ABSTRACTS OF PAPERS PUBLISHED IN OTHER JOURNALS

CHEMISTRY

ANALYTICAL

Ascorbic Acid, Determination of. G. di Bacco. (*Boll. chim.-farm.*, 1953, **92**, 115.) The usual method of determining ascorbic acid with iodine cannot be used in the presence of vitamin B_1 and the author recommends the reduction of picric acid to picramic acid in alkaline solution and estimating the amount of the latter colorimetrically. 1 g. of ascorbic acid will reduce 0·43 g. of picric acid to produce 0·3768 g. of picramic acid. The reagents required are:—a 1 in 1000 solution of picric acid, a 2 per cent. solution of sodium hydroxide, and a standard solution of 0·235 g. of picramic acid in 400 ml. of hot water with 25 ml. of sodium hydroxide solution, cooled and made up to 1 l. This solution keeps well in dark glass bottles. Weigh exactly about 50 mg. of ascorbic acid and dissolve in sufficient water to make 50 ml. Place 5 ml. of this solution, 3 ml. of picric acid solution and 2 ml. of sodium hydroxide solution in a 10 ml. graduated flask. Place 8 ml. of picramic acid solution, 1·8 ml. of sodium hydroxide solution and 0·2 ml. of water in another 10 ml. flask. Heat the two flasks for exactly 3 minutes in a boiling water bath, cool in running water, adjust to 10 ml. and compare the two solutions in a colorimeter. 5 mg. of ascorbic acid produce 1·884 mg. of picramic acid, which is the quantity contained in the 8 ml. of standard solution used. When testing solutions of ascorbic acid they should be suitably diluted; the presence of sulphites or bisulphites does not interfere. For tablets, a number corresponding to about 100 mg. is powdered in a mortar with 30 ml. of a 2 per cent. solution of metaphosphoric acid in boiled and cooled distilled water. After a minute the solution is decanted, the residues treated with another 30 ml. of acid solution, and then with distilled water and the united solutions neutralised carefully and made up to 100 ml.; 5 ml. are used for the estimation. If lactose or glucose are present the method is not applicable. If vitamin B_1 is present this can be precipitated with picric acid. For example, with a solution containing 50 mg. of ascorbic acid and 5 mg. of aneurine per ml., 1 ml. is mixed with 5 ml. of saturated picric acid solution, allowed to stand for 10 minutes and then centrifuged for 10 minutes. 3 ml. of the clear liquid is diluted to 25 ml., 5 ml. of this solution, 0·4 ml. of distilled water, 2·60 ml. of picric acid solution and 2 ml. of sodium hydroxide solution are mixed and treated as for pure ascorbic acid. H. D.

Digitalis, Chemical Assay of. D. H. E. Tattje and F. H. L. van Os. (*Pharm. Weekbl.*, 1953, **88**, 237.) By determinations carried out before and after enzymatic hydrolysis of the glycosides, it is possible to determine separately the primary glycosides, secondary glycosides and aglycones in digitalis leaf. For the final determinations both the Baljet reaction and the Lindewald reaction are used. The former gives all the aglycones, free and combined, and the colour is measured at 498 mμ. With the second reaction only the aglycones combined with digitoxose (i.e. glycosides) are determined, the colour being determined at 580 mμ. In both cases a standard of pure digitoxin is used. Details are as follows: 0·5 g. of dried leaf is allowed to stand for 15 minutes with water, and made up to 50·5 g. with water. After shaking for 1 hour, 5 g. of lead acetate solution (15 per cent.) is added, and the mixture is filtered: 36·67 g. of the filtrate

is shaken 3 times for 1 minute with 20 ml. quantities of chloroform. The chloroformic solution is dried, filtered and the filter is washed with 3 quantities, each of 5 ml. chloroform. The chloroformic solution is divided into two portions, the chloroform is distilled off and the residues are used for the two colorimetric determinations, giving the two values B_1h and L_1h. Another 0·5 g. of the leaf is treated with water and 10 drops of an ethanolic solution of methyl *p*-hydroxybenzoate (25 per cent.) and made up to 50·5 g. After standing for 3 days at 30° C. the mixture is shaken for 1 hour and the determination is continued as above, giving the values B_2d and L_3d. The results are calculated as follows: percentage of primary glycosides (purpurea glycosides A and B) $= x = 1{\cdot}96\ (B_3d - B_1h)$ or $1.59\ (L_3d - L_1h)$. Percentage of secondary glycosides (digitoxin + gitoxin) $= L_3d - 0{\cdot}825 \times$ Percentage of aglycones (digitoxigenin + gitoxigenin $= 0{\cdot}4\ (B_3d - L_3d)$. In the case of stabilised leaf it is necessary to add 0·5 g. of an enzyme powder, the weight being made up to 51·0 g., and the mixture macerated for 6 days at 40° C. This enzyme preparation is prepared by a modification of the method of Stoll and Kreis: 3 kg. of finely-cut fresh leaf is shaken for 2 hours with 4·5 l. of ethanol, and the mixture is pressed out. The pressed cake is cut fine and transferred to 7 l. of ethanol (50 per cent.). After shaking for 1 hour the mixture is again pressed out and the residue is broken up and dried carefully at 35° C. It is then converted to a coarse powder and the extraction is repeated as before. This process is repeated again. After careful drying the final product is brought to a B 30 powder. G. M.

Morphine in Poppies, Determination of. H. Baggesgaard-Rasmussen. (*Ann. pharm. franç.*, 1952, **10**, 693.) A simple, rapid method for the determination of morphine in whole plants, leaves, stems and capsules, giving reproducible results with an error of less than 5 per cent. on samples containing as little as 0·1 mg. of morphine, was required. Assays depending on the formation of nitrosomorphine were investigated because they can be used with small samples and are unaffected by the presence of other alkaloids of opium with the exception of laudinine and codamine which occur in negligible proportions in poppy plants. The 2-nitrosomorphine formed may be determined colorimetrically in the visible or ultra-violet, or polarographically. The following method was used. To 5 ml. of a solution containing 0·005 to 0·1 per cent. of morphine in N hydrochloric acid add 2 ml. of M potassium nitrite. Allow to stand for exactly 5 minutes and add 3 ml. of a 20 per cent. solution of methylcellulose. Pass nitrogen through the solution to remove dissolved oxygen and determine the nitrosomorphine polarographically, using a standard curve prepared with known quantities of morphine similarly treated at the same temperature. Potassium nitrite and hydroxide gave better results than the corresponding sodium salts by providing a better parallelism between residual and diffusion currents. Methylcellulose was employed to adjust the surface tension to a suitable value. The temperature had to be carefully controlled, because the diffusion current rises about 1·6 per cent. per ° C. The assays indicated that the morphine yield of poppy plants is greatly affected by climatic conditions at the time of harvest, and exposure of the harvested plants to rain removes some of the morphine. Poppies yielded 2 to 3 per cent. of morphine, rising to 5 to 6 per cent. when conditions of cultivation were very good. Figures are given for the morphine content of leaves, stem and capsules of the plant during growth as well as for the various parts of the ripe capsules. G. B.

***iso*Nicotinic Acid, Determination of.** E. F. G. Herington. (*Analyst*, 1953, **78**, 174.) A rapid colorimetric method of analysis is described which permits the estimation of *iso*nicotinic acid in samples of mixed pyridine carboxylic acids containing nicotinic, picolinic and dipicolinic acids. A solution containing trisodium pentacyanoamminoferrate, glycerol and acetic acid is used for the determination; the reagent solution is green, whereas *iso*nicotinic acid produces a reddish colour, and hence it is necessary to use a photoelectric absorptiometer with Ilford No. 604 filters to measure the depth of colour. The colour reaction is sensitive to hydrogen-ion concentration and is therefore carried out in the presence of a large controlled excess of acetic acid, glycerol being added to the reagent both to intensify the colour and to keep the complexes in solution. The method of preparation of the trisodium pentacyanoamminoferrate is given and results of the analyses of synthetic mixtures are quoted; picolinic acid does not interfere with the determinations. R. E. S.

Organic Acids, Circular Paper Chromatography of. J. W. Airan, G. V. Joshi, J. Barnabas and R. W. P. Master. (*Analyt. Chem.*, 1953, **25**, 659.) The method described makes use of circular paper chromatography for the identification of 5 organic acids. An airtight glass tank, 30 cm. in diameter, was used with, inside, a filter paper disc supported 2 cm. from the bottom arranged over a 10 ml. circular Petri dish. At the centre of the filter paper a 2 cm. radius circle was drawn and 5 μl. of 1 per cent. aqueous solutions of tartaric, citric, malic, malonic, and succinic acids were separately spotted at 5 points on this circle. The spots were air-dried, and through a slit made at the centre of the disc a small strip of paper was inserted to take up the solvent onto the paper. Within 3 hours the solvent front had reached the edge of the disc and, after air-drying, the chromatogram was sprayed with bromophenol blue reagent, yellow bands developing against the blue background. A 0·1 per cent. ethanolic solution of mercurochrome was also used as a spray reagent, white bands developing against a pink background. R. E. S.

Oxalate and Calcium, Determination of. F. Burriel-Martí, J. Ramirez-Munoz and E. Fernandez-Caldas. (*Analyt. Chem.*, 1953, **25**, 583.) Attempts were made to apply the decrease in optical density when ferric salicylate is treated with oxalate, to the determination of oxalate ion by indirect colorimetry. Measurements must be done in an acetic acid medium, the unknown solutions added to the coloured reagent being neutral, weakly acid with acetic acid, or slightly alkaline with ammonium hydroxide; errors of ± 4 per cent. encountered in recovery experiments, but the method was reliable in the concentration range $2{\cdot}5 \times 10^{-6}$ to $2{\cdot}5 \times 10^{-5}$ mole of oxalate. While tartrate did not interfere even when in greater concentration than oxalate, citrate gave positive errors by decrease in the optical density, which reached approximately 7 per cent. when the concentration of citrate is 1·6 times that of oxalate. The method was extended to the determination of calcium by precipitation as oxalate followed by indirect colorimetric determination of the oxalate which remains in the solution. Calcium in the presence of magnesium could also be determined by this procedure the amount of oxalate being increased owing to the slow precipitation of calcium due to the interaction of the magnesium and calcium ions which increases the solubility of the calcium oxalate. The results compared satisfactorily with those obtained by volumetric methods in the range 1·2 to 2·2 mg. of calcium. R. E. S.

Particle Size Distributions, Determination of. J. S. Smith and R. Gardenier. (*Analyt. Chem.*, 1953, **25**, 577.) A simple apparatus for the determination of particle size distributions as functions of the Stokes diameter in liquid media is described. The data provided by sedimentation experiments using any apparatus of this sort give the weight fraction of the particles which have settled out from the suspension as a function of the time. A graphical method due to Oden gives the cumulative weight fractions oversize as the intercepts on the weight fraction axis of tangents drawn to the curve of weight fraction versus time at times equivalent to the appropriate Stokes diameters. It is suggested in this paper that in many cases the cumulative fraction oversize as a function of the Stokes diameter is not the most cogent information obtained from the experiment. It is also suggested that the true distribution plot of fraction per unit diameter versus the diameter obtained from the cumulative curve is also not the best information, as these distribution curves imply that a great deal more information is available than is justified by the precision of the measurement. Details of the type of apparatus used are given together with the mathematical theory involved. The liquid chosen was the 2-ethylhexyl monoether of ethylene glycol. Tables are given of the results of 6 measurements of a powder of unknown distribution. R. E. S.

Quinidine Sulphate and Strychnine Nitrate, Assay of. D. Köszegi and E. Salgo. (*Pharm. Zentralh.*, 1953, **92**, 157.) Potassium mercuritetrathiocyanate, which has the formula $K_2Hg(SCN)_4$, contains only two titratable thiocyanate groups. Since it precipitates alkaloids, it may be used for the assay of alkaloidal salts by a titration procedure. The reagent solution is prepared by rubbing down 21·6 g. of yellow mercuric oxide with 39 g. of potassium thiocyanate and 200 g. of water, adding dilute nitric acid until the mercuric oxide has almost completely dissolved, and making up to 1 l. The reaction must remain somewhat alkaline and should be checked with litmus paper. For the assay, about 0·25 g. of quinidine sulphate or strychnine nitrate is dissolved, with warming, in 20 ml. of water. After cooling, an excess (25 ml.) of reagent is added, the mixture is made up to 50 ml. and filtered: 25 ml. of the filtrate is treated with 20 ml. of 0·05N silver nitrate solution, and the excess of silver is determined, after acidification, by titration with 0·05N thiocyanate using ferric alum as indicator. The weight of alkaloidal salt is then equal to $[a - (b - 2c)]f$, where a = number of ml. of 0·05N mercurithiocyanate solution, b = number of ml. of 0·05N silver nitrate solution, c = ml. of 0·05N thiocyanate solution, and f = amount of alkaloidal salt equivalent to 1 ml. of reagent (0·01987 g. of strychnine nitrate or 0·019562 g. of quinidine sulphate). G. M.

Theobromine in Cocoa Residues, Determination of. K. W. Gerritsma and J. Koers. (*Analyst*, 1953, **78**, 201.) A study has been made of the methods available for the determination of theobromine in cocoa residues and a new method is proposed. The residues are shaken for 5 minutes with chloroform in an ammoniacal medium; after dehydration with anhydrous sodium sulphate, the chloroform solution is filtered and the residue and filter are washed with chloroform. The chloroform is removed from the filtrate by distillation and the residue is dissolved in water, after which silver nitrate is added and the liberated nitric acid is titrated with alkali. Details of the method are given together with the results obtained in a comparison with Wadsworth's method (*Analyst*, 1921, **46**, 32); good agreement was shown between the two methods and in 20 replicate determinations, the results obtained by the new method lay between 3·00 and 3·02 per cent. R. E. S.

FIXED OILS, FATS AND WAXES

Oils and Fats, Preservation of. B. Siegfried and R. Schneider. (*Pharm. Acta Helvet.*, 1953, **28**, 131.) Three antioxidants, propyl gallate, nordihydroguaieretic acid and ascorbyl palmitate, were tested on samples of almond and olive oils, and lard, which had already undergone a certain amount of oxidation and showed peroxide values of 4 to 8. For vegetable oils, which contain a certain amount of natural antioxidants, the effect of the additions was not great, best results being attained with ascorbyl palmitate (0·05 per cent.). In lard only the phenolic antioxidants were effective, an addition of 0·05 per cent. of propyl gallate or of nordihydroguaieretic acid being sufficient to preserve the material for 6 months. G. M.

GLYCOSIDES, FERMENTS AND CARBOHYDRATES

Borate Complexes of Sugars and Related Compounds, Separation of, by Ion Exchange Chromatography. L. P. Zill, J. X. Khym and G. E. Cheniae. (*J. Amer. chem. Soc.*, 1953, **75**, 1339.) Further studies were made on the separation of sugars and related compounds by the ion exchange chromatography of their borate complexes on strong-base anion exchangers. The sugars which are known to occur in complex mixtures, such as those obtained from plants, were studied with regard to their elution characteristics. The following compounds were investigated; raffinose, rhamnose, stachyose, sorbose, gentiobiose, melibiose, melezitose, turanose, sedoheptulose, sedoheptulosan, dulcitol, sorbitol and mannitol. A number of separations are reported. The borate from the isolated sugar-borate complexes was removed by distillation as volatile methyl borate. The structure of the sugars in relation to the affinity of the sugar-borate complexes for the ion exchange resin is discussed. A. H. B.

Starches from Peas, Fractionation of. A. L. Potter, V. Silveira, R. M. McCready and H. S. Owens. (*J. Amer. chem. Soc.*, 1953, **75**, 1335.) Smooth-seeded Alaska and wrinkled-seeded Perfection pea starches were isolated by sedimentations from their aqueous suspensions and then fractionated with amyl alcohol and *n*-butanol into amylose and amylopectin. These starches have 35 and 66 per cent. amylose content respectively. The following properties of the separated amyloses and amylopectins were then examined: (*a*) molecular weights by osmotic pressure measurements, (*b*) end-group assays by periodate oxidation, (*c*) iodine affinities and blue values, and (*d*) limiting viscosity measurements. The average molecular weight of Alaska pea amylose was found to be 125,000 and Perfection pea amylose to be 100,000, while the molecular weights of the corresponding amylopectins were 2,000,000 and 140,000. From periodate oxidation and molecular weight data, the degree of branching of these pea-starch amyloses is low and similar to those of other amyloses prepared by similar methods from other plant sources. Alaska pea amylopectin has an average of 25 glucose residues per terminal non-reducing glucose unit, while Perfection pea amylopectin has 36. Besides the difference in molecular weight and end-group assay of these amylopectins, the iodine potentiometric titration curve of Perfection pea amylopectin is different from that of other amylopectins. The results of the investigation show that starch from smooth-seeded Alaska peas is similar to cereal and root starches, while that from wrinkled-seeded Perfection peas is different. Besides having a higher amylose content, the amylopectin fraction of the wrinkled pea starch has a much smaller molecular weight and a smaller degree of branching than amylopectins from other plant sources. A. H. B.

ORGANIC CHEMISTRY

Urea and Thiourea, "Insertion" Compounds of. W. Schlenk. (*Chim. et Industr.*, 1953, **69**, 454.) X-ray studies show that when certain hydrocarbons of the urea or thiourea crystal enlarges, forming a honeycomb structure. The react with urea or thiourea to form addition compounds the original lattice hydrocarbon molecules are accommodated in the canals of the lattice. Straight-chain hydrocarbons, benzene, *cyclo*hexane and aliphatic compounds with 1 or 2 lateral methyl groups may thus be distinguished from branched chains and tetra-substituted carbon compounds which do not form addition compounds with urea or thiourea. Certain very thin molecules are incapable of causing enlargement of the urea or thiourea lattice, but form compounds when wider molecules are present in addition. Mixtures of paraffins, fatty acids, etc., may be fractionated by filtration through urea or thiourea and the method is applicable to the separation of isomers such as *n*- and *iso*octane. Separation is not always complete, depending on the equilibrium between the addition compound and the liquid phase. When this method was applied to the analysis of ozokerite the material was divided into 4 fractions (1) forming an addition product with urea, (2) forming an addition product with thiourea, (3) with both and (4) with neither. Examination of the physical constants of the fractions enabled the following composition to be attributed to the ozokerite: paraffins, average about C_{35}, the greater unbranched, 60 per cent., *cyclo*paraffins, 20 to 30 per cent.; condensed cyclic systems, not more than 20 per cent. and hydrocarbons having lateral groups longer than $-CH_3$, not more than 20 per cent. G. B.

PLANT ANALYSIS

Growth Substances in Plant Extracts, Chromatography of. T. A. Bennett-Clark and N. P. Kefford. (*Nature, Lond.*, 1953, **171**, 645.) Plant extracts from a variety of etiolated seedlings and roots were studied including pea (*Pisum sativum*) shoots and roots, broad bean (*Vicia faba*) shoots and roots, sunflower (*Helianthus annuus*) shoots, maize (*Zea mays*) roots, potato tubers and shoots, and *Ægopodium* rhizomes. Macerated plant tissues were extracted with ethanol for 24 hours at $-5°$ C., the extract being centrifuged, concentrated under reduced pressure, and the aqueous residue extracted with ether at *p*H 3. After purification the ether-soluble acidic substances were suitably concentrated and applied to the starting line of a chromatogram as a spot or a strip, from a dropping pipette in a stream of nitrogen. The chromatograms were developed in *iso*propanol: water:: 10: 1, with ammonia in the base of the tank; indole-3-acetic acid and indol-3-acetonitrile were run simultaneously and their positions detected at the conclusions of the development by spraying with a ferric chloride–perchloric acid mixture and nitrous acid–nitric acid mixture respectively. Biological testing, using subapical sections of *Avena* coleoptiles and sections from the top of the pea, was performed on developed chromatograms. On all the chromatograms of the acidic fractions of ether extracts of shoot and root material which were bioassayed, 3 active areas were clearly detected. The central active area (R_F = approx. 0·3) corresponded with indole-3-acetic acid; the term accelerator-α was suggested for the substance with R_F value less than that for indole-3-acetic acid and the term inhibitor-β for that with R_F value greater. All shoot and root materials studied contained hormones α and β and indole-3-acetic acid, although no information is available at present on the nature of either α or β is available.

R. E. S.

BIOCHEMISTRY

GENERAL BIOCHEMISTRY

Œstrone and β-Œstradiol, Biosynthesis of, in the Perfused Ovary. N. T. Werthessen, E. Schwenk and C. Baker. (*Science*, 1953, **117**, 380.) In order to investigate whether the ovary may synthesise œstrone or β-œstradiol from acetate, some ovaries were perfused with sodium acetate labelled with ^{14}C in the carboxyl group. Two perfusions of long duration were carried out with ovaries obtained from one pregnant and one non-pregnant animal. Immediately after the perfusion was terminated, the mixture of ovaries and perfusion liquid was converted into a mash, and extracted. The details of the method of isolation of the radioactive œstrone, β-œstradiol and cholesterol are recorded. Thus isolated surviving sow ovaries, when perfused with sodium acetate labelled in the carboxyl group, produce labelled œstrone, β-œstradiol and cholesterol. The experiments described do not allow any conclusion as to whether the hormones are derived from the cholesterol, or whether cholesterol and the hormones are produced from a common precursor. A. H. B.

Procaine and Succinylcholine Diiodide, Substrate Competition between, for Plasma Cholinesterase. F. F. Foldes, P. G. McNall, D. L. Davis, C. H. Ellis and A. L. Wnuck. (*Science*, 1953, **117**, 383.) Because both procaine and succinylcholine are hydrolysed by the same enzyme it was considered possible that the additive effect of the simultaneous administration of these two substances on respiratory depth might be due to substrate competition between the two agents for the plasma cholinesterase. *In vitro* hydrolysis studies were carried out for the quantitative determination of this substrate competition. Aliquot portions of heparinised human plasma were treated with (*a*) procaine in different concentrations; (*b*) the same procaine concentrations as in (*a*) with succinylcholine diiodide added, and (*c*) constant procaine concentration with varying succinylcholine diiodide concentrations. All plasma samples were incubated at 37° C. for 10 minutes and then the procaine and *p*-aminobenzoic acid concentrations determined. The results show that increasing the procaine concentration from 50 to 400 μg./ml. had very little effect on the quantity of procaine hydrolysed. However, when 100 μg./ml. of succinylcholine diiodide was added before incubation, the quantities of procaine hydrolysed increased with increasing procaine concentrations. Similarly, when the procaine concentration was kept constant at 100 μg./ml. and the succinylcholine diiodide concentration was increased, the quantity of procaine hydrolysed decreased with increasing succinylcholine diiodide concentrations. Other experiments using Warburg's micromanometric technique and Ting's method for the determination of procaine showed that procaine hydrochloride inhibits the enzymatic hydrolysis of succinylcholine dichloride and succinylcholine inhibits the enzymatic hydrolysis of procaine hydrochloride. The inhibitory effect of procaine hydrochloride on the hydrolysis of succinylcholine dichloride was shown to be greater than that of succinylcholine dichloride on the enzymatic hydrolysis of procaine hydrochloride, which indicates that, although the hydrolysis rate of succinylcholine dichloride is greater than that of procaine hydrochloride, the affinity of the latter to the enzyme is considerably greater than that of the succinylcholine dichloride. The additive effects of succinylcholine and procaine were also demonstrated with mammalian sciatic-gastrocnemius preparations of dogs and cats. The practical importance of the observation that competition exists between procaine and succinylcholine for the plasma cholinesterase lies in the fact that they might be employed simultaneously in anæsthetised patients.

A. H. B.

BIOCHEMICAL ANALYSIS

Cholesterol in Serum, Determination of. A. Zlatkis, B. Zak and A. J. Boyle. (*J. Lab. clin. Med.*, 1953, **41**, 486.) A method is described for the direct estimation of cholesterol in serum which is more sensitive and less time consuming than previous methods. It consists of adding a fixed volume of concentrated sulphuric acid, glacial acetic acid and ferric chloride solution to 0·1 ml. of serum in 3 ml. of glacial acetic acid. A purple colour is formed in 1 minute, the absorption being measured in a spectrophotometer at 560 mμ. The concentration is read from a predetermined response curve using standard cholesterol solutions. The colour obeys Beer's law and remains stable over several hours. Comparisons were made with the other methods of Kingsley-Schaffert and Schoenheimer-Sperry procedures and the results were in good agreement. G. F. S.

Corticosteroids, Paper Chromatography of. E. H. Sakal and E. M. Merrill. (*Science*, 1953, **117**, 451.) The simple procedure described is applicable to both descending and ascending paper chromatography, and the application to the latter method is outlined. A sheet of Whatman No. 1 filter paper (43 × 43 cm.) is folded into a cylinder in the manner indicated by Wolfson *et al.* (*Science*, 1949, **109**, 541). Small droplets of the solutions to be chromatographed are applied to the paper on the starting line and the paper then placed in a cylindrical glass jar (15 cm. diameter and 46 cm. high) containing a one-phase solvent mixture of xylene (225 ml.) and absolute methanol (75 ml.), and the jar then closed by a gas-tight cover. The solvent is allowed to ascend on the paper to a distance of about 25 cm. from the starting line (a period of about 2½ hours required). The paper is then removed and air-dried and the locations of the various steroids found by normal procedures. Advantages of the above procedure over those reported in the literature are as follows. A one-phase solvent system is used, rendering unnecessary the equilibration of the paper in the solvent vapour prior to development; pretreatment of the paper with any solvent is unnecessary; lateral diffusion of steroid spots during development is quite limited, thus rendering unnecessary the precutting of the paper into a pattern of separated strips; the development period required for the resolution of mixtures of corticosteroids is not greater than 2 to 3 hours; air-drying of the paper afterwards is completed in less than 30 minutes. A. H. B.

Ethanol in Biological Fluids, Determination of. I. Sunshine and R. Nenad. (*Analyt. Chem.*, 1953, **25**, 653.) A modification of the determination of ethanol in blood by oxidation with potassium dichromate is described. The method uses potassium dichromate in the central wall of a Conway cell, complete diffusion (evaporation) taking 20 minutes. The amount of ethanol present is determined colorimetrically, the contents of the centre well being compared with prepared standards, or the optical density being measured photoelectrically. Known amounts of ethanol were added to blood and urine and the samples were assayed for ethanol; results indicated that the recovery of ethanol was within ±0·02 per cent. of the amount added. R. E. S.

Mannosidostreptomycin and Dihydromannosidostreptomycin, Determination of. J. Levine, G. Selzer and W. W. Wright. (*Analyt. Chem.*, 1953, **25**, 671.) The method proposed depends on the methanolysis of the sample to give

methyl streptobiosaminide dimethyl acetal or methyl dihydrostreptobiosamide, streptidine and methyl mannoside. Methanolysis was effected by refluxing in absolute methanolic sulphuric acid for 2 hours; the presence of water resulted in separation of streptidine sulphate, discolouration of the solution, and destruction of carbohydrate. Of the products of methanolysis, all except methyl-mannoside were removed by ion exchange; passage of the solution over a mixture of anion and cation exchangers removed cations completely; eluates prepared from methanolysed mannosido-free streptomycin being free from carbohydrate. Removal of intact streptomycin for the determination of the free sugar blank was accomplished by using mixed cation and anion exchange resins in which the anion exchange portion had been converted to the chloride form; use of the anion exchange resin in the usual hydroxide form resulted in the loss of free sugar. It was found that mannose reacted quantitatively in acid solution with a small excess of 2·4-dinitrophenylhydrazine when the solution was evaporated to complete dryness at a slow rate; the residue from evaporation dissolved in aqueous ethanolic alkali to give a stable purple solution having an absorption maximum at 556 mμ and this colour was used for spectrophotometric estimation. R. E. S.

Urea, Determination of. H. S. Friedman. (*Analyt. Chem.*, 1953, **25**, 662.) A modification of the quantitative Fearon reaction for urea in blood and other body fluids is presented in which fuming hydrochloric acid is eliminated. Attempts to reproduce a standard curve using potassium persulphate to develop the colour were unsuccessful owing to the photo-sensitivity of the reaction. A procedure was developed using a solution of arsenic in 1:1 sulphuric acid; details of the method are given together with a specimen calibration curve.

R. E. S.

CHEMOTHERAPY

***N*-Naphthylmethyl-2-haloethylamine Derivatives, Antihistamine and Anti-adrenaline Properties of.** J. D. P. Graham and G. P. Lewis. (*Brit. J. Pharmacol.*, 1953, **8**, 54.) A series of *N*-naphthylmethyl-*N*-(aryl or alkyl)-2-haloethylamines have been examined for their antihistamine, anti-adrenaline, anti-acetylcholine and antipituitrin actions. None of the compounds antagonised the depressor response to acetylcholine or the pressor response to pituitrin. Most of the compounds antagonised the pressor response to adrenaline and its excitatory actions on the cat's pregnant uterus, rabbit uterus and cat nictitating membrane, but did not affect the inhibitory actions of adrenaline on the cat non-pregnant uterus, rabbit intestine or the effects of adrenaline on the heart. In small doses the haloethylamines potentiated the pressor response to adrenaline and stimulated isolated perfused rabbit hearts. While these compounds reversed the pressor effects of adrenaline they were much less active against sympathetic nerve stimulation. Some of the compounds competitively antagonised the effects of histamine, and structural configurations which favoured anti-adrenaline action also favoured antihistamine action. The presence of 1-naphthylmethyl in the molecule was favourable, while the 2-naphthylmethyl lowered activity. If the halogen atom in the haloethyl side-chain was fluorine the molecule was inactive while the presence of bromine conferred the greatest activity. G. F. S.

PHARMACY

NOTES AND FORMULÆ

Oxytetracycline (Terramycin). (*New and Nonofficial Remedies; J. Amer. med. Ass.*, 1953, **151**, 1291.) Oxytetracycline is an antibiotic produced by the growth of the actinomycete *Streptomyces rimosus* on suitable media. It is the dihydrate of 4-dimethylamino-1:4:4a:5:5a:6:11:12a-octahydro-3:5:6:10:12:12a-hexahydroxy-6-methyl-1:11-dioxo-2-naphthacenecarboxamide and occurs as a dull yellow, odourless, slightly bitter, crystalline powder, m.pt. 179·0° to 182·0° C., with decomposition. It is soluble in acids and alkalis, very slightly soluble in acetone, ethanol, chloroform and water, and practically insoluble in ether. It gives a dark brown colour with ferric chloride, a precipitate of copper when warmed with Fehling's solution, and a red colour with sodium hydroxide and diazobenzenesulphonic acid. When a solution in diluted hydrochloric acid is treated with an ethanolic solution of α-naphthol and superimposed on sulphuric acid, a reddish brown colour appears at the interface. The specific rotation of a 1 per cent. solution of the anhydrous substance in 0·1 N hydrochloric acid is −208° to −216°. A 0·00125 per cent. solution of the anhydrous substance buffered at *p*H 2·0 exhibits ultra-violet absorption maxima at about 269 mμ, and 353 mμ ($E^{1\,\text{per cent.}}_{1\,\text{cm.}}$, 300 to 312), minima at about 232 and 299 mμ, and a slight inflection at about 313 mμ; the ratio of the absorptions at 269 and 353 mμ is 1·32 to 1·38. Oxytetracycline contains not more than 25 p.p.m. of heavy metals and yields not more than 0·6 per cent. of sulphated ash. The loss of weight on drying in a vacuum oven at 75° C. for 6 hours is not more than 7·75 per cent. It is assayed spectrophotometrically by measuring the absorption at 353 mμ of a 0·00125 per cent. solution buffered at *p*H 2·0, and contains 96·0 to 104·0 per cent. of oxytetracycline. G. R. K.

Potassium Penicillin O (Cer-O-Cillin Potassium). (*New and Nonofficial Remedies; J. Amer. med. Ass.*, 1953, **151**, 1491.) Penicillin O is allylmercaptomethyl penicillin and is obtained by growing the mould in a medium containing allylmercaptoacetic acid. It is stable in the dry form at room temperature for at least 3 years, and does not need refrigeration. Solutions may be kept for 3 days in a refrigerator without significant loss of potency. Clinically, penicillin O has been shown to be as effective as benzylpenicillin (penicillin G) and less likely to cause sensitivity or allergic reactions. It has a similar range of antibacterial activity, and is particularly useful as a substitute in the treatment of patients sensitive to benzylpenicillin. Allergic reactions to penicillin O have been observed in less than 1 per cent. of patients who have no history of previous allergic reactions to benzylpenicillin, while about 90 per cent. of patients sensitive to benzylpenicillin tolerate therapeutic doses of penicillin O without the development of allergic phenomena. Some patients may lose their sensitivity to benzylpenicillin during a short course of penicillin O. When given by mouth, penicillin O may produce an onion-like odour of the breath, which subsides shortly after the drug is discontinued. It produces blood levels comparable to those obtained with benzylpenicillin administered by similar routes. Potassium penicillin is given by mouth or by intramuscular injection (intermittent or continuous infusion). In general the dosage is the same as that recommended for benzylpenicillin. G. R. K.

PHARMACOLOGY AND THERAPEUTICS

Amœbicides, Evaluation of. J. S. Vegas. (*J. Amer. med. Ass.*, 1953, **151**, 1059.) This study involves 264 cases of amœbiasis with follow-up physical and stool examinations ranging from 6 to 24 months. In order to evaluate the effectiveness of the amœbicides to be investigated the series was studied under 8 treatment groups, as follows:—bismuth glycolylarsanilate, aureomycin, aureomycin and bismuth glycolylarsanilate, chloramphenicol, chloramphenicol and bismuth glycolylarsanilate, oxytetracycline, oxytetracycline and bismuth glycolylarsanilate, and chloroquine and bismuth glycolylarsanilate. Of these treatments the combination of bismuth glycolylarsanilate and chloroquine was found the most effective; of 102 patients treated, 91 (89·2 per cent.) finally became free of cysts. Treatment consisted in the administration of 2 tablets daily, spaced at 12-hourly intervals, each tablet containing 500 mg. of bismuth glycolylarsanilate and 150 mg. of chloroquine, the course lasting 15 days. The few side-effects occasionally observed (nausea, vomiting, pruritus ani, lower left quadrant pain and diarrhœa) do not necessitate interruption of treatment; side-effects may be further reduced by administration of vitamin B complex. The combination of the non-absorbable arsenical, bismuth glycolylarsanilate, with the highly absorbable chloroquine is a logical and effective treatment for amœbiasis, which should be considered as a systemic and not a purely intestinal disease. S. L. W.

Carbimazole, Antithyroid Activity of. A. G. Macgregor and H. Miller. (*Lancet*, 1953, **264**, 881.) The antithyroid activity of this drug (neomercazole) and methimazole were compared by the technique of Stanley and Astwood (*Endocrinology*, 1947, **41**, 66), a dose of the antithyroid drug being given after administration of a tracer dose of radioactive iodine, and the effect on the normal curve of accumulation of iodine in the thyroid gland observed. In doses of 10 mg. both drugs caused complete inhibition of uptake of radioactive iodine by the thyroid gland during the whole period of observation and a similar effect was observed with doses of 2·5 mg. of each drug. The effect is comparable to 200 to 300 mg. of methylthiouracil. In doses below this level carbimazole appears to be consistently rather more potent than methimazole. Clinical experience suggests that both drugs are effective antithyroid agents, but considerable caution should be exercised in the transfer of the results of potency trials of this type to the clinical field. These tests suggest a potency possibly 50 times greater than that of methylthouracil, but this does not imply that the therapeutic dose is correspondingly low, since it is now agreed that, weight for weight, methimazole is probably only about 10 times as active clinically as methylthiouracil. S. L. W.

Carbimazole (Neomercazole), Treatment of Thyrotoxicosis with. H. Poate. (*Lancet*, 1953, **264**, 879.) Experience in the treatment of 9 cases suggests that this drug is superior to any of the thiouracil compounds, not only for control of thyrotoxicosis but also for reducing vascularity of the gland before operation. The usual dosage employed was 10 mg. thrice daily until thyrotoxicosis was controlled, after which it was reduced to 10 mg. twice daily, or even 5 mg. twice daily. It reduces the basal metabolic rate more slowly than the thiouracils but does not induce or increase thyroid hyperplasia and does not interfere with the leucocytes. It therefore appears to be a safer remedy and there is reason to think that once control of a primary thyrotoxicosis is obtained a comparatively short period of maintenance therapy will result in a complete cure. S. L. W.

Carbimazole, Treatment of Thyrotoxicosis with. D. Doniach. (*Lancet*, 1953, **264**, 873.) The author presents a clinical evaluation of this antithyroid

compound (neomercazole, 2-carbethoxythio-1-methylglyoxaline) in the treatment of 120 thyrotoxic patients, 30 of whom received the drug pre-operatively and 90 as curative treatment. The patients were followed for periods of up to 21 months. Of the 120 patients 93 became euthyroid within 2 to 8 weeks, and 27 responded more slowly. Of the latter 9 were operated on and the rest were controlled in 3 to 6 months. The optimum initial dosages were 15, 30 and 45 mg. daily by mouth for mild, average and severe cases respectively. After 4 to 12 weeks this dosage could be reduced in early remission to two-thirds or half, and after 6 months' treatment 5 to 10 mg. a day sufficed for maintenance in average cases; mild cases were maintained on 2 mg./day within 3 months. Treatment was stopped when the patient was able to remain euthyroid on 2 to 5 mg. daily for about 6 months; to date, about 12 months' treatment has been found necessary. Goitrogenic reactions were avoided, in all except 7 cases, by careful control of the maintenance dose; no toxic reactions were seen. Weight for weight carbimazole seems to be at least as effective as, and very much less toxic than, methimazole. It is probably hydrolysed to methimazole before it exerts its antithyroid action, and its increased efficiency must be due to the maintenance of a steadier blood level produced by the slow release of methimazole. S. L. W.

Cortisone, Effect of, on Experimental Corneal Tuberculous Lesions. H. Greenburgh, J. M. Robson and D. R. C. Willcox. (*Brit. J. Pharmacol.*, 1953, **8**, 120.) The effects of intravitreous injections of cortisone have been studied against intracorneal infection of rabbits with *Mycobacterium tuberculosis*. Cortisone depressed the severity of the lesions during its administration, but after discontinuance of therapy there followed a rapid increase in the severity of the lesions. Promethazine, given subcutaneously, had no effect on the development of the corneal lesions, although it penetrated the anterior chamber. It is suggested that cortisone may act by rendering the cornea resistant to enzymatic action. G. F. S.

Diphenan in the Treatment of Oxyuriasis. L. M. Dowsett and A. E. Brown. (*Lancet*, 1953, **264**, 1070.) An investigation into the efficacy of diphenan (*p*-benzylphenylcarbamate) as a vermifuge was carried out on 174 school children aged from 3 to 10 years. The presence of thread worms was demonstrated in these cases by the application of a strip of cellophane tape to the skin in the anal region by means of a wooden applicator. The cellophane was then stuck on a microscope slide and examined under a low power objective, when the ova could be seen. Cellophane swabs were examined daily for 9 days before treatment and if any of these were positive, diphenan was given for 10 days; swabs were again examined for 9 days after treatment. The dosage of diphenan was from 0·5 to 1 g. according to age thrice daily and was far in excess of that recommended by the manufacturers. Only 35 (20 per cent.) of the children were subsequently free of infestation; 44 per cent. were improved in the sense that they gave fewer positive swabs after treatment than before, 18 per cent. were worse and 18 per cent. gave the same number of positive swabs before and after treatment. All 35 "cures" had been only lightly infested, 31 of them having given only one, two or three positive swabs out of the nine taken for diagnosis. Of 15 children who gave 9 diagnostic negatives, only 6 remained negative after receiving the same dosage of diphenan. No toxic reactions were noted. H. T. B.

Ethanol, Distribution of, in the Human Body. H. Handovsky, W. Van Hecke and F. Thomas. (*Acta pharm. tox. Kbh.*, 1952, **9**, 18.) Ethanol determinations were made on a series of organs and body fluids from 93 healthy

persons killed by accident when under the influence of ethanol. Blood samples were taken from the femoral vein and all analyses were carried out by Widmark's method. The ethanol concentrations found in the organ or body fluid were plotted against the ethanol concentration in blood from the same individual, and the ratios are shown in the following table, together with S, the estimated value of the standard deviation of a single observation.

Organ or body fluid	Total No. ratios	Mean ratio and standard deviation of mean	S = standard deviation of a single observation
Urine	80	0·77	±0·17
Cerebrospinal fluid	25	0·79	±0·20
Bile	55	0·89	±0·15
Testes	43	1·18	±0·33
Brain	48	1·48	±0·35
Cardiac muscle	51	1·30	±0·25
Skeletal muscle	57	1·28	±0·31
Spleen	33	1·50	±0·32
Kidney	60	1·32	±0·27
Liver	57	1·92	—

From these figures it is seen that the body fluids have a higher ethanol concentration and the organs a lower ethanol concentration than the blood. From the ratios it is possible to calculate the concentration of ethanol in the blood from the ethanol concentrations in organs (except the liver) and body fluids, and *vice versa*. S. L. W.

Hyaluronidase, Bioassay on Rabbits. V. M. Venturi. (*Acta pharm. tox., Kbh.*, 1953, **9**, 93.) A cannula was inserted into the subcutaneous tissue of the dorsal area of the forelegs of unanæsthetised adult albino rabbits and a 0·9 per cent. sodium chloride solution infused through it under constant pressure from a 100 or 200 ml. burette, the pressure being adjusted to an infusion rate of about 1 ml./minute. The hyaluronidase preparation used contained 2·5 V.R. units per ampoule: the contents of an ampoule were dissolved in 2·5 ml. of 0·9 per sodium chloride solution. Solutions of the contents of not less than 2 ampoules were mixed and the mixture diluted to 1:10 or 1:100. Thus 3 different hyaluronidase solutions were obtained containing 1·0, 0·1 and 0·01 V.R. units/ml. For control purposes 0·9 per cent. sodium chloride solution without hyaluronidase was used. After the infusion rate had been adjusted to about 1 ml./minute the average infusion rate was calculated for several periods of 10 minutes each. The rate was expressed in ml./minute. After the infusion rate had been constant over several periods hyaluronidase was injected and the average infusion rate calculated for a series of 10 minute periods extending over one or more hours. The value measured before the enzyme injection was in each case taken as 1·0 and the values measured after the injection expressed in terms of this; these are therefore referred to as infusion rate (relative values). In the following table are set out means, their standard deviation and fiducial limits (P = 0·05) of the infusion rate (relative values) 20 minutes after injecting different amounts of hyaluronidase or control physiological saline.

Injected solution	Means	Fiducial limits
Physiological saline	0·738 ± 0·096	0·953 — 0·523
Hyaluronidase 0·01 V.R. units	1·136 ± 0·290	1·792 — 0·480
Hyaluronidase 0·10 V.R. units	1·825 ± 0·133	2·118 — 1·523
Hyaluronidase 1·00 V.R. units	3·010 ± 0·182	3·417 — 2·603

S. L. W.

Local Anæsthetics, Specificity of. S. Wiedling. (*Acta pharm. tox., Kbh.*, 1953, **9**, 75.) The local anæsthetic and spasmolytic activities against acetylcholine, histamine, and barium-induced spasms were determined for some 20 commercially available compounds of the general type aromatic residue-intermediate chain-amino group. The compounds are used clinically as local anæsthetics, antispasmodics, antihistaminics, and analgesics, and include such substances as syntropan, procaine, amethocaine, lidocaine, cinchocaine, diethazine, promethazine, diphenhydramine, methadone, adiphenine, antistine, antergan, mepyramine and caramiphen. Determinations of local anæsthetic activity were carried out by a comparative method on the rabbit's cornea using 2 per cent. lidocaine solution as standard. Determinations of activity against spasms produced by acetylcholine, histamine or barium were carried out by a comparative "curative" method on isolated guinea-pig small intestine, using diphenhydramine as the standard substance. A high degree of specificity was shown to be exhibited only by certain compounds, such as cinchocaine and lidocaine, used clinically as local anæsthetics. The least specific substances are certain antihistamines, such as diphenhydramine, antergan and promethazine, and certain antispasmodics, such as diethazine. While compounds of the general type aromatic residue-intermediate chain-amino group show very different properties, local anæsthetic activity of some degree is common to all of them. Some of them also exert a spasmolytic effect against acetylcholine, histamine and barium-induced spasms. There is, however, no connection between the acetylcholine potency and the local anæsthetic action; the antihistamine effect is also independent of the local anæsthetic action, and no general connection between this action and the antibarium effect can be established. There would appear to be a certain parallelism between the antiacetylcholine and the antibarium activities. S. L. W.

Primidone in Treatment of Refractory Epilepsy. B. H. Smith and F. L. McNaughton. (*Canad. med. Ass. J.*, 1953, **68**, 464.) Primidone (mysoline, 5-ethyl-5-phenylhexahydropyramidine-4:6-dione) was given to a series of 66 patients, 23 females and 43 males ranging in age from 4 to 63 years, all of whom had been treated for a considerable time with hitherto available drugs, singly or in combination, with little success. No particular type of epilepsy was studied, the chosen cases including 17 with idiopathic epilepsy, 35 with focal seizures and 14 with unlocalised cerebral seizures. More than half had had seizures for more than 10 years, 10 of them for over 30 years. Primidone was administered in 0·25 g. tablets, the dosage being 1 or 2 tablets daily according to age; this treatment was given in addition to previous medication. After 4 days the dosage was increased by one tablet, the other medicament being correspondingly reduced, and thereafter at weekly intervals this was continued. No patient received more than 8 tablets a day, some showing good response on less. When possible, primidone was given alone in order to assess its value, but where a good result was obtained on a combined treatment of, say, primidone and sodium diphenylhydantoinate further alteration was not tried. In 14 successful cases, primidone was being used alone in dosages of 4 to 8 tablets daily. The side effects produced by the drug included transient drowsiness and dizziness as the most common; vertigo, nausea, ataxia and skin rashes occurred in some cases, but no serious toxic effects were noted. Of the 61 cases treated for more than 4 months, 23 (35 per cent.) had the number of their attacks reduced by half or more. 16 of these patients had been on primidone for more than a year. All types of epilepsy appear to derive benefit, cases of major convulsion, petit mal and automatism being included among those benefited. H. T. B.

1-Phenyl-1-*cyclo*pentyl-3-piperidino-1-propanol Hydrochloride in the Treatment of Parkinsonism. D. W. Mulder. (*Proc. Mayo Clin.*, 1953, **28**, 210.) This compound (designated compound 08958) is chemically related to benzhexol. Pharmacological studies indicate that it is an active antispasmodic with fewer side-effects than atropine. It is slightly more toxic than atropine in mice and rats by either oral or intravenous administration. Of 102 patients with parkinsonism treated with the compound and observed originally for a 3 to 5-day period, 75 showed sufficient objective and subjective improvement to warrant continuation of the drug. The improvement included increased rapidity of finger movements, diminution of tremor, improvement of gait and of handwriting, and increased ability to perform well-learned motor patterns. The evidence of improvement continued to be demonstrated over follow-up periods of from 6 months to 2 years. Patients with oculogyric crises were particularly benefited. The remaining 27 said that their previous medication seemed more satisfactory or the side-effects were more distasteful than with other medicaments. Gastro-intestinal symptoms, including transient nausea, with associated anorexia, and occasionally dryness and soreness of the mouth, occurred in 21 patients; mental symptoms (lightheadedness, stupidity or confusion) occurred in 22 patients, and were most marked in older patients, particularly those with arteriosclerotic changes. The initial dose of the drug is 1·25 mg. thrice daily, gradually increased over a period of several days to 2·5 mg. thrice daily. The optimal dose for most patients is from 7·5 to 15·0 mg. daily. This preliminary clinical trial suggests that the effectiveness of the compound is approximately equal to that of benzhexol and that it may be employed for the partial relief of parkinsonism. S. L. W.

1-Propyl-3-ethyl-6-aminothiouracil; Clinical Trial of Diuretic Properties. A. G. Spencer and H. G. Lloyd-Thomas. (*Brit. med. J.*, 1953, **1**, 957.) This oral diuretic (designated Compound S.C. 2614) was tested 57 times in 36 subjects. The compound was given by mouth in 125 mg. tablets, 2 every 3 hours until 1250 mg. had been taken. In normal subjects there was a satisfactory diuresis in 9 out of 10 tests. In 22 tests on 10 œdematous patients with heart disease there was a good diuretic response in 19. The compound usually failed to produce a satisfactory diuresis in any other group of patients; there were 19 failures in 25 tests on 17 patients. No useful diuresis occurred in cardiac patients without pitting œdema, in patients with a low plasma sodium, a serum albumin below 2 g./100 ml. or a blood urea over 150 mg./100 ml. In renal disease there were a few useful diuretic responses, but the evidence was insufficient to assess value. The diuretic action of the compound was seriously offset by the high incidence of gastro-intestinal disturbances (epigastric discomfort, anorexia, nausea and vomiting). Though rarely severe, these were sufficient to elicit a spontaneous complaint from 23 per cent. of the test subjects. The authors conclude that the results of this experiment do not justify extensive trials of the compound at the present time. S. L. W.

Strychnine, Effect of Vitamin B_2 Deficiency on the Toxicity of. C. F. Poe and J. F. Suchy. (*Arch. int. pharmacodyn.*, 1953, **93**, 244.) It has been previously shown that severe vitamin B_1 deficient rats are more susceptible to strychnine than normal rats. This paper reports that vitamin B_2 deficient rats are not more susceptible and confirms a previous observation that strychnine is more toxic for female rats than for male rats. G. F. S.