055	sv —	5	1.6
2 0 0	α_1	5409	5405	m	56	68
2 0 2	—	—	5588	—	0	0
2 0 4	α_1	6137	6137	sv —	9	8
0 0 12	α_1	6585	6585	sv	18	14
2 1 1	α_1	6801	6802	sv	18	13
1 0 11	α_1	6885	6884	sv	24	14
2 0 6	—	—	7051	—	0	0
2 1 3	α_1	7170	7168	sv +	61	84
1 1 10	α_1	7273	7275	m	97	113
2 1 5	α_1	7903	7899	m	68	111
2 0 8	α_1	8334	8332	m	63	85
2 1 7	α_1	8997	8997	m	48	59
1 0 13	α_1	9078	9079	sv	30	44

MoB: $a = 3.105$ Å, $c = 16.97$ Å, $V = 163.9$ Å^3
WB: $a = 3.115$ Å, $c = 16.93$ Å, $V = 164.6$ Å^3

With a cell content of 8 MeB the calculated densities will be 8.77 for MoB, and 16.0 for WB, in good agreement with the observed values 8.3 and 15.3 respectively.

Reflections $h\ k\ l$ were observed only for $h + k + l = 2n$, $h\ k\ 0$ for $h = 2n$ and $k = 2n$, $h\ h\ l$ for $l = 2n$ and $2h + l = 4n$ and $0\ k\ l$ for $k + l = 2n$, which is characteristic of the space group D_{4h}^{19}—$I\ 4/amd$. From space considerations all positions are excluded for eight metal atoms except 8 : (*e*) having the coordinates:

8 : *(e)* 0, 0, z; 0, 0, $\bar{z}$; 0,1/2, 1/4 + z; 0,1/2, 1/4—z; 1/2, 1/2, 1/2 + z; 1/2, 1/2, 1/2—z; 1/2, 0,3/4 + z; 1/2, 0, 3/4—z.

* Referred to α_1 for all lines.

Table 5. Mo—B, and W—B, δ-phases.

h k l	$p\|F\|^2$ (Mo)				$p\|F\|^2$ (W)			
	obs.	calc. z =			obs.	calc. z =		
		0.194	0.197	0.200		0.194	0.197	0.200
2 0 4	18	10	20	31	64	38	75	116
0 0 12	37	21	37	54	125	77	139	204
2 1 1	37	38	34	31	128	143	128	116
1 0 11	37	67	38	15	171	250	143	58

The value of z was determined by means of systematic variations and calculations of $p|F|^2$, starting with values derived from space considerations. As will be seen from tables 3 and 4, the agreement is good for a value of $z = 0.197$.

The limits of z are $0.194 < z < 0.200$ (Table 5).

In Fig. 1 the structure is projected on (010) and (100). The distances, calculated for the W—B lattice are:

A—B = C—D = D—G = 2.89 Å,
A—C = A—D = A—F = 2.83 Å.

If z is varied from 0.197 to 0.196 the distances become:

A—B = 2.86 Å
A—C = 2.86 Å.

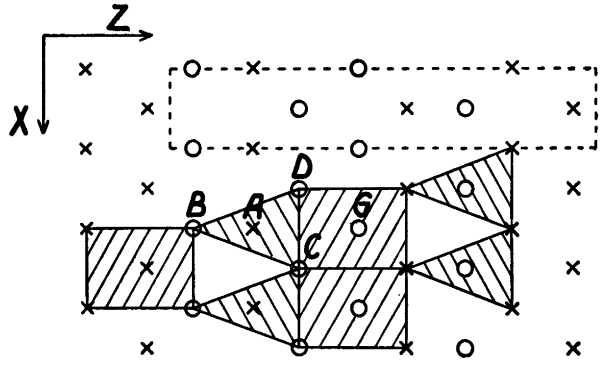

Fig. 1a. The metal lattice of the δ phase, projected on (0 1 0). (The unit cell and the prisms of metal atoms are indicated.)
x = metal atoms in x 0 z
O = metal atoms in x ½ z

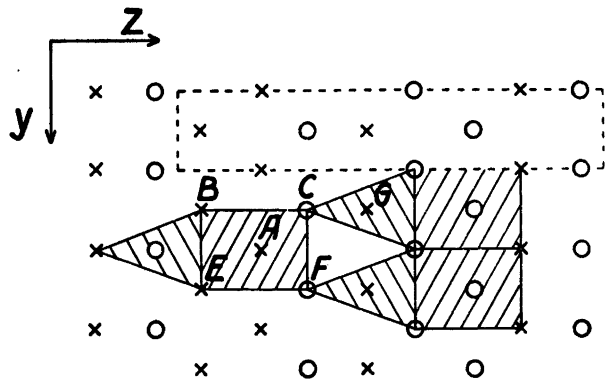

Fig. 1b. The same structure as in 1a projected on (1 0 0).
x = metal atoms in 0 yz
O = metal atoms in ½ yz

This variation of z lies within the limits of error (Table 5) and gives a very regular arrangement of the metal atoms. Every metal atom, for instance A (Fig. 1), is surrounded by six neighbours at the same distance, 2.86 Å. The surrounding atoms lie in the corners of a trigonal prism with A in the centre (Fig. 2). The height of the prism, BE, is $= a = 3.115$ Å as is the base (CD) of the triangular face BCD. The height (BH) of this face is $= c/4 = 4.22$ Å. (a and c are the axes of the tetragonal cell.)

The metal lattice may be described as a packing of trigonal prisms in a manner indicated by Fig. 1.

The boron atoms must be situated in the holes of this lattice. As may be seen from Fig. 1 the lattice is crossed by a system of channels. These channels are of two kinds. Those parallel to the a axis have their centre at z coordinates 3/8 and 7/8. Those, running at right angles to the first, are parallel to the b axis and have z coordinates 1/8 and 5/8.

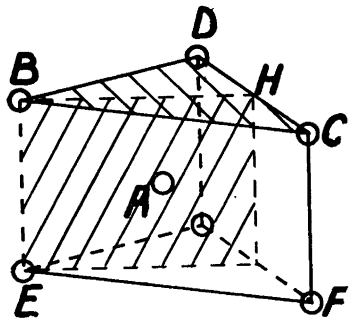

Fig. 2. Prism of metal atoms (compare fig. 1).

There are 8 boron atoms per cell and in D_{4h}^{19} they may be placed in the channels if they possess the same eightfold position, 8 : (*e*), as the metal atoms but with a value of $z = (5/12 - z_{Me}/3) = 0.352$. This position corresponds to the largest holes in the lattice. Each boron atom lies in the center of a trigonal prism of six metal atoms, each at the distance 2.22 Å from the center of the boron atom. The boron atoms in each channel are connected to zig-zag-lines running through the channel, the distance boron-boron being 1.74 Å. Thus the radius of the boron atom will be 0.87 Å and the radius of the metal atom 1.35 Å. The chains of boron atoms are indicated in Fig. 3. The coordination of metal atoms around the boron atom is the same as has been found for FeB and CoB [9].

The δ phases in both the molybdenum and tungsten systems show an extended homogeneity range. The limits were determined from the variation of the *a* axis with the composition of the systems. For the molybdenum phase the limits were 48.8 atomic % boron $<\delta<$ 51.5 atomic % boron. The *a* and *c* axes for 48.8 atomic % boron are 3.105 Å and 16.97 Å respectively, while at 51.5 atomic % the *a* and *c* axes are 3.119 Å and 16.92 Å respectively.

For the tungsten phase the limits were 48.0 atomic % boron $<\delta<$ 50.5 atomic % boron, with the *a* and *c* axes varying from 3.096 Å and 16.96 Å to 3.120 Å and 16.92 Å respectively. The extended homogeneity ranges probably depend on possible variations in the number of boron atoms in the channels.

Thus the δ phases crystallize in space group D_{4h}^{19}—$I\ 4/amd$ with the metal atoms in

8 : (*e*) 0, 0, *z*; 0, 0, $\bar{z}$ 0, 1/2, 1/4 + *z*; 0, 1/2, 1/4—*z*; 1/2, 1/2, 1/2 + *z*; 1/2, 1/2, 1/2—*z*; 1/2, 0, 3/4 + *z*; 1/2, 0, 3/4—*z*.

The boron atoms are also in the position 8 : (*e*), the values of the parameters being $z_{Me} = 0.196$ and $z_B = 0.352$.

THE ε PHASES

In both the Mo—B and the W—B systems a third intermediate phase could be obtained by preparing the phases in evacuated quartz tubes at 1200° C. These phases seemed to be homogeneous at a composition of about 70 atomic % boron. Though not isomorphous, because of their similarity in structure they will be described together.

The physical properties were not thoroughly investigated, but the ε phases did not show the marked hardness of the γ and the δ phases.

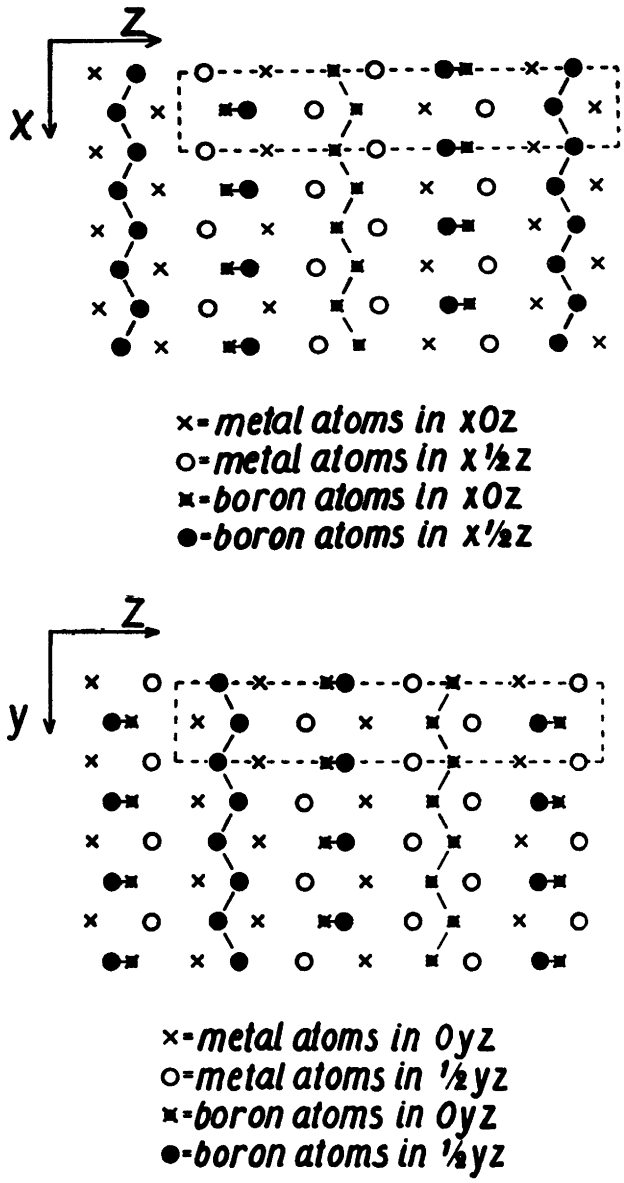

Fig. 3. Chains of boron atoms in the δ phases.
a/Projection on (0 1 0).
b/Projection on (1 0 0).

Table 6. Mo—B, ε-phase, Cr-K radiation.

Hexagonal $h\ k\ i\ l$	R	$\sin^2\Theta$ obs.	$\sin^2\Theta$ calc.	I obs.	$p\|F\|^2$ obs.*	$p\|F\|^2$ calc. $z = 0.075$
0 0 0 6	a	0.1074	0.1077	st	3	8
0 1 $\bar{1}$ 1	a	1954	1957	st	14	19
1 0 $\bar{1}$ 2	a	2052	2047	sv	3	8
0 1 $\bar{1}$ 4 0 0 0 9	a	2416	2406 2423	sv	3	2.1 1.5
1 0 $\bar{1}$ 5	a	2679	2675	m +	8	11
0 1 $\bar{1}$ 7	a	3397	3393	st	26	19
1 0 $\bar{1}$ 8	a	3846	3842	m +	17	12
0 0 0 12	a	4311	4308	sv	7	4
0 1 $\bar{1}$ 10	—	—	4919	—	0	0
1 0 $\bar{1}$ 11	a_1	5553	5548	sv—	6	3
1 1 $\bar{2}$ 0	a_1	5784	5782	st	32	15
1 1 $\bar{2}$ 3	a_1	6060	6052	sv—	2	0.7
0 0 0 15	a_1	6733	6732	sv—	5	2
1 1 $\bar{2}$ 6	a_1	6861	6859	st	38	25
0 1 $\bar{1}$ 13	a_1	6984	6984	m	19	13
2 0 $\bar{2}$ 1	a_1	7738	7740	m	19	10
1 0 $\bar{1}$ 14	a_1	7783	7792	m	18	11
0 2 $\bar{2}$ 2	—	**	7830	—	**	4
2 0 $\bar{2}$ 4	—	—	8189	—	0	1.2
1 1 $\bar{2}$ 9	a_1	8198	8206	sv	9	5
0 2 $\bar{2}$ 5	a_1	8453	8458	sv +	10	6
2 0 $\bar{2}$ 7	a_1	9172	9176	m	14	11
0 1 $\bar{1}$ 16	—	—	9587	—	0	1.0
0 2 $\bar{2}$ 8	a_1	9621	9625	m	9	7
0 0 0 18	—	—	9694	—	0	1.2

The metal lattice of the Mo-B phase

The interpretation of the powder photographs (Cr-K and Cu-K radiation) gave for the Mo-B phase a rhombohedral lattice with the constants:

$$r = 7.190 \text{ Å}, \quad \alpha = 24° 10'$$

Because of the analogy with the hexagonal W—B phase, however, hexagonal axes will be used in the discussion of the structure. The dimensions of the hexagonal cell are:

* Referred to a_1 for all lines.

** 0 2 $\bar{2}$ 2 is concealed by Ka_2 of 1 0 $\bar{1}$ 14.

Table 7. Mo—B, ε-phase.

$h\ k\ i\ l$	$p\|F\|^2$			
	obs.	calc. z =		
		0.072	0.075	0.078
0 1 $\bar{1}$ 7	125	191	186	176
1 0 $\bar{1}$ 8	82	141	118	94
0 0 0 12	34	26	37	48
0 1 $\bar{1}$ 10	0	5	0	5
1 0 $\bar{1}$ 11	31	12	32	57
1 1 $\bar{2}$ 0	150	150	150	150
1 1 $\bar{2}$ 3	11	13	7	3

$a = 3.011$ Å, $c = 20.93$ Å, $V = 164.3$ Å^3

If the composition of the phase is assumed to be $MoB_{2.5}$ (71.4 atomic % boron) the observed density of 7.01 most closely corresponds to a cell content of 6 $MoB_{2.5}$ (calculated density 7.48).

Reflections $h\ k\ i\ l$ were observed only for $h—k + l = 3n$ and $h\ h\ 2\bar{h}\ l$ for $l = 3n$. This is characteristic of the space groups D^5_{3d}—$R\ \bar{3}m$, D^7_3—$R\ 32$ and C^5_{3v}—$R\ 3m$.

The only possible positions for 6 Mo atoms are:

in D^5_{3d} the sixfold position 6 : (*c*), one parameter,
in D^7_3 the sixfold position 6 : (*c*), one parameter,
in C^5_{3v} two threefold positions 3 : (*a*), one parameter in each.

For all these positions the metal lattice will possess the symmetry of D^5_{3d}, the space group with the highest symmetry, and it will be enough to discuss 6 : (*c*) in D^5_{3d} to discover the positions of the metal atoms. The coordinates are as follows:

6 : (*c*) 0, 0, z; 0, 0, $\bar{z}$; 1/3, 2/3, 1/3 + z; 1/3, 2/3, 1/3—z; 2/3, 1/3, 2/3 + z; 2/3, 1/3, 2/3—z.

Good agreement between observed and calculated $p|F|^2$ values is found for $z = 0.075$ (Table 6). The limits of z seem to be $0.072 < z < 0.078$ (Table 7).

The metal atoms (Fig. 4a) are situated in triangular closepacked planes. Two such planes with the same x and y coordinates are in near contact with each other, thus forming a double layer. Double layers of this kind are piled upon each other with a shift in the x and y coordinates similar to that in a

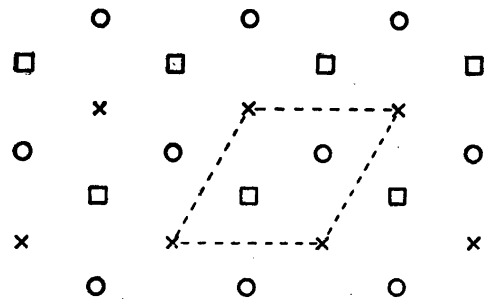

Fig. 4a. The metal lattice of the ε phase of the Mo-B system, projected on (0 0 1). (The unit cell is indicated.)

x = *Mo in* $00z$, $00\bar{z}$
○ = *Mo in* $1/3\ 2/3\ 1/3+z$, $1/3\ 2/3\ 1/3-z$
□ = *Mo in* $2/3\ 1/3\ 2/3+z$, $2/3\ 1/3\ 2/3-z$

cubic close packing. If in the ordinary cubic close packing the sequence of layers is described as A B C A B C A . . . the sequence of layers of molybdenum atoms in the direction of the c axis in this structure will be AA BB CC AA BB CC AA . . . The distance between the center of two adjacent Mo-atoms in the same A, B, or C layer will be $a = 3.01$ Å. The distance A—A, B—B or C—C will be $2zc = 3.13$ Å, and the distance A—B, B—C, or C—A will be $c(1/3-2z) = 3.82$ Å, where a and c are the lengths of the hexagonal axes.

The possibility of another value of z within the limits given above may, to a small extent, alter the distances between the atoms, but will interfere with neither the fundamental unit of structure nor the positions of the boron atoms, the arrangement of which is discussed below.

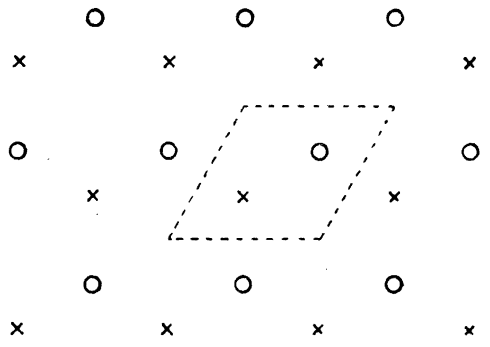

Fig. 4b. The metal lattice of the ε phase of the W-B system, projected on (0 0 1). (The unit cell is indicated).

○ = *W in* $1/3\ 2/3\ z$, $1/3\ 2/3\ 1/2-z$
x = *W in* $2/3\ 1/3\ \bar{z}$, $2/3\ 1/3\ 1/2+z$

Table 8. W—B, ε-phase, Cr-K radiation.

h k i l	R	$\sin^2\Theta$ obs.	$\sin^2\Theta$ calc.	*I* obs.	$p\|F\|^2$ obs.*	$p\|F\|^2$ calc. $z = 0.139$
0 0 0 4	α	0.1095	0.1090	st	7	12
1 0 $\bar{1}$ 0	α	1966	1965	sv +	4	9
1 0 $\bar{1}$ 1	α	2034	2033	st	10	31
1 0 $\bar{1}$ 2	—	—	2238	—	0	0.5
0 0 0 6	α	2456	2452	sv —	1.1	2.8
1 0 $\bar{1}$ 3	α	2582	2578	m	7	12
1 0 $\bar{1}$ 4	α	3053	3055	sv +	9	14
1 0 $\bar{1}$ 5	α	3670	3668	m +	26	37
0 0 0 8	α	4364	4358	sv	8	6
1 0 $\bar{1}$ 6	—	—	4417	—	0	3
1 0 $\bar{1}$ 7	—	—	5302	—	0	1.1
1 1 $\bar{2}$ 0	α_1	5895	5896	st	33	24
1 1 $\bar{2}$ 2	—	—	6168	—	0	1.4
1 0 $\bar{1}$ 8	α_1	6322	6324	sv —	2.4	7
0 0 0 10	α_1	6810	6810	sv —	4	5
1 1 $\bar{2}$ 4	α_1	6987	6985	st	49	40
1 0 $\bar{1}$ 9	α_1	7480	7481	sv +	24	33
2 0 $\bar{2}$ 0	α_1	7859	7861	sv	7	5
2 0 $\bar{2}$ 1	α_1	7926	7929	sv +	11	19
2 0 $\bar{2}$ 2	—	—	8133	—	0	0.3
1 1 $\bar{2}$ 6	α_1	8343	8347	sv	10	10
2 0 $\bar{2}$ 3	α_1	8476	8474	sv —	5	8
1 0 $\bar{1}$ 10	α_1	8770	8775	sv —	2	6
2 0 $\bar{2}$ 4	α_1	8952	8950	sv —	5	9
2 0 $\bar{2}$ 5	α_1	9559	9563	st	21	25

The metal lattice of the W—B phase

The interpretation of the powder photographs (Cr-*K* and Cu-*K* radiation) gave for the W—B phase a hexagonal cell:

$$a = 2.982 \text{ Å}, \quad c = 13.87 \text{ Å} \quad \text{and} \quad V = 107.0 \text{ Å}^3$$

With an assumed composition of $WB_{2.5}$ the observed density of 11.0 corresponds to a cell content of 4 $WB_{2.5}$ (calculated density 13.1). The fact,

* Referred to α_1 for all lines.

Table 9. W—B, ε-phase.

$h\ k\ i\ l$	$p\|F\|^2$				Resulting limits for z
	obs.	calc. z =			
		0.136	0.139	0.142	
0 0 0 8	76	67	55	42	
1 0 $\bar{1}$ 6	0	8	34	47	$z<0.142$
1 0 $\bar{1}$ 7	0	33	11	2	
1 1 $\bar{2}$ 6	104	65	102	140	
2 0 $\bar{2}$ 3	51	88	75	63	$0.136<z<0.142$
1 0 $\bar{1}$ 10	20	41	59	75	

that the c axis of the W—B phase is 2/3 of the c axis of the Mo-B phase is an additional indication of 4 W atoms per cell.

Reflections $h\ h\ 2\bar{h}\ l$ were observed only for $l = 2n$, which is characteristic of the space groups D_{3d}^2—$C\ \bar{3}1c$, C_{3v}^4—$C\ 31c$, D_{6h}^4—$C\ 6/mmc$, C_{6v}^4—$C\ 6mc$ and D_{3h}^4—$C\ \bar{6}2c$. From space considerations and from the fact that $h\ k\ i\ l$ were observed for both odd and even values of l the remaining possibilities of placing four tungsten atoms are:

in D_{3d}^2, the fourfold position 4 : (*f*),
in C_{3v}^4, two twofold positions 2 : (*b*),
in D_{6h}^4, the fourfold position 4 : (*f*),
in C_{6v}^4, two twofold positions 2 : (*b*).

For all these positions the metal lattice will obtain the symmetry of D_{6h}^4, the space group with the highest symmetry, and it will be sufficient to discuss 4 : (*f*) in D_{6h}^4 to learn the positions of the metal atoms. The coordinates of the position will be the following:

4 : (*f*) 1/3, 2/3, z; 2/3, 1/3, $\bar{z}$; 2/3, 1/3, 1/2 + z; 1/3, 2/3, 1/2—z.

Good agreement between observed and calculated values is found for $z = 0.139$ (Table 8). The limits for the possible variations of z are $0.136<z<0.142$ (Table 9).

In this case the metal lattice (Fig. 4b) may be described as an arrangement similar to hexagonal close packing. If in the ordinary hexagonal close packing the sequence of layers is described as A B A B A B . . ., the sequence of tungsten atoms in the direction of the c axis will be . . .AA BB AA BB AA . . . The distance between the center of two adjacent tungsten atoms in the same A

or B layer will be $a = 2.98$ Å. The distance between two adjacent layers are. A—A or B—B $= c(1/2 - 2z) = 3.07$ Å and A—B or B—A $= 2zc = 3.85$ Å:

As was the case for the Mo-phase, the error in the determination of z has no influence on the fundamental unit of structure.

The boron positions

The boron atoms must be situated in holes of the metal lattices, and as these holes are of the same type in both the Mo- and the W-lattices, they may be considered together. In the structures there are three different types of holes.

1. The holes between the metal atoms of a doublesheet A—A, B—B or C—C.

In D^5_{3d}, the space group of the Mo-B phase, these holes correspond to the sixfold position:

6 : (*c*) 0, 0, *z*; 0, 0, $\bar{z}$; 1/3, 2/3, 1/3 + *z*; 1/3, 2/3, 1/3—*z*; 2/3, 1/3, 2/3 + *z*; 2/3, 1/3, 2/3—*z*,

where $z = 1/3$.

The distance center of boron-center of metal atoms for this position would be $\sqrt{a^2/3 + z_M^2 c^2} = 2.34$ Å.

In D^4_{6h} (W—B phase) the corresponding positions are:

2 : (*b*) 0, 0, 1/4; 0, 0, 3/4, and
2 : (*d*) 1/3, 2/3, 3/4; 2/3, 1/3, 1/4,

and the distance boron-metal would equal 2.31 Å ($\sqrt{a^2/3 + (1/4 - z_W)^2\ c^2}$). The arrangement of metal atoms around the boron atom would be the same as was found for the δ phase; the boron lying in the center of a trigonal prism with six metal atoms at the corners. The boron atoms will then form a plane hexagonal network, similar to that of the carbon atoms in graphite (fig. 5a).

The distance boron-boron in the Mo-B phase is 1.74 Å and in the W—B phase 1.72 Å. If the boron atoms are assumed to be spherical and in contact their radius will be 0.86—0.87 Å in good agreement with the value given by Pauling and Weinbaum [17] for boron in CaB_6. Such plane hexagonal networks have been found for the boron atoms in AlB_2^{10} and Zr B_2^{11}.

In the interspaces between the double sheets there are two additional types of holes.

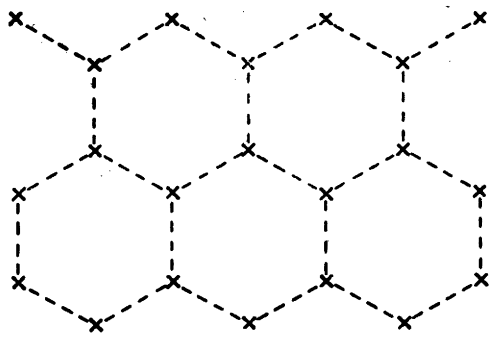

Fig. 5a. Network of boron atoms, occupying holes of type »1».

2. Holes in the center of a tetrahedron of metal atoms. In the Mo-B phase there are six such holes per unit cell with the center in

6 : (*c*) 0, 0, *z*; 0, 0. $\bar{z}$; 1/3, 2/3, 1/3 + *z*; 1/3, 2/3 1/3-*z*; 2/3, 1/3, 2/3 + *z*; 2/3, 1/3, 2/3—*z* (space group D_{3d}^{5}).

where $z = 1/6 + a^2/6\ c^2(1/3—2z_{Mo}) = 0.186$.

The distance center of boron-center of metal = 2.32 Å [$c\ (z_B—z_{Mo})$].
In the W—B phase there are four which are in

4 : (*f*) 1/3, 2/3, *z*; 2/3, 1/3, $\bar{z}$; 2/3, 1/3, 1/2 + *z*; 1/3, 2/3, 1/2—*z* (space group D_{6h}^{4}).

where $z = — a^2/12\ z_W^2\ c^2 = — 0.028$.

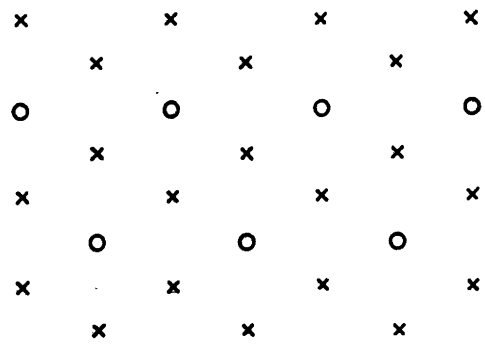

Fig. 5b. Sheet of boron atoms, occupying holes of type »2» and »3».
x = boron in hole of type »2».
O = boron in hole of type »3».

The distance boron-tungsten = 2.32 Å [$c\ (z_W - z_B)$].

3. Finally there is a third type of hole. The center for this type of hole have the position:

in D_{3d}^5 (Mo-B phase) 3 : (*b*) 0, 0, 1/2; 1/3, 2/3, 5/6; 2/3, 1/3, 1/6,
in D_{6h}^4 (W—B phase) 2 : (*a*) 0, 0, 0; 0, 0, 1/2.

A boron atom placed in such a hole would be surrounded by six metal atoms, all at a distance of 2.59 Å ($\sqrt{a^2/3 + c^2 z_W^2}$) from the center, and arranged at the corners of a deformed octahedron. If all the holes of type »2» were filled, a boron atom in »3» would not be in direct contact with any metal atom but with six other boron atoms, and the atoms of »2» and »3» would form a slightly puckered, close packed hexagonal sheet of boron atoms, lying in the interspaces between the doublesheets of metal atoms (Fig. 5b). The distances »2»—»3» = 1.76 Å and »2»—»2» = 1.92 Å.

Thus, from space considerations boron atoms may be placed in all the holes of the metal lattice described above. Such an »ideal» ε phase would have the formula Me_2B_5 corresponding to a boron content of 71.4 atomic %, and in these »ideal» phases, the arrangement of layers along the *c* axis would be ...

$\overbrace{\text{AHAKBHBKCHCK}}^{c}\text{AHAKB}\ldots$ for the Mo-B phase and

$\overbrace{\text{AHAKBHBK}}^{c}\text{AHAKBHBK}\ldots$ for the W—B phase. Here A, B, and C are the metal sheets described above, H are the hexagonal networks of boron atoms (holes of type »1») and K are the sheets of boron atoms (holes of types »2» and »3»).

The exact boron content of the phases was very difficult to determine because of the difficulties of isolating and analysing the pure phases. In both the systems, however, it seemed to be less than 71.4 atomic %. A study of the photographs of these phases, starting with different compositions Me-B indicated that for the Mo-B phase the reflections always occurred at fixed angles. The boron content of the phase was found to be about 70 atomic %. In the case of the W—B phase, however, there was a small variation of the $\sin^2\Theta$ values (Table 10) which indicates an extended homogeneity range with the limits $66.7 < \varepsilon < 68.0$ atomic % boron.

The corresponding values of the axes are $a = 2.982$ Å, $c = 13.87$ Å and $a = 2.984$ and $c = 13.87$ Å respectively. The values of the limits, however, are rather uncertain because of the small variation and the possible errors of the composition. It is impossible to determine which holes of the metal lattices are occupied by boron atoms and which are not. The phases thus must be

Table 10. W—B, ε-phase.

h k i l	$\sin^2\Theta$ obs. Boron content (atomic %)					
	64.0	66.7	67.3	67.9	71.5	82.3
1 1 $\bar{2}$ 0	5894	5896	5893	5887	5886	5884
1 1 $\bar{2}$ 4	6987	6987	6981	6974	6972	6972
1 0 $\bar{1}$ 9	—	7482	7479	7480	7475	7480
2 0 $\bar{2}$ 0	7865	7865	7858	—	7849	7849
2 0 $\bar{2}$ 1	7932	7926	7924	7918	7916	7912

described in relation to the ideal ε phase, described above. Of the boron positions in the ideal phase, 93 % are occupied in the Mo-B phase and 80—86 % in the W—B phase.

The ε phase in the Mo-B system thus crystallizes in D_{3d}^5—$R\,\bar{3}m$ with the metal atoms in the sixfold position

6 : (*c*) 0, 0, *z*; 0, 0, $\bar{z}$: 1/3, 2/3, 1/3 + *z*; 1/3, 2/3, 1/3—*z*; 2/3, 1/3, 2/3 + *z*; 2/3, 1/3, 2/3—*z*,

where $z = 0.075$.

Six boron atoms may be placed in 6 : (*c*) with $z = 1/3$, six in 6 : (*c*) with $z = 0.186$ and three in

3 : (*b*) 0, 0, 1/2; 1/3, 2/3, 5/6; 2/3, 1/3, 1/6.

About 93 % of these positions are occupied by boron atoms.

The ε phase in the W—B system crystallizes in D_{6h}^4—$C\ 6/mmc$ with the metal atoms in

4 : (*f*) 1/3, 2/3, *z*; 2/3, 1/3, $\bar{z}$; 2/3, 1/3, 1/2 + *z*; 1/3, 2/3, 1/2—*z*,

where the parameter $z = 0.139$. Four boron atoms may be placed in 4 : (*f*) with $z = -0.028$, and two boron atoms in each of the following three positions

2 : (*b*) 0, 0, 1/4; 0, 0, 3/4,
2 : (*d*) 1/3, 2/3, 3/4; 2/3, 1/3, 1/4,
2 : (*a*) 0, 0, 0; 0, 0, 1/2.

About 80—86 % of these positions are occupied.

GENERAL CONCLUSIONS

The formation of interstitial compounds in the binary systems transition element-boron, carbon, nitrogen or hydrogen without serious distortion of the metal lattice, according to Hägg [12,13], is possible only when the ratio of boron radius: metal radius is less than 0.59. In discussing the radius of the boron atom the γ phases are excluded because of the variation of r_B in the isomorphous compounds Me_2B. From the distance boron-boron in the chains of boron atoms of the δ phases the value $r_B = 0.87$ Å is obtained, and from the hexagonal networks in the ε phases (holes of type »1») the values $r_B = 0.87$ Å (Mo-B) and $r_B = 0.86$ Å (W—B) are obtained. These values are in good agreement with the value 0.86 Å, reported by Pauling and Weinbaum [17] for boron in CaB_6, which seems to be the most accurate value from earlier experiments. Assuming 0.87 Å to be a correct value, the ratio $r_B : r_{Me}$ will be 0.62 for molybdenum and tungsten (1.40 and 1.41 Å beeing the value of r_{Mo} respectively r_W corresponding to 12-fold coordination). In fact, none of the intermediate phases in the systems Mo-B and W-B has the simple type of metal lattice which exists if $r_B : r_{Me}$ less than 0.59. The value 0.62, however, is close to the critical value and all the metal lattices are rather simple. The ε phase, for example, may be considered as consisting partly of a simple hexagonal lattice with the boron atoms in the interspaces.

The boron atoms seem to have a marked tendency to form first chains and then rings as the boron content of the system increases. Upon studying the arrangement of the boron, the following three cases may be distinguished:

1. In the γ phases, Me_2B, the boron atoms are present as isolated units in the lattice.

2. In the δ phases with 48—51 atomic % boron they form zig-zag shaped lines running through the channels of the lattice.

3. In the ε phases with 67—70 atomic % boron they form partly hexagonal networks and partly two-dimensional sheets.

All the metal borides previously recognized, may be classified in one of these groups, or in a fourth group with a three-dimensional network of boron atoms.

Fe_2B[7,8], Co_2B, and Ni_2B[9], having isolated boron atoms, belong to group 1.

In FeB and CoB[9] the boron atoms form zig-zag chains as in the δ phases above. The coordination around the boron is the same too, but the chains are all parallel. These borides belong to group 2.

AlB_2[10] and ZrB_2[11] belong to group 3, the boron atoms forming plane hexagonal networks, all of which are parallel.

The borides of type CaB_6 (D 2_1) belong to group 4 [14—17].

It is of interest to note that most of these stages can be distinguished in the configuration of the carbon atoms in the carbides. For example, in Fe_3C [18] and the carbides of manganese and nickel, the carbon atoms lie in isolated positions in the metal lattice, while in the chromium carbide Cr_3C_2 [19], the carbon atoms form zig-zag chains throughout the lattice. Potassium graphite compounds may be regarded as analogous to group 3, since they are compounds of potassium and infinitely extended hexagonal networks of carbon atoms. Carbides analogous to group 4 have not been observed.

It would be of great interest to learn whether or not this analogy may be extended to the formation of different boron hydrides, starting with borides from different groups above. Such an experiment, however, would be difficult to perform because of the problems involved in isolating the boron hydrides for study.

SUMMARY

The systems Mo-B and W—B have been studied by X-ray methods, and the following boride phases were found to exist:

γ phases Mo_2B and W_2B. Unit dimensions are given and the phases are shown to be isomorphous with Fe_2B, Co_2B, and Ni_2B.

δ phases, isomorphous and existing in a homogeneity range of about 48—51 atomic % boron. Unit dimensions are given and a complete structure determination has been made.

ε phases, whose ideal boron content is 71.4 atomic % boron, but which in reality exist at about 70.0 atomic % boron for Mo-B and whose range is 67—68 atomic % boron for W-B. Unit dimensions are given and a complete structure determination has been accomplished. The phases are not isomorphous but a close relationship exists between them. The ideal formula of both phases is Me_2B_5 but some of the boron atoms corresponding to this formula are missing.

A boron preparation with a purity of 98—99 % B, which was used in this investigation, was obtained by thermic dissociation of BBr_3 in a heated quartz tube at about 700° C in the presence of hydrogen.

The radius of the boron atom has been found to be 0.87 Å.

The structures are discussed according to the relation $r_B : r_{Me}$.

The boron atoms has a marked tendency to form first chains and then rings or sheets as the boron content of the system increases, and all the known borides of the metals may be classified according to the distribution of the boron atoms.

Similarity between the borides and some carbides is noted.

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Investigations of Barley and Malt-Amylase

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When fractionating protein components of malt, Osborne [1] found that the amylase activity follows the albumin fraction. He isolated the albumins by precipitation with ammonium sulphate followed by dialysis. Out of the albumin solutions obtained in this way the albumins were precipitated with alcohol and dried over H_2SO_4. By this means a product of powerful amylase activity was obtained.

For the experiments described in this paper, Osborne's method of preparation has been mainly followed. Modifications have been introduced based on the experiences which were met with during earlier experiments to isolate the globulins from barley and malt [2,3]. Albumin is obtained from both barley and malt in exactly the same way, according to the method stated below.

METHOD OF PREPARATION

500 g of barley (or malt) are thoroughly ground and extracted at + 4° C with 2000 ml 1 *M* NaCl, buffered to pH = 7 by primary and secondary sodium phosphate. After centrifuging and filtering until clear, part of the proteins are precipitated by 4/10 saturation with anhydrous ammonium sulphate [2]. The precipitate obtained is discarded because it contains no albumin. The rest of the proteins are precipitated by 9/10 saturation with ammonium sulphate and the precipitate so obtained is separated by centrifugation and dissolved in 0.2 *M* NaCl at pH 7.0. The solution is then dialysed in a cellophane sack against distilled water at the lowest possible temperature (2—4° C) for 48 hours. The precipitate obtained by the dialysis consists of globulins, and has no amylase activity, being therefore centrifuged down and discarded. The solution from the dialysis is 7/10 saturated with ammonium sulphate and the precipitate formed is dissolved in buffer at pH 7.0 and dialysed anew. This procedure is repeated until a precipitate is no longer obtained on dialysis. The solution is then quite free from globulins. The preparation proceeds according to the following scheme of preparation.

1. Initial materials
2. Extraction with 1 *M* NaCl, pH 7
3. Precipitation by $(NH_4)_2SO_4$; 4/10 saturation

Precipitate | Solution

4. Precipitation by $(NH_4)_2SO_4$; 9/10

Precipitate | Solution

5. Dissolving in buffer at pH 7.0
6. Dialysis against distilled water

Precipitate | Solution

7. Precipitation by $(NH_4)_2SO_4$; 7/10

Precipitate | Solution

8. Dissolving in buffer at pH 7.0
9. Dialysis against distilled water

Precipitate | 10. Solution containing albumin

The solution obtained at stage 10 is treated according to stages 7—9 several times, until a precipitate is no longer obtained on dialysis against water.

The albumin fractions from barley and malt, prepared according to the scheme stated above, were tested in the ultracentrifuge (*cf.* Svedberg and Pedersen [4]). Exactly the same sedimentation diagram was obtained from both albumins (see Fig. 1).

The sedimentation constants were also identical. In earlier investigations of the albumin of barley, Quensel [5] determined the sedimentation constant of the albumin to be 3.5 S, but pointed out that the albumin fractions were not homogeneous. We also obtain this value of the sedimentation constant for those fractions which have not been precipitated a sufficient number of times. By careful purification of the albumin the value 4.6 S is obtained. This value remains constant for different preparations, and is independent of divergences from the scheme of preparation stated above, (*e. g.* precipitation at another concentration and dialysis at another pH).

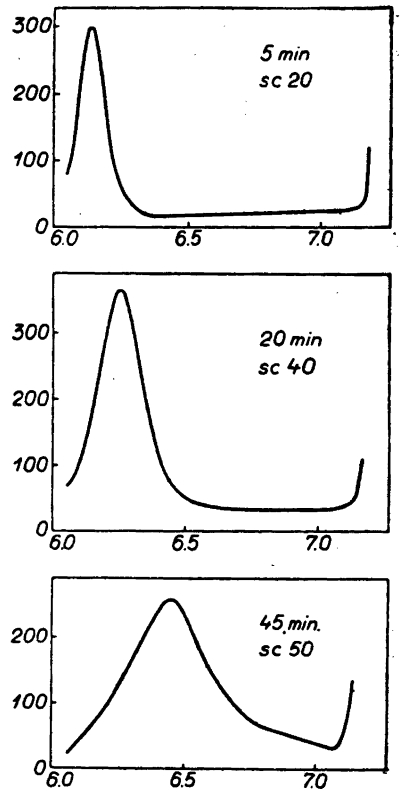

Figure 1. Sedimentation diagrams for barley and malt albumin.

DETERMINATION OF THE AMYLASE ACTIVITY

Despite the fact that the sedimentation constants of barley and malt albumin are identical, the amylase activities are quite different. The determination of the saccharification power has been carried out according to Windisch-Kolbach [6]. The value given by this test, which can be considered as a measure of the activity of β-amylase, is given as the number of grams of maltose formed from soluble starch by 100 g of enzyme preparation acting for 30 minutes at 20° C and pH $= 4.3 \pm 0.1$.

The determination of the dextrinizing power has been carried out according to a modified Wohlgemuth method [7], and is primarily a measure of the α-amylase activity. The result is given as the number of grams of starch which are broken down to dextrins by 100 g of enzyme preparation acting for 15 minutes at 30° C and pH $= 5.0$.

In those cases for which the enzyme activity was calculated from the dry

weight of the material the moisture determinations were carried out by means of drying in vacuum at 50° C.

Table 1. Effect of albumin purification on amylase activity.

	Dry substance %	Saccharification power (dry substance)	Dextrinizing power (dry substance)
Malt (from six-rowed barley)	93.0	680	770
Barley albumin solution no. 1	1.30	5400	0
» » » » 2	0.82	7800	0
Malt albumin » » 1	1.30	12600	12800
» » » » 2	1.27	13400	15400
» » » » 3	1.15	20500	25600
» » » » 4	0.85	32600	37500
» » » » 5	0.72	45600	51400
» » » » 6	0.55	59800	67100
» » » » 7	0.52	63700	60400

Through continued purification by precipitation and dialysis the enzyme activity reaches a maximum. Further manipulation tends to diminish the activity. The activity of α-amylase in malt albumin no. 7 is lower than in malt albumin no. 6 and it has in general been found that α-amylase in these preparations is more unstable than β-amylase, despite the fact that the pH is kept constant.

THE CHANGES OF THE ACTIVITY ON STORAGE

The aqueous solutions of malt albumin could be stored in a refrigerator (+ 1° C) for several weeks without any significant change of the activity being detectable. On longer storage ca. 3—4 weeks, a small part of the albumin precipitates in the form of extremely small amorphous particles. The precipitate easily redissolves on warming to room temperature, but appears again on recooling.

In two cases this precipitate was centrifuged down and redissolved, after which the activity was again determined.

Table 2. Amylase activity of albumin solutions and precipitates after storing at + 1° C for 4 weeks.

	Saccharification power (dry substance)	Dextrinizing power (dry substance)
Original solution no. 1	59800	67100
Precipitate by cooling	38400	112600
Original solution no. 2	63700	60400
Precipitate by cooling	96900	240800

By storage in the cold in this way the α-amylase is precipitated to a considerably higher degree than β-amylase. Consequently, it appears possible that by means of fractional precipitation in the cold, α-amylase may be separated from β-amylase. However, hitherto the precipitation has only been done by cooling to 1° C and at this temperature the separation is very slow and gives a low yield. Further attempts will therefore be made, by means of an improved method, to separate quantitatively both the amylases from each other and at the same time to obtain, if possible, a concentration.

CHANGES OF ACTIVITY ON PROLONGED STORAGE

The solutions of the enzymes have been kept in a refrigerator at + 4° C for a period of 3 months. At this temperature no precipitate was formed. Determinations of the activity were made at the beginning and end of this period. In the first column of Table 3 the original activity is given, in the second column the activity which was found after three months. In the third column appears the percentage of the original activity remaining after three months.

Table 3. Changes in amylase activity of albumin solutions after 3 months at + 4°C.

	Saccharification power			Dextrinizing power		
	Before	After	% Remaining	Before	After	% Remaining
Solution no. 1	418	324	78	522	429	82
» » 2	233	186	80	167	147	87
» » 3	—	—	—	2667	2224	83

The activity has consequently decreased by the same extent for all the solutions, or to about 80 % of the original value, in 3 months. The enzyme solutions are therefore relatively stable.

ULTRAFILTRATION OF ALBUMIN SOLUTIONS

In order to obtain concentrated solutions of malt albumin, experiments have been made with ultrafiltration [8] of the solutions. For this purpose a filter has been used which was obtained by dipping an alundum tube into a solution of collodion in glacial acetic acid. With a suitable thickness of the collodion membrane a filter is obtained which does not allow the albumin molecules to pass through. During the tests below the protein did not, on any occasion, pass through the filter. In Table 4 is presented the result of two tests with malt albumin.

Table 4. Effect of ultrafiltration on amylase activity.

	Saccharification power (dry substance)	Dextrinizing power (dry substance)
Untreated solution 1	29800	37300
Filtrate	0	0
Remainder	90000	55700
Untreated solution 2	29900	37200
Filtrate	0	0
Remainder	59100	75000

Ultrafiltration, therefore, offers two advantages: (1) concentration of the albumin and (2) increased enzyme activity.

DRYING OF ALBUMIN

The albumin solutions which had been concentrated by ultrafiltration can, after cooling down to $-16°$ C, be dried in vacuum over $CaSO_4$. By this means the albumin is obtained in the form of a brown, amorphous powder, which dissolves immediately and completely in distilled water. Chemical investigations of these dried albumin fractions show that the nitrogen content is about 11 %. Further investigations regarding the chemical composition will be made. In the dried condition the albumin is of unlimited stability. Both α- and β-amylase activities are largely retained. The drying entails some diminution of the amylase activities (see Table 5), but this is compensated by the fact that the enzyme becomes stable and easier to keep.

Table 5. Effect of drying on amylase activity.

	Saccharification power (dry substance)	Dextrinizing power (dry substance)
Albumin, no. 1		
before drying	59100	75000
after drying	45000	57400
Albumin, no. 2		
before drying	47500	62500
after drying	39200	55000

ELECTROPHORETIC EXPERIMENTS

The first problem with the electrophoretic experiments was to establish whether the α- and the β-amylase had the same mobility in an electric field. Since the enzymes could not be separated by centrifugation, it was hoped that this could be carried out with the help of electrophoresis.

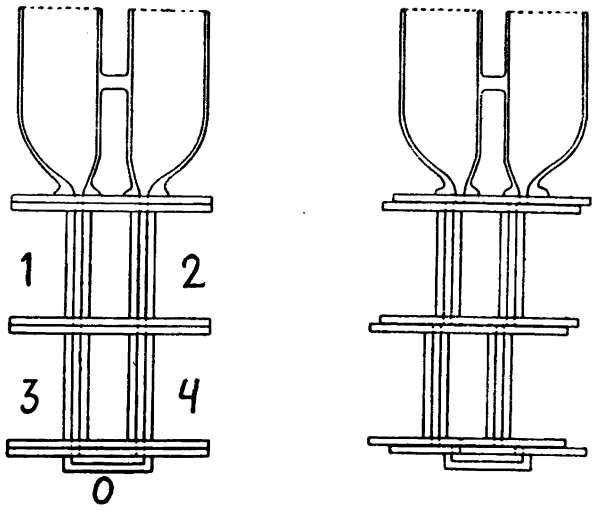

Figure 2. Electrophoretic cell (Tiselius).

The Tiselius apparatus [9] with the modifications indicated by Svensson [10] for the collection and chemical analysis of samples was used.

The method is as follows. At the beginning of the experiment the solution is placed in the cells 0, 3 and 4 (see Fig. 2). The cells 1 and 2 hold the solvent. The boundary between the solution and solvent is produced by the displacement of cells 3 and 4. When a direct current is passed through the solution, the active substance migrates under the influence of the current.

The experiment continues for a definite period, during which the current and voltage are determined. Conductivity and pH are also measured. At the end of the period, the cells are displaced in relation to each other, so that the solution in each one can be taken out with a syringe, after thorough mixing to equalize all the differences in concentration. The determination of the enzyme activity is then done for the 5 fractions, and in such a way that one obtains a relative measure of the enzyme concentration. In all the tests, all the samples have as far as possible, been treated in exactly the same way.

At the beginning of the experiment the enzyme activity is the same in cells 0, 3 and 4. In the cells 1 and 2 the concentration (a) is zero, since these cells contain pure solvent. After the time t the enzyme has migrated by the extent l into cell 1. If the volumes of the cells 1 and 3 are v_1 and v_3, cross sections $q_1 = q_3 = q$, and concentrations a_1 and a_3, one can write:

$$a_1 = \varrho_1 \cdot a_3 \qquad 0 < \varrho_1 < 1: \tag{1}$$
$$\varrho_1 = \frac{a_1}{a_3}$$

but

$$l_1 = \frac{\varrho_1 \; v_1}{q} = \frac{a_1 \; v_1}{q a_3} \tag{2}$$

now,

$$l_1 = \frac{u_1 \cdot i \cdot t}{q \cdot \varkappa} \tag{3}$$

where

u_1 = mobility measured in cell no. 1
i = current strength
t = time
$\varkappa$ = conductivity

one gets

$$u_1 = \frac{l_1 \cdot q \cdot \varkappa}{i \cdot t}$$

Substitution of l_1 from (2) gives

$$u_1 = \frac{a_1 \; v_1 \; \varkappa}{a_3 \cdot i \cdot t} \tag{4}$$

In this equation one can put $a_3 = a_0$, since the experiment is stopped before any change of concentration in cell 0 has taken place.

By observing the change in cell 4 a control value for the velocity is obtained. In an analogous way to the above is obtained:

$$a_4 = \varrho_2 \cdot a_0 \qquad 0 < \varrho_2 < 1 \tag{1'}$$
$$\varrho_2 = \frac{a_4}{a_0}$$

$$l_4 = \frac{(1 - \varrho_2) \; v_4}{q} \tag{2'}$$

From (1') and (2')

$$l_4 = \frac{v_4}{q} \cdot \frac{a_0 - a_4}{a_0} \tag{3'}$$

but

$$l_4 = u_4 \cdot \frac{i \; t}{q \; \varkappa}$$

and

$$u_4 = \frac{v_4 \; \varkappa}{i \cdot t} \cdot \frac{(a_0 - a_4)}{a_0} \tag{4'}$$

where u_4 is the mobility of the active substance, measured in cell 4.

With the help of these equations one can determine the mobility for both the α- and the β-amylase activities. The following example may be given:

Electrophoresis no. 17

Cell no. 1 positive pH 6.80

$i = 23.1$ mA

$\varkappa = 3.809 \cdot 10^{-3}$ ohm$^{-1} \cdot$ cm^{-1}

$t = 14400$ s

$v_1 = v_4 = 3.60$ ml

Concentrations were determined to be

$a_1^{\alpha} = 187$	$a_1^{\beta} = 292$
$a_3^{\alpha} = 414$	$a_3^{\beta} = 720$
$a_0^{\alpha} = 414$	$a_2^{\beta} = 729$
$a_4^{\alpha} = 213$	$a_4^{\beta} = 397$
$a_2^{\alpha} = 0$	$a_2^{\beta} = 0$

Formulae (4) and (4′) give with these values,

$u_1^{\alpha} = 1.67 \cdot 10^{-5}$ cm$^2 \cdot$ volt^{-1} s^{-1}

$u_4^{\alpha} = 1.87 \cdot 10^{-5}$ »

$u_1^{\beta} = 1.86 \cdot 10^{-5}$ »

$u_4^{\beta} = 2.00 \cdot 10^{-5}$ »

If the averages are taken,

$u^{\alpha} = 1.77 \cdot 10^{-5}$ cm$^2 \cdot$ volt^{-1} s^{-1}

$u^{\beta} = 1.93 \cdot 10^{-5}$ »

In this case the enzymes migrated towards the positive electrode, and were consequently negatively charged. The above-mentioned mobilities should therefore have negative signs.

By an exactly analogous way the values stated in Table 6 have been obtained.

Table 6. Electrophoretic mobility of amylase activity.

Buffer	pH	$u^{\alpha} \cdot 10^5$	$u^{\beta} \cdot 10^5$
0.175 *M* HAc 0.100 *M* NaAc	4.38	—	+ 1.81
0.002 *M* Na_2HPO 0.094 *M* NaH_2PO_4 0.05 *M* NaCl	5.26	+ 0.78	+ 0.78
0.00125 *M* Na_2HPO_4 0.0112 *M* NaH_2PO_4 0.05 *M* NaCl	5.98	— 0.44	— 0.51
0.00125 *M* Na_2HPO_4 0.0112 *M* Na_2HPO_4 0.05 *M* NaCl	6.15	— 0.68	— 0.64
0.0075 *M* Na_2HPO_4 0.005 *M* NaH_2PO_4 0.05 *M* NaCl	6.80	— 1.77	— 1.93
0.0075 *M* Na_2HPO_4 0.005 *M* NaH_2PO_4 0.05 *M* NaCl	6.85	— 1.25	— 1.19
0.004 *M* H_3PO_3 0.00125 *M* $Na_2B_4O_7$ 0.05 *M* NaCl	7.90	— 4.73	— 3.90
0.004 *M* H_3BO_3 0.00125 *M* $Na_2B_4O_7$	8.22	— 5.09	— 4.61

The results of Table 6 are shown graphically in Fig. 3.

Consequently α-amylase has exactly the same mobility in an electric field as β-amylase, over most of the pH range examined. A certain tendency to a different mobility appears at pH 8, but the differences are very small. It has not as yet been possible to separate the amylases from each other by the electrophoretic method. It appears from the diagram that the iso-electric point for both enzymes lies at pH 5.75. It is interesting to compare these determinations of the mobility of the two amylases with the values which have been obtained from electrophoretic experiments on the α-amylase from pancreas[11,12]. By means of optical determinations of the mobility these authors found $u = 3.09 \cdot 10^{-5}$ for the crystallized enzyme at pH 7.9. At pH 6.5 they

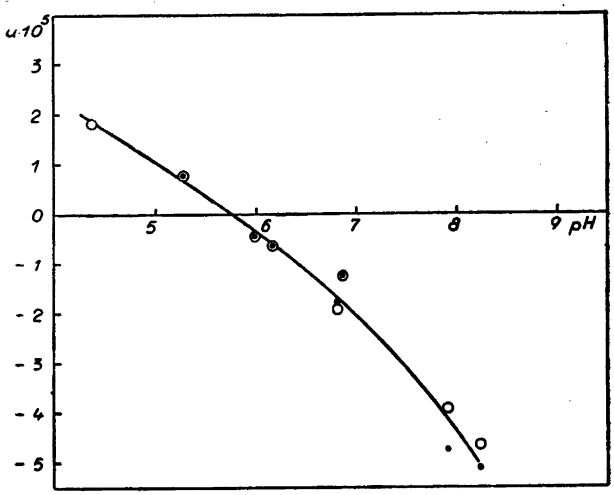

Figure 3. Electrophoretic mobility of amylase activity.

○ *Saccharification activity.*
● *Dextrinizing activity.*

found $u = 1.8 \cdot 10^{-5}$. From Fig. 3 the value $u = 4.3 \cdot 10^{-5}$ is given for malt amylase at pH 7.9, and at pH 6.5 the value is $u = 1.5 \cdot 10^{-5}$.

SUMMARY

1. The amylase activities of barley and malt have been localized in the albumin fractions. The amylase activity of the preparation increases with increasing purity of the albumin. Barley and malt albumins behave exactly alike in ultracentrifugation and both have a sedimentation constant of $s_{20} = 4.6$ S.

2. If aqueous solutions of pure malt albumin are kept in the cold (+ 1° C), α-amylase is precipitated after a time to a considerably higher degree than β-amylase. The aqueous solutions of albumin kept at + 4° C lose only 20 % of their activity after three months.

3. The albumin solutions can be concentrated and purified by means of ultrafiltration and by cooling and drying in vacuum over $CaSO_4$. From albumin solutions purified in this way a stable amylase preparation is obtained with high enzyme activity.

4. Electrophoretic experiments with malt albumin prove that the mobility for the α- and the β-amylase activity is practically the same in an electric field throughout the range pH = 4.4 to pH = 8.2. Only a very small difference is seen to be present at pH 8 and above. It is therefore probable that the α- and the β-amylase cannot be separated by means of electrophoresis.

5. The isoelectric point for the α- and the β-amylase has been determined to be pH 5.75.

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The Structure of Molecules Containing Cyclohexane or Pyranose Rings

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The development of methods which make it possible to determine in some detail not only the molecular structures in crystals, but even the structure of free molecules in the vapour state, has revealed very interesting details throwing light on the forces acting between not directly linked atoms within the same molecule.

In 1943 [1] one of the present authors, starting with the structure of ethane, discussed the structure of some of its derivatives and of a series of cyclic compounds drawing attention to the importance of the secondary intramolecular forces just mentioned. It was assumed that the stable form of ethane is the *trans* form in which the smallest distance between hydrogen atoms not belonging to the same methyl group is about 2.5 Å, a value corresponding to the smallest distance between hydrogen atoms belonging to different molecules in crystals. As a first approximation the simple conception of definite van der Waals' radii was adopted although the fundamental objections which may be raised were of course recognized.

In the case of the cyclohexane molecule [2] it is then obvious that the molecule must be considerably more stable in the symmetrical »staggered» form than in the so called »boat» form in which four pairs of hydrogen atoms would be in *cis* position and the distance between a fifth pair of hydrogen atoms would be 1.83 Å only (if normal valency angles are assumed). It was further pointed out [3] that the model proposed by Mohr [4] for the *cis* form of decalin containing two cyclohexan rings of the »boat» type would probably have to be rejected in favour of a new model based on two staggered rings. The correctnes of this assumption has since been verified by electron diffraction investigations based on the sector method [5]. The discussion was extended to cycloparaffins with a number of carbon atoms smaller than six, and it was

pointed out that in cyclopropane and cyclobutane the *cis* position of hydrogen atoms resulting from a planar arrangment of the carbon atoms would not tend to increase the instability of the molecules. It was assumed, however, that in cyclobutane[6] the very small distances between 1,3 carbon atoms of about 2.18 Å may explain the relatively great increase in heat of combustion per CH_2 group when the C-C-C-angle is decreased from 110° to 90° (in cyclobutane) compared with the figure corresponding to a further decrease to an angle of 60° (cyclopropane). In molecules like cyclopropane [7] and phosphorus (P_4) [8] the very small valency angle of 60° does not result in abnormally small distances between atoms which are not directly linked together.

The discussion of the structure of chlorinated hydrocarbons was based on the results obtained for ethane derivatives [1], in which the Cl-atoms introduced into one methyl group is never found in *cis* position relative to hydrogen atoms of the other methyl group, which would correspond to a H-Cl distance of 2.57 Å. The H-Cl distance of the equilibrium configuration is about 2.9 Å. This observation explains the fact, that the $\varkappa$ form of monochloro-cyclohexane is energetically more stable than the ε form [9]. If all valency angles were tetrahedral, the distances from the ε Cl atom to the two nearest H atoms would have values very little different from 2.57 Å calculated for the *cis* distance in chlorinated ethanes.

The designations $\varkappa$ ($\varkappa\varepsilon\grave{\iota}\mu\varepsilon\nu o\varsigma$ = lying), and ε ($\grave{\varepsilon}\sigma\tau\eta\varkappa\acute{\omega}\varsigma$ = erected), were introduced by one of us in 1943[2]. To avoid confusion it should be mentioned that Beckett, Pitzer and Spitzer in 1947[25] introduced their own terms equatorial and polar for $\varkappa$ and ε respectively.

CYCLOHEXANE AND DERIVATIVES

In the case of 1,2 disubstituted cylohexanes, one of the chemically separable forms, the $\varkappa,\varepsilon$ form, (or *cis* form according to the older conception) consists of a mixture of equal quantities of *d* and *l* molecules which are readily transformed into each other by a conversion of the ring. The separation of the *d* and *l* molecules is therefore impossible. The $\varkappa,\varkappa \rightleftharpoons \varepsilon,\varepsilon$ form (or *trans* form) on the other side will also consist of optically active molecules, but in this case the conversion process does not lead to racemisation and a separation of the optically active antipodes is always possible, at least in principle. It would be of great interest to determine the proportion of $\varkappa,\varkappa$ and ε,ε molecules in the equilibrium mixture, but this is a difficult task, and only rough estimates have so far been possible.

The arguments which seem to explain the preponderance of $\varkappa$ molecules observed in the case of monosubstituted cyclohexanes, like cyclohexylchloride [9]

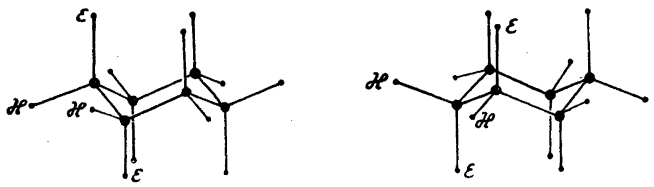

Fig. 1. The stepformed cyclohexane ring.

and cyclohexylmercaptan [10], namely the small distance between an ε substituend and the nearest hydrogens might lead to the assumption that the 1 $\varkappa$, 2 $\varkappa$ form would be energetically more stable than the 1 ε, 2 ε form. It must be born in mind, however, that the distance between two 1,2 substituends, both in $\varkappa$ positions, will in many cases be considerably smaller than the sum of their van der Waals radii if the valency angles retain their normal values. The interaction of the dipole moments of the two groups may also tend to increase the energy of the 1 $\varkappa$, 2 $\varkappa$ form.

It is therefore not very surprising that the results obtained by electron diffraction methods indicate equilibria in the case of 1,2-dibromocyclohexane [10] and cyclohexanediol-1,2 [11] in which the concentration of the 1 ε, 2 ε form is somewhat greater than fifty per cent.

When considering 1,3-disubstituted cyclohexanes the *trans* form should theoretically correspond to the 1 $\varkappa$, 3 ε form, the *cis* form to 1 $\varkappa$, 3 $\varkappa \rightleftharpoons$ 1 ε, 3 ε form. In the case of halogen derivatives it should be expected that the 1 $\varkappa$, 3 $\varkappa$ configuration is considerably more stable than the 1 ε, 3 ε configuration. An electron diffraction investigation [12,13] of the dibromide and diiodide carried out nearly ten years ago using the visual method confirms this view. In the latter case dipole measurements lead to the same result. It is also to be expected that the *cis* form (in the 1 $\varkappa$, 3 $\varkappa$ configuration) has a smaller energy than the *trans* form (1 $\varkappa$, 3 ε form).

In the case of 1,4-disubstituted cyclohexanes the 1$\varkappa$, 4$\varkappa \rightleftharpoons$ 1ε, 4ε form should correspond to the *trans* compound, the 1 $\varkappa$, 4 ε form to the *cis* compound. X-ray crystallografic work [14, 15] indicates that the 1$\varkappa$, 4$\varkappa$ configuration is the more stable. Here also the internal energy of the 1$\varkappa$, 4$\varkappa$ configuration should in general be expected to be smaller than that of the $\varkappa$, ε configuration.

So far we have omitted from our discussion the question of hydrogen (hydroxylic) bond formation. It must be admitted that such phenomena may render the use of a concept like that of van der Waals radii rather illusory. For example, the identity period of 2.5 Å only, reported for polyvinylalcohol seems to indicate that such bonds may bring the two oxygen atoms at a considerably smaller mutual distance than the distance permitted by the van

der Waals radii (about 2.9 Å). If this is so, there should be nothing to prevent the hydroxyl groups in cyclohexanediol-1,3 from having the ε,ε configuration. In the case of 1,2 hydroxyl groups, however, it seems less likely that hydrogen bonds should be formed (between 1 $\varkappa$, 2 $\varkappa$ and 1 $\varkappa$, 2 ε oxygens) because this should give C—O . . . O angles of about 60°.

Before discussing further structures containing alcoholic groups we should like to mention the studies of cyclohexane derivatives containing more than two halogen atoms. In these cases it seems reasonable to assume that configurations containing two halogen atoms in positions 1 ε, 3 ε will be relatively unstable because of the short distance of 2.52 Å between the atoms, if tetrahedral angles are conserved. We do not think, however, that molecules containing 1 ε, 3 ε halogen atoms are strictly forbidden, though in such cases considerable deformations of valency angles must be expected.

Turning to the benzene hexachlorides this would mean that more than four stereoisomeric forms may be expected. The possible configurations beeing:

Table 1. A survey of the eight possible isomers of benzenehexachloride. 1 ε — 3 ε = the number of 1 ε — 3 ε pairs of chlorine atoms. III and VI are optically active, conversion of the carbon ring, however, transposes the d-form of VI into the l-form and vice versa. The table presented by Kauer, DuVall and Alquist [24] shows that these authors are not familiar with the newer theory of cyclohexane isomerism [2].

	1 ε — 3 ε				1 ε — 3 ε	Dipole moment
I	0	$\varkappa,\varkappa,\varkappa,\varkappa,\varkappa,\varkappa$	$\rightleftharpoons$	$\varepsilon,\varepsilon,\varepsilon,\varepsilon,\varepsilon,\varepsilon,$	6	zero
II	0	$\varepsilon,\varkappa,\varkappa,\varkappa,\varkappa,\varkappa$	$\rightleftharpoons$	$\varkappa,\varepsilon,\varepsilon,\varepsilon,\varepsilon,\varepsilon$	4	
III	0	$\varepsilon,\varepsilon,\varkappa,\varkappa,\varkappa,\varkappa$	$\rightleftharpoons$	$\varkappa,\varkappa,\varepsilon,\varepsilon,\varepsilon,\varepsilon$	2	
IV	0	$\varepsilon,\varkappa,\varkappa,\varepsilon,\varkappa,\varkappa$	$\rightleftharpoons$	$\varkappa,\varepsilon,\varepsilon,\varkappa,\varepsilon,\varepsilon$	2	zero
V	1	$\varepsilon,\varkappa,\varepsilon,\varkappa,\varkappa,\varkappa$	$\rightleftharpoons$	$\varkappa,\varepsilon,\varkappa,\varepsilon,\varepsilon,\varepsilon$	3	
VI	1	$\varepsilon,\varepsilon,\varkappa,\varepsilon,\varkappa,\varkappa$				
VII	3	$\varepsilon,\varkappa,\varepsilon,\varkappa,\varepsilon,\varkappa$				
VIII	1	$\varepsilon,\varepsilon,\varepsilon,\varkappa,\varkappa,\varkappa$				

Four different substances obtained by addition of chlorine to benzene have been known for a long time. One of these, the so-called β-benzenehexachloride, has been shown to have the structure I. Dipole measurements carried out in this laboratory has shown that of these four substances the β-compound only has zero dipole moment. This indicates that the structure IV does not correspond to any of the modifications α, γ or δ. It is interesting that one of the new hexachlorocyclohexanes (having the m. p. 145°) prepared in this laboratory by chlorination of cyclohexane or chlorocyclohexane has dipole moment zero. This can hardly be explained otherwise than by assuming this new

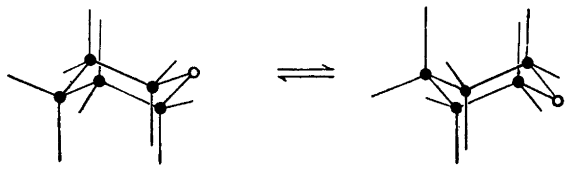

Fig. 2. The stepformed pyranose ring.

substance to be a benzenehexachloride corresponding to structure IV. It seems clear, however, when considering the electron diffraction curves of the α, β, γ and δ compounds, that slight deviations from tetrahedral angles occur, resulting in a flattening of the six-membered ring. This, of course, does not interfere with the designation of the individual bonds which we are using.

The only halogen derivatives containing four substituends so far thoroughly studied is the 1,2,4,5 tetrabromocyclohexane (m. p. 185°)[16] which was found to have the configuration 1 $\varkappa$, 2 $\varkappa$, 4 ε, 5 ε. This substance contains optically active molecules like the 1 $\varkappa$, 2 ε dihalogenides mentioned above, but here a conversion of the ring transforms d-molecules into d-molecules and vice versa, and a separation of the active forms is possible at least in principle.

THE PYRANOSE RING

All experimental evidence indicates that the six-membered pyranose ring found in many sugars will generally have the staggered form corresponding to the symmetrical cyclohexane ring discussed above. The discussion of these sugars, which were originally based on a plane ring, will therefore have to be revised to some degree. In addition to the well known configuration proofs of E. Fischer, the configuration at the first C-atom corresponding to the α and β forms respectively has been settled by the following convention: The compound, in which the two OH-groups linked to the C-atoms 1 and 2 are in *cis* position, is called the α form; the other compound in which these two groups occupy *trans* positions is the β form. Remembering what has been stated above concerning 1,2 disubstituted cyclohexanes, it is clear that the β compound may correspond to both of the configurations 1 $\varkappa$, 2 $\varkappa$ and 1 ε, 2 ε. Which of these two configurations predominates in the equilibrium mixture will depend very much on the positions of the three other substituends attached to the pyranose ring. In the α form the OH groups will have either the configuration 1 $\varkappa$, 2 ε or 1 ε, 2 $\varkappa$. From these considerations it will be clear that each pyranose sugar will, at least theoretically, be able to occur in two different configurations. It will hardly ever be possible to separate the two forms

Table 2. The possible structures of the normal hexoses.

C-atom no.	1	2	3	4	5	⇌	1	2	3	4	5
Substituend	OH	OH	OH	OH	CH_2OH		OH	OH	OH	OH	CH_2OH
α — *d*-allose	ε	ϰ	ε	ϰ	ϰ		ϰ	ε	ϰ	ε	ε
β —»—	ϰ		—»—				ε		—»—		
α — *d*-altrose	ϰ	ε	ε	ϰ	ϰ		ε	ϰ	ϰ	ε	ε
β — —»—	ε		—»—				ϰ		—»—		
α — *d*-glucose	ε	ϰ	ϰ	ϰ	ϰ		ϰ	ε	ε	ε	ε
β — —»—	ϰ		—»—				ε		—»—		
α — *d*-mannose	ϰ	ε	ϰ	ϰ	ϰ		ε	ϰ	ε	ε	ε
β — —»—	ε		—»—				ϰ		—»—		
α — *d*-gulose	ε	ϰ	ε	ε	ϰ		ϰ	ε	ϰ	ϰ	ε
β — —»—	ϰ		—»—				ε		—»—		
α — *d*-idose	ϰ	ε	ε	ε	ϰ		ε	ϰ	ϰ	ϰ	ε
β — —»—	ε		—»—				ϰ		—»—		
α — *d*-galaktose	ε	ϰ	ϰ	ε	ϰ		ϰ	ε	ε	ϰ	ε
β —»—	ϰ		—»—				ε		—»—		
α — *d*-talose	ϰ	ε	ϰ	ε	ϰ		ε	ϰ	ε	ϰ	ε
β —»—	ε		—»—				ϰ		—»—		

because of the great velocity of conversion. Chemically one must expect the mixture to behave like a single compound, but it is evident that the conditions just mentioned may be of fundamental importance to the chemical reactivity and physical behaviour of the pyranose sugars.

Anomal optical activity in respect to the rules of Hudson may find an explanation in the different rotatory power of the two equilibrium forms of the pyranose sugars.

Table 2 shows the theoretically possible structures of the normal pyranose sugars. Before discussing which of the possible structures of Table 2 are realised, we will consider the behaviour of the step-formed six-membered ring in more detail.

During the transposing one or more of the angles of this ring must increase from 109.5° up to 120°. The structures of 1,2-epoxy-cyclohexane [17] and the benzenehexachlorides show that the substituends may cause a more or less pronounced deformation of the tetrahedral angles in the six-membered ring. Between the atoms of the molecule forces of different types and strength are acting. Some of them produce a stabilising effect on the stepform of the six-membered ring, others tend to distort the tetrahedral angles. A quantitative

evaluation of all these forces is not possible, but experience from precision determinations of related molecular structures allows us to make the following general statements about these forces, in an empirical way [18]:

1. Deformation of the valency angles generally does not alter the length of the chemical bonds in question.

2. The four atoms attached to the same C-atom repel each other (compare methyl halides etc.).

3. The atoms attached to either of two C-atoms which are in turn linked to each other with a single bond will generally tend to occupy *trans*-positions (compare ethane and derivatives).

When we transfer these experiences to six-membered rings we must, of course, also take into consideration the forces between substituends linked to C-atoms further apart. Generally, only substituends in ε position on the same side of the six-membered ring need to be considered.

In the molecules all the forces producing these effects balance each other and the molecule oscillates about an equilibrium configuration. In the interferometric molecular structure determinations this equilibrium configuration only is observed. Deformation of the valency angles by introduction of new substituends means a shift of the equilibrium configuration. In the case of six-membered rings the oscillations are of special importance. Without any definite statements about the nature of the oscillations we must assume that they are able to cause considerable temporary deformations of the valency angles in the six-membered ring. Angular deformations corresponding to those, which when sufficiently great, cause the ring to be converted, should be more probable than deformations taking place in the opposite direction. In other words: The potential energy of the molecule taken as a function of the valency angles should be expected to display two minima corresponding to the equilibrium configurations in the two possible transposition forms of the six-membered ring. We must expect the potential curve to rise less steeply on the side where the conversion takes place. The energy difference between the two minima and the height of the barrier will of course depend on the nature of the substituends present.

It should be kept in mind that a beginning conversion will necessarily increase the mean value of the valency angles in the six-membered ring. It will be shown later that the picture just given makes it possible to explain certain chemical reactions taking place in molecules containing six-membered rings. Some caution is needed, however, when discussing the stability of local atomic arrangements in the molecule: An arrangement which is favourable in a certain molecule may be unfavourable in other molecules, due to the presence of other substituends within the molecule.

The structure of cyclohexanediol-1,2 [11] indicate that the angle O—C—C is somewhat larger than the tetrahedral angle (about 112.5°). This is probably a manifestation of the effect mentioned under 2. If this deformation of the angle O—C—C is taken into consideration the following distances between the O-atoms in the hydroxyl groups are obtained:

$O_1\varkappa - O_2\varkappa$	2.85 Å		
$O_1\varkappa - O_2\varepsilon$	2.96 »	$\angle$ C — $O_1\varkappa$	$O_2\varkappa \sim 60°$
$O_1\varepsilon - O_2\varepsilon$	3.73 »	$\angle$ C — $O_1\varkappa$	$O_2\varepsilon \sim 60°$
$O_1\varepsilon - O_3\varepsilon$	2.78 »	$\angle$ C — $O_1\varepsilon$	$O_3\varepsilon \sim 84°$

Remembering what has been said about the cyclohexanediols we think it probable that OH-groups linked to neighbouring C-atoms may occupy any combination of $\varkappa$ and ε positions in the pyranose ring and we even think it probable that the configuration $(OH)_{1\varepsilon}$—$(OH)_{3\varepsilon}$ is possible. Although allose is the only hexose based on a pyranose ring, which must necessarily contain 1 ε, 3 ε OH groups its existense is sufficient to demonstrate the possibility of such an arrangement, if we suppose that the ring has retained the staggered form. In pentoses containing pyranose rings it thus seems little reason to reject any one of the transposition forms. The relative stability of the configurations 1 $\varkappa$, 3 $\varkappa$ and 1 ε, 3 ε of the hydroxyl groups might be determined by examining the structure of the *cis* 1,3-cyclohexanediol or the corresponding 1,3,5-cyclohexanetriol.

In the normal hexoses it seems reasonable to assume that configurations placing both the CH_2OH group and one OH group in ε positions on the same side of the ring are energetically unfavourable. If this argument holds, it settles the energetically preferred configuration of the following aldopyranoses:

β-allose, α-altrose, α- and β-glucose, α- and β-mannose, β-gulose, α-idose, α- and β-galactose, α- and β-talose. The configurations are printed in heavy types in Table 2. In the case of α-allose, β-altrose, α-gulose and β-idose this argument is without consequence.

In a few cases only experimental evidences are at hand, but then confirming the view just presented; compare x-ray analyses of substances like cellulose, sucrose and glucosamin.

POLYSACCHARIDES AND OTHER POLYMERS

In certain cases the determination of fiber-periods of polysaccharides may give valuable information concerning the configuration of the six-membered rings. If the chain is formed by 1,4-bonds the possible fiber periods are easily recognised: Each molecule may contribute with two $\varkappa$-bonds, two ε-bonds or

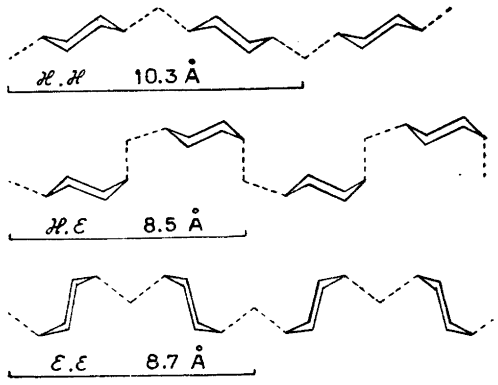

Fig. 3. Polysaccharide chains with 1,4-glycosidal linkages.

one $\varkappa$-and one ε-bond. In the first case the computed fiber-period is about 10.3 Å and in the two other cases nearly identical and about 8.7 Å (the identity period comprises two rings). Well known examples of the 10.3 Å period are those of cellulose and chitin [19].

Astbury [20] has made a x-ray investigation of alginic acid. He finds an identity period of 8.7 Å and favours the view that the alginic acid contains units of β-mannuronic acid molecules linked together in a way corresponding to the use of ε-bonds only (Fig. 3). The structure of β-mannuronic acid, however, excludes such a possibility. If the alginic acid is built up from β-mannuronic acid units the only alternative is a structure in which each mannuronic acid unit makes use of one $\varkappa$- and one ε-bond. It may easily be shown, however, that an identity period of about 8.7 Å may be obtained in this way also (Fig. 3). If 1 ε, 4 ε linkages are present in alginic acid, the chain unit must be α-mannuronic acid and not the β-acid. The chemical evidences, however, seem to indicate that the units are β-mannuronic acid rather than α-mannuronic acid.

Astbury, without knowledge of our work from 1943 [2], points out that the alginic acid may change through a conversion of the six-membered ring into a structure with an identity period corresponding to that of cellulose. It is easily recognised, however, that a transposing of the six-membered ring will not alter the identity period if the units are β-mannuronic acid. In cellulose, however, a conversion of the ring will necessarily change the period, but it is difficult to foresay if this will be possible to carry out in practice. Probably a conversion will take place only by chemical changes in the substituends at the six-membered ring. The x-ray investigations of cellulose esters show that in these cases also the 1 $\varkappa$, 4 $\varkappa$ configuration is the more stable.

In polymers containing open carbon chains fiber periods of 2.5 Å or simple multipla of this distance have often been reported. In polymethylen chains

this is very easy to understand because it places H-atoms of adjacent methylene groups into *trans*-position and the 1.3 H-atoms at a distance of 2.5 Å. In the case of polyvinyl alcohol the short period of 2.5 Å is more surprising because a O—O-distance of 2.5 Å only seems somewhat small even when the possibility of hydrogen bond formation is taken into consideration. The period of about 5 Å in the case of polyvinyl chloride seems very probable considering the experiences so far obtained concerning van der Waals forces. The question in which way the different chains are linked together in the crystalline part of this chloride is much more involved. In each case the explanation given by Mark [21] assuming a real hydrogen bond formation between a methylene H-atom of one chain and a chlorine atom of a neighbouring chain has to be modified.

ORTHOESTERS OF SUGARS CONTAINING PYRANOSE RINGS

A very interesting survey of the recent advances in this field has been given by Pacsu [22]. Experience has shown that under normal conditions only the α- or only the β-form of the acetylglycosyl halides in the d and l series are formed. Of these only one in each epimeric pair is able to give orthoesters by the reaction of Koenigs-Knorr. This has been experimentally verified in three of the four epimeric pairs. The studies of this reaction by Isbell and Frush [23], were based on the modern conceptions of substitution reactions. On the basis of a plane six-membered ring they are able to give an explanation of the reaction. The normal Koenigs-Knorr reaction is a substitution reaction of the SN1 type, usually accompanied by a complete inversion at C-atom no. 1. When the acetyl glycosyl halide (Fig. 4, I) is heated with dry silver carbonate in anhydrous methyl alcohol at room temperature, usually the halogen atom is substituted by a metoxyl-group. If the inversion is incomplete a mixture of α- and β-glycosides is formed.

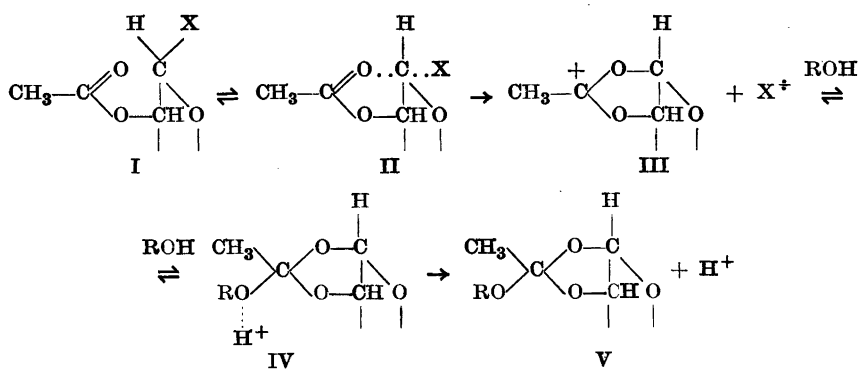

Fig. 4. Formation of the orthoester by the reaction of Koenigs-Knorr.

When, however, the acetoxyl group at C-atom no. 2 is situated on the side of the ring opposite to the halogen atom at C_1, the carbonyl oxygen of the acetyl group may take part in a substitution reaction at the SN2 type. This results in the formation of a intermediate carbonium ion (Fig. 4. III), which in turn may react with the solvent molecule under formation of a possible mixture of two diastereoisomeric ortoacetates (Fig. 4, V). Accordingly in this case the Konigs-Knorr reactions may result in a mixture of normal α- and β-glycosides and two diastereoisomeric ortoesters.

The investigations of these reactions have stated that orthoesters are only formed when the substituends in 1,2-positions are *trans*-situated, in the case of *cis*-position no orthoester is formed.

Taking into consideration that the six-membered ring is step-formed, the explanation of Isbell and Frush is not quite so evident. In an undeformed, step-formed ring (all angles beeing tetrahedral) the distances between two substituends in 1 $\varkappa$, 2 $\varkappa$ and the same substituends in 1 $\varkappa$, 2 ε positions are identical. If however, in the acetyl glycosyl halides the carboxylic oxygen in the acetyl group at C-atom no. 2 shall be able to substitute the chlorine atom at C atom no. 1 according to an SN2 reaction scheme with Walden inversion, the carboxylic oxygen must be brought into a favourable position by deformation of the six-membered ring. In the case of the 1 $\varkappa$, 2 $\varkappa \rightleftharpoons$ 1 ε, 2 ε compound this deformation is consistent with a beginning conversion of the six-membered ring. In the case of an 1 $\varkappa$, 2 $\varepsilon \rightleftharpoons$ 1 ε, 2 $\varkappa$ compound the necessary deformation would mean a decrease of the valency-angles in the six-membered ring. The movement is thus opposite to that leading to a conversion of the ring. Accordingly the necessary activation energy of the deformation will be smaller in the case of an 1 $\varkappa$, 2 $\varkappa$ compound. Accepting this view it is reasonable to assume that only the 1 $\varkappa$, 2 $\varkappa \rightleftharpoons$ 1 ε, 2 ε acetyl glycosyl halides (*trans*-forms) are able to give ortho-esters.

The assumption of stepformed six-membered rings or plane rings thus leads to the same conclusion concerning the ability of the halogenoses in forming orthoesters (Fig. 4, V). The new point of view involves the readiness with which the ring is deformed in the process of conversion, thus bringing the carboxylic oxygen atom in a position favourable to the reaction in question. It also appears reasonable that a beginning conversion of the ring facilitates the inversion at the C_1-atom, because it increases the angle O—C_1—C_2. Such an increase is necessary if the change of configuration at the C_1-atom shall take place.

THE ACETYL GLYCOSYL HALIDES

Pacsu points out that under normal conditions only one of the acetyl glycosyl halides in the hexose series, either the α or the β-form, is stable. In

many cases the unstable form may be obtained by taking special precautions, but it will always tend to mutarotate and give the stable form. Experience has further shown that in the cases where the normal stable halogenoses do not give ortho-esters by the Koenigs-Knorr reaction, orthoesters are obtained from the corresponding unstable forms. This has been used in order to determine the structure of the halogenoses. With regard to the stability of the halogenoses Pacsu points out that a satisfactory explanation of the experimental facts is still lacking. The experiences from the cyclohexane derivatives, however, draws the attention to the possibility of steric hindrance caused by substituends in ε-position on the same side of the six-membered ring.

The stable acetyl glycosyl halides are usually formed by the action of hydrogen halides on the completely acetylated sugars. This substitution of a »negative» group caused by a negative ion is normally accompanied by a Walden inversion. The transformation of the unstable form of the halogenoses into the stable form may also be interpreted as a substitution reaction with Walden's inversion.

To start the reaction the nucleophilic Cl^-ion must come in a favourable position to the C_1-atom. This is the case if the molecule has the form shown in Fig. 5 a, with the H_1-atom in $\varkappa$ position. In the transition state (5 b) the

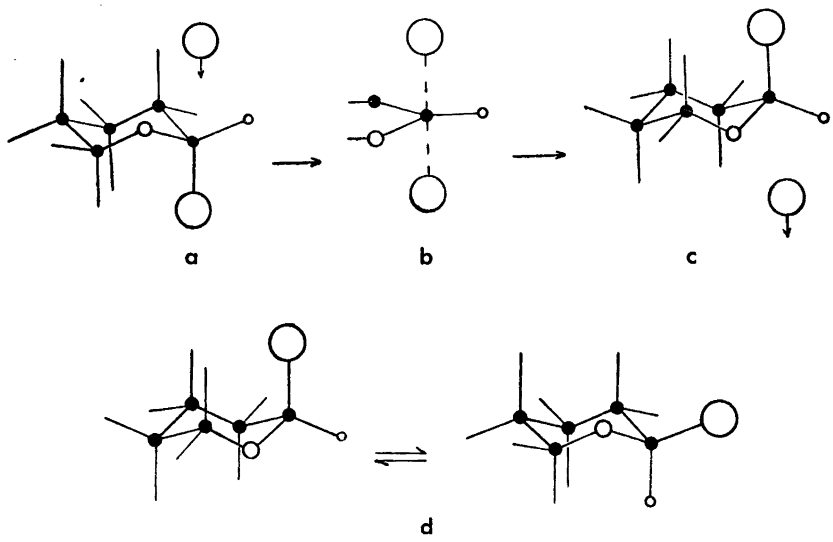

Fig. 5. Substitution reaction with Walden inversion at C-atom no. 1 of the stepformed pyranose ring.

atoms H_1, C_2 and the ring-oxygen must be brought into a plane with the central atom C_1. This means a deformation of the six-membered ring which must nearly correspond to the transition state between the two possible forms of the six-membered ring. When the inversion is completed and the ring has retained the staggered from, it must have passed through a conversion, in order to give sufficient space for the escaping ion. The important result of this picture is that the new substituend in the first instance takes an ε-position in the new-formed molecule (5 c).

In the case of mutarotation the old and the new substituends are of the same sort. If one of the two possible endconfigurations with the new substituend in ε-position is energetically unfavourable, this will be the »unstable» one. If the old and the new substituend are different the formation of the unstable modification may be possible if the bond energy and space requirement of the two substituends favours this. If an unstable modification is produced in the 1 ε form, it will immediately transpose into the 1 $\varkappa$ form (5 d).

If the halogen atom in the unstable halogenoses shall take part in a new substitution reaction, it must first occupy the ε-position. Accordingly we may expect the activation energy of the so-called unstable modifications to be rather high if substitution reactions involving the C_1-atom are considered.

THE HALOGENOSES OF THE HEXOSE, PENTOSE AND HEPTOSE SERIES

In the acetylated sugars of the hexose series the CH_2-link of the CH_2OAc group at carbon atom 5 requires more space than the other substituends. Accordingly all the normal »stable» forms of the corresponding halogenoses have the configuration:

$$Cl_{1\varkappa},\ CH_2OA_{\ 5\varepsilon} \rightleftharpoons Cl_{1\varepsilon},\ CH_2OAc_{\ 5\varkappa}$$

The unstable forms have the configuration:

$$Cl_{1\varkappa},\ CH_2OAc_{\ 5\varkappa} \rightleftharpoons Cl_{1\varepsilon},\ CH_2OAc_{\ 5\varepsilon}$$

Since the formation of the unstable forms will usually pass through the energetically unfavourable 1 ε, 5 ε configuration these are not produced under normal conditions. Under special circumstances the unstable form may be obtained, for instance in the case of an SN1 reaction with an unshielded carbonium ion or in cases where the nature of the old and new substituends are sufficiently different.

Such a case is that of the β-acetyl glucosyl halides which in confirmity with the view just developed show little reactivity.

In the acetyl glycosyl halides of the pentose series based on a pyranose ring there is no steric hindrance at C-atom 5. In this case according to our theory the acetyl group at C-atom 3 must play the dominant role. Experimental evidences confirm this view. All the stable halogenoses of the pentose series have the configuration:

$$Cl_{1\varkappa},\ OAc_{3\varepsilon} \rightleftharpoons Cl_{1\varepsilon},\ OAc_{3\varkappa}$$

The unstable forms will have the configuration:

$$Cl_{1\varkappa},\ OAc_{3\varkappa} \rightleftharpoons Cl_{1\varepsilon},\ OAc_{3\varepsilon}$$

In the heptoses the stable forms of the halogenoses should correspond to the stable halogenoses of the hexose series, which have the same configuration at the pyranose ring.

We are of course aware of the complexity of the problems related to the rather complicated molecules which we have been discussing; so far our knowledge of the deformations from tetrahedral angles (which no doubt occur) is too limited. We hope, however, that the general ideas developed here may serve as a basis for further work dealing with the structure and chemical reactions of molecules containing the pyranose ring.

SUMMARY

The molecular structure of a series of organic substances containing single bonds only is discussed, and the importance of considering the interaction between atoms which are not directly linked together is pointed out. Starting with results previously obtained in the case of halogenated ethanes and some ring systems, chiefly sixmembered rings, the structure of cyclohexane derivatives and substances containing the pyranose ring are discussed in some detail. In the latter case the mechanism of chemical reactions not opening the sixmembered ring has been dealt with, the special conditions arising from the non-planarity of the pyranose ring being taken into consideration.

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Investigations in Serum Copper

I. Nature of Serum Copper and its Relation to the Iron-binding Protein in Human Serum

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Since the finding of Warburg and Krebs [1] that blood serum has a fairly constant copper content of about 100 γ per 100 ml, the nature and functions of this serum copper have been subjects of many investigations. Our knowledge about serum copper is however still very incomplete.

Already Krebs [2] has shown that in man there is a physiological rise in serum copper during pregnancy; and mainly through the work of Heilmeyer and collaborators [3] it has been shown that also infections are associated with a rise. The latter pointed out that serum copper often increases under conditions which lead to a decrease in serum iron.

Abderhalden and Möller [4] have shown that serum copper is non-dialyzable. Warburg and Krebs [1], Locke *et al.*[5] and Boyden and Potter [6] have shown that the binding of serum copper is broken by lowering pH.

When serum proteins are precipitated with trichloroacetic acid the copper goes quantitatively with the filtrate.

Eisler, Rosdahl and Theorell [7] have studied the behavior of serum copper during electrophoresis and conclude from their experiments that it is bound to albumin.

Mann and Keilin [8] prepared from blood corpuscles a crystalline copper protein with a copper content of about 0.34 % which they named haemocuprein. A similar compound was also prepared from horse serum. Haemocuprein is a blue protein with a molecular weight of about 35,000. Cohn *et al.*[9] report that crystalline haemocuprein is precipitated from horse serum by increasing the ammonium sulphate concentration from 0.62 to 0.68 saturation.

Cohn *et al.*[10] found that the iron combining β_1-globulin in serum also combines with copper and that this globulin is responsible for the transport of

both iron and copper, perhaps of zinc too. They refer to the old finding of Heilmeyer regarding the interrelation of iron and copper in serum, and their idea seems to be that this interrelation should be explained as a competition between the two metals for the transport protein.

Holmberg [11] has shown that the assumption of Eisler, Rosdahl and Theorell that copper is bound to albumin cannot be quite correct. Part of the serum copper was found by him in Green's P_1-globulin.

The function of serum copper is still obscure. Probably at least a part of it is to be regarded as transport copper. Mann and Keilin were not able to show any catalytic activity of haemocuprein. When this compound is reduced by hydrosulphite, the blue color disappears and does not reappear when the solution is oxidized by shaking. Holmberg, however, found that the blue color of the copper-containing P_1-globulin, which also disappears on reduction, reappears when the solution is left in contact with the air. He also found that this copper protein catalyzes the oxidation of paraphenylendiamine.

EXPERIMENTS WITH SERUM AIMING TO EXPLAIN THE RELATION BETWEEN SERUM IRON AND COPPER

When, about three years ago, in this laboratory it was found that serum had a latent iron binding capacity of a fixed value, our interest in the finding of Heilmeyer [3] that a decrease in serum iron often runs parallel with an increase in serum copper was at once awakened. The hypothesis which immediately suggested itself to us was that, when the iron binding capacity remained unchanged, the variations in serum iron could be explained by assuming that copper and iron competed for the same protein.

This hypothesis, which has now been taken up by Cohn [10] was however proved to be erroreous by the following experiments.

1. a) *When an excess of copper was added to serum this did not effect the iron binding capacity of the serum.*

b) *If the iron binding capacity was saturated, addition of copper did not result in a breaking up of the iron complex.*

These experiments show that if copper enters into combination with the same protein as iron, the affinity of this protein for iron must be greater than the affinity for copper.

2. Experiments were made with addition of sodium diethyldithiocarbamate to serum (normal serum and pregnancy serum with high copper content).

a) By photometric analysis it could be shown that the serum copper in no instance reacted with the sodium diethyldithiocarbamate at physiological and slightly alkaline pH.

b) When small amounts of copper as copper sulphate was added to serum, it could be shown that this copper reacted quantitatively with sodium diethyldithiocarbamate. These results were independent of the primary concentration of copper in the serum employed. Even serum from newborns with a very low copper content was unable to bind any copper added, so that it did not react with sodium diethyldithiocarbamate.

c) Addition of excess iron could not expel copper from firm binding in serum so that it could react with sodium diethyldithiocarbamate.

These experiments prove that *the capacity of serum to bind copper contrary to its iron binding capacity is in all instances used up to a hundred per cent;* they also prove that *iron has no stronger affinity than copper for the copper binding protein* (it is to be emphasized that iron was allowed to react with serum for a considerable length of time).

From the experiments here reported we concluded that *the hypothesis about iron and copper competing for the same protein in serum cannot be correct.*

EXPERIMENTS CONCERNING THE NATURE OF SERUM COPPER

Up to present but very little has been published about the behavior of the copper protein in serum on salt precipitation. As pointed out already Cohn et al. state that haemocuprein in horse serum is precipitated between 0.62—0.68 ammonium sulphate saturation.

Wishing to see if the additional copper which appears in serum during pregnancy and infections behaves in the same way as the normal serum copper, we have performed some salting out experiments on different types of human sera.

For these experiments we used ammonium sulphate which had been freed from copper by treatment with sodium diethyldithiocarbamate. Each serum, diluted with an equal amount of glass-distilled water, was precipitated with an equal amount of neutralized saturated ammonium sulphate. The precipitates were washed twice with half-saturated ammonium sulphate. Copper determinations, after wet combustion, were performed on serum and globulin precipitate. In most cases double determinations were made.

The results of these experiments are recorded in Table 1, from which *it is evident that most of the copper in serum is precipitated by 50 % saturation with neutralized ammonium sulphate.* It is also evident *that the increase in serum copper during pregnancy and infections is due to an increase in a fraction which can be precipitated with 50 % ammonium sulphate saturation* (globulin copper). As a matter of fact, the amount of copper which cannot be precipitated under these conditions in normal sera is less than 10 %. It is quite possible that it

Table 1. Amount of copper precipitable by 50 % saturation with ammonium sulphate at different serum copper concentrations.

Serum number	Total Cu γ/100 ml	Globulin Cu γ/100 ml	Albumin Cu γ/100 ml
1.	157	138	19
2.	119	109	10
3.	109	112	—3
4.	94	84	10
5.	116	109	7
6.	282	256	26
7.	328	288	40
8.	288	214	74
9.	258	214	44
10.	300	242	58
11.	201	168	33
12.	226	173	53
13.	222	178	44

1— 5. Normal sera.
6—10. Pregnancy sera.
11—13. Infection sera.

might be attached to a globulin which cannot be completely separated from the albumin in this way. The proportional increase in albumin copper with increase in total copper supports this view. In this connection *it should be pointed out that the iron binding component in plasma is not precipitated by 50 % ammonium sulphate.*

Luetscher [12] and Pedersen [13] have described a blue globulin which was found in a fairly high concentration in pig serum, probably identical with the one described by Holmberg (1944) in human serum. Later Cohn *et al.* have found the same globulin, which they describe as an α-globulin. In this laboratory we have purified this blue globuline by repeated precipitations at different pH's and different alcohol concentrations, and the purified preparations we found to have a copper content of about 0.03 %. More recently we found that if this globulin is treated with alcohol and chloroform in the same concentrations as were used by Mann and Keilin in the preparation of haemocuprein, the main part of the protein is rendered insoluble and settles as a bulky colorless precipitate. The supernatant fluid is a clear blue solution with a very small protein content. By fractional precipitation with ammonium sulphate it was

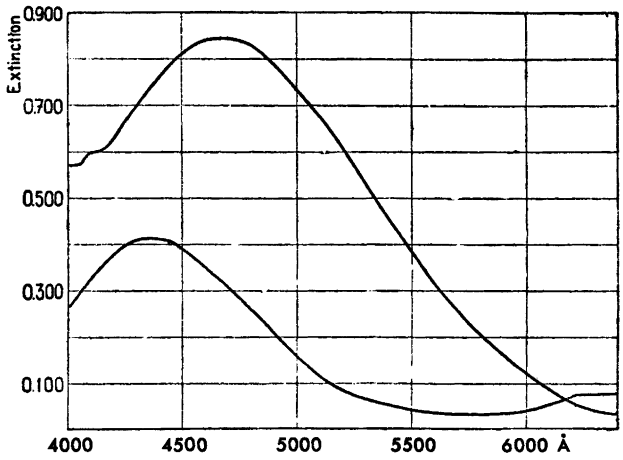

Fig. 1. Absorption curves for iron and copper complexes with metal combining protein. The curves have been obtained by reading a solution of metal free protein against a solution of protein saturated with metal at pH 7.5. Upper curve iron and lower curve copper. Binding of 100 γ % iron corresponds to an increase in extinction of 0.0435 at 4600—4700 Å (1 cm layer). For copper the corresponding extinction at 4300—4400 Å is 0.0202.

possible from this solution to prepare a copper protein with a copper content of 0.35 % (protein determination after Kjeldahl method). This protein is not precipitated by half saturation with ammonium sulphate, but comes down when the concentration is increased from 50 to 65 % saturation, and might be identical with the haemocuprein of Mann and Keilin.

On correlating these results with our finding that most of the serum copper is precipitated by 50 % ammonium sulphate, *it seems reasonable to assume that at least the main part of the serum copper is to be found in the α-globulin and that very little or no free haemocuprein exists in native serum.*

EXPERIMENTS WITH THE PURIFIED IRON-COMBINING PROTEIN

Using the pure iron-combining protein prepared by Laurell [14] we have been able to confirm the finding of Cohn *et al.* that this protein combines with copper. It gives a yellow complex which shows a broad absorption maximum at 4400 Å. A given amount of protein can bind an amount of copper equivalent to the amount of ferrous iron that can be taken up. Fig. 1 shows the absorption curves for the copper and iron complexes of this protein. The curves have been plotted by reading solutions of the metal-saturated protein against a solution of protein which has been freed from metals.

If a solution of this protein is saturated with copper, and iron then is added, this iron will quantitatively expel the copper, and an iron complex is formed, which can be shown by spectrographical analysis. If, on the other hand, the protein is first saturated with iron, and copper is then added, nothing happens. We have also tried if zinc interferes with the formation of copper and iron complexes by this protein. This is not the case. It seems therefore clear that the affinity of the protein for iron is greater than its affinity for copper, and that its affinity for both iron and copper is greater than for zinc. All these experiments were carried out at pH 7.5.

Even if the protein is only half-saturated with copper and sodium diethyldithiocarbamate is added the copper complex is broken up, and the copper combines rapidly with diethyldithiocarbamate.

These facts as well as the finding in the serum experiments go against the view of Cohn *et al.* that this protein should serve as a carrier of copper and zinc in serum.

If copper is added to serum with a high iron-binding capacity and a low iron content, it should be possible to show an increase in light absorption at 4400 Å. If Cohn's idea about copper being taken up by the same protein as iron were correct, the amount of this increase in absorption could be calculable from the experiments on the pure protein.

Such experiments were made, but no increased light absorption was found. The most probable explanation of this fact is that the copper complex with the iron-binding protein at the pH of serum is dissociated in at least the same degree as the complexes formed between copper and other serum proteins, and that therefore only a very small amount of the added copper forms a complex with the iron binding protein in serum. If this holds true for copper, it is evident from the competitional experiments with zinc and copper that it also applies to zinc.

The absorption at 4400 Å of a complex between iron combining protein and copper disappears upon addition of serum. This proves that other serum proteins have a greater affinity for copper than has the iron-binding protein.

SUMMARY

It has been shown that at least 90 % if not all of the serum copper is to be found in the globulin fraction (precipitated by 50 % saturation with ammonium sulphate) and that this also applies to the increase in copper during pregnancy and infections. It is probable that the haemocuprein of Mann and Keilin forms an integrating part of the blue α-globulin. The idea of Cohn *et al.* that the variations in serum iron and serum copper can be explained

by assuming the two metals to compete for the same protein is not tenable. Even though this protein combines with copper in vitro, it can be shown that in serum it is not combined with copper to any appreciable extent. The same must hold true for zinc.

In view of these facts we suggest that the new metal-combining protein (iron-binding component) in serum be called transferrin.

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