Summary

1. A modification of the Lefèvre-Tollens method for glucuronic acid has been applied to the determination of galacturonic acid in pectic substances. The method is described and results for several pectin preparations are given.

2. The same samples were extracted with 70% alcohol and the furfural yields of the residues were determined. Comparison of the results by the two methods shows that apparently no simple relation exists between galacturonic acid and furfural yield so obtained.

3. A possible interpretation of the data is discussed with reference to Nanji, Paton and Ling's hexa-ring structure for the basal unit of pectic substances.

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STUDIES ON ATMOSPHERIC OXIDATION. I. THE OXIDATION OF GLUCOSE AND RELATED SUBSTANCES IN THE PRESENCE OF SODIUM FERRO-PYROPHOSPHATE

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It has recently been shown[1] that various monosaccharides, disaccharides and polyatomic alcohols are oxidized by atmospheric oxygen in the presence of a complex iron phosphate catalyst and disodium phosphate. These oxidation reactions have been further studied with the thought that they would serve as a "model" of carbohydrate oxidation and throw some light on the intricate chemical reactions comprising respiration. The significance of iron, as well as manganese and copper, in respiratory oxidation has long been recognized; the kinetics of the reactions involved remains to be solved. The substances oxidized (alcohols, carbohydrates, acids) are not autoxidizable. It is the iron which reacts with molecular oxygen, and in turn with the organic substance. For iron to act thus as a catalyst certain conditions pertaining to the form of the iron as well as the dissociation of the oxidizable substances must be met. The problem has been approached from two sides. The first method is a study of the nature and action of the catalyst, with which this paper deals; the second is an investigation of the action of sodium phosphates on the dissociation of various carbohydrates, the results of which will be presented later.

The Iron Catalyst

The catalyst used is sodium ferro-pyrophosphate, made by dissolving ferrous sulfate in sodium pyrophosphate. Some of the properties of this

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[1] Spoehr, This Journal, 46, 1494 (1924).

substance have already been described in the earlier publication cited. It became necessary to know how rapidly a solution of sodium ferro-pyrophosphate is oxidized by air to sodium ferri-pyrophosphate. The solution of the catalyst was made by dissolving sodium pyrophosphate decahydrate (9.62 g.), ferrous sulfate heptahydrate (1.0 g.), and ferric sulfate nonohydrate (1.01 g.) in 100 cc. of water. As soon as the solution was prepared it was assayed for ferrous and total iron. After the solution had been shaken for five minutes with free access to the air it was again assayed for ferrous iron and none was found. This gives some idea of how rapidly the ferrous iron is oxidized in this solution. To determine the velocity constant of this reaction, physicochemical measurements are now being made and will be described in a later publication.

To estimate the ferrous iron, the solution was acidified with sulfuric acid and then titrated with 0.02 *N* potassium permanganate solution. Total iron was determined by the usual method of passing an acidified solution of the iron through a Jones reductor and titrating the solution with permanganate. Ferric iron was then obtained by difference. The analytical results follow.

	Initial amt. present, g.	Found, g.
Ferrous iron	0.205	0.1157
Total iron	.410	.410
After shaking for 5 min., ferrous iron	.205	.000

This shows that even in the preparation of the catalyst solution a large percentage of the ferrous iron is oxidized, and that great precaution must be taken to exclude oxygen if oxidation is undesirable. Repeated attempts to isolate the catalyst in pure form were unsuccessful.

As has been shown previously, this catalyst utilizes the oxygen of the air for the oxidation of carbohydrates and certain related products. It is not patent what the mechanism of the oxygen transfer is, but there are three ways possible in which it may occur, namely, (1) by means of surface catalysis, (2) by an electronic oxidation and reduction of the catalyst, (3) by auto-oxidation, a hemoglobin-like oxygenation.[2]

1. It was assumed that if the iron would diffuse through a semipermeable membrane it is present in a molecularly dispersed condition; therefore diffusion experiments were set up which showed not only that the iron is present in a molecularly dispersed form, but also that it is part of a complex radical.

Two solutions were made, one containing sodium ferro-pyrophosphate which had been oxidized by shaking the solution with air, and the other containing sodium ferri-pyrophosphate made by adding ferric sulfate to a solution of sodium pyrophosphate. That neither of the two contained ferrous iron was shown by the fact that when the solutions were acidified

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[2] Conant, *J. Biol. Chem.*, **57**, 401 (1923).

and treated with a 0.02 N solution of potassium permanganate they showed no reduction action. These solutions were put in fish-skin sacs, which were shown to contain no leaks, and dialyzed against distilled water for four days. The dialysate from each was tested for iron in the following manner.

In one portion the absence of ferric ion was demonstrated by the fact that it gave no test with potassium thiocyanate. Another portion, boiled with dil. sulfuric acid in order to break up the complex ion, gave the characteristic coloration for ferric ions. From these diffusion experiments we may conclude that the iron is in a complex molecule which is molecularly dispersed. Hence, the assumption that the oxidation is one of surface catalysis becomes improbable.

2. Many substances are oxidized by either the ferro or ferric compound. If, therefore, it can be proved by electrochemical means that the reaction, ferro $\rightleftharpoons$ ferri $+\ e$, is possible, then the second hypothesis will give a logical explanation of the oxidation. The experiments, which will be described, show that this is a possibility.

The Oxidation Potential of the Catalyst

To study the electronic reversibility of the oxidized and reduced forms of the catalyst, a cell was constructed similar to that employed by Forbes[3] and his collaborators in their oxidation-reduction studies. The potential was measured between a 0.1 N calomel electrode and a platinum electrode dipping into a solution of the catalyst.[4]

$$\mathrm{Pt}\left|\begin{matrix}\mathrm{Na[Fe(P_2O_7)]}\\ \mathrm{Na_2[Fe(P_2O_7)]}\end{matrix}\right., \mathrm{KCl}\ 0.1N \left| \mathrm{KCl}\ 0.1N,\ \mathrm{Hg_2Cl_2}\ (\text{solid}) \right| \mathrm{Hg};\ E = 0.540$$

This, in the form of the ferro compound, was partially oxidized by means of atmospheric oxygen, the solution was stirred, the potential measured, and two samples were withdrawn into tared weighing bottles containing dil. sulfuric acid. The ferrous iron was estimated in one and total iron in the other by the methods outlined above. From these values the ratio of ferric to ferrous iron was calculated. A value for the standard potential, E_0, of the cell was then calculated by the well-known formula, $E_0 = E + K \log (\mathrm{Fe}^{+++}/\mathrm{Fe}^{++})$, where E is the measured electromotive force, K is a constant, Fe^{+++} and Fe^{++} are the concentration of the ferric and ferrous anions. Since the ferrous anion is of higher valence than the ferric, its activity will be less for an equivalent concentration than that of the ferric, and consequently the ratio in parenthesis will be greater than the gross concentrations would indicate. To compensate for this, K must be smaller than the theoretical value 0.05915. This we find to be the case. The value for K was found to be 0.0574 from the slope of

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[3] Forbes, This Journal, **46**, 385 (1924), and bibliography.

[4] Lewis and Randall, "Thermodynamics and the Free Energy of Chemical Substances," McGraw-Hill Book Co., Inc., **1923**, p. 389.

the curve obtained by plotting E against log (Fe^{++}/Fe^{+++}). It was not deemed necessary to include this plot in the text. Thus we get some idea of the relative activities of the two anions. From Table I we see that E_0 is very nearly constant, showing that we are dealing with a true electronic equilibrium which substantiates the second hypothesis given above. Furthermore, the reaction, $^{=}(FeP_2O_7) \rightleftarrows {}^{-}(FeP_2O_7) + e$, appears to be represented by unit electronic change which agrees with the structure proposed by Roseheim[5] for complex pyrophosphates of other trivalent atoms such as manganese and chromium, rather than that of Pascal,[6] namely, $^{8-}[Fe_2(P_2O_7)_3] \rightleftarrows {}^{6-}[Fe_2(P_2O_7)_3] + 2e$. It is impossible to establish a standard potential for this oxidation reaction from the data obtained, for no account was taken of the hydrogen-ion concentration, but work is now in progress on such a study. The results of this investigation are summarized in Table I. All measurements were made at 25°.

TABLE I

E.m.f.	Ratio Fe^{++}/Fe^{+++}	0.0574 × log ratio	E_0(total cell)
0.520	0.419	−0.0217	0.542
.562	2.381	.0216	.540
.559	2.400	.0218	.537
.527	0.539	− .0154	.542
.479	.082	− .0623	.541

For the purpose of the present investigation it is necessary only to show that the catalyst is electronically reversible. That such is the case is proved beyond a doubt by the constancy of the value E_0. Therefore, it may be said with reasonable assurance that one mechanism by which the oxidation is carried on,[7] is through the reversibility of the reaction, catalyst oxidized = catalyst reduced —e.

Regarding the third possible mode of oxidation, that of autoxidation or moloxide formation, it is pertinent that some substances are oxidized by the ferro compound but not affected by the ferri-derivative. In view of the special treatment required for this subject, it is described separately in the next paper.

Mechanism of the Oxidation

The shape of the oxidation curve for glucose (reproduced in the previous paper) shows an initial relatively low rate of formation of carbon dioxide which rises rapidly during the first 48 hours. No definite theory can as yet be advanced to account for this lag, but it is probable that intermediate products, either of oxidation or compound formation which subsequently

[5] *Ber.*, **48,** 582 (1915).

[6] Pascal, *Ann. chim.*, [8] **16,** 386 (1909). *C. A.*, **2,** 1394, 1937 (1908); **3,** 1505 (1909). Oliveri-Mandalà, *C. A.*, **15,** 3038 (1921).

[7] It has been calculated that the single electrode potential of this reaction (P_H = 9, approximately) is +0.21 volts. We do not, however, wish to give any value as correct at the present time.

split off carbon dioxide, are being formed. In order to gain some light on the mechanism of oxidation, a number of carboxylic acids which are known oxidation products of glucose, and related acids were tried with the same oxidation method with air. Thus the sugar acids, *d*-gluconic and *l*-arabonic, have a very much shorter period of induction than glucose. This indicates that the induction period may at least partially be accounted for by the oxidation of the aldehyde group in the glucose. It was thought that the period of induction of *d*-gluconic acid may be due to the oxidation of the primary alcohol group. While this was apparently supported by the fact that tartaric acid shows no induction period, it was made improbable when it was found that *d*-saccharic acid is oxidized only very slowly.

As yet we do not feel justified in formulating a theory of the mechanism of the oxidation, as more knowledge is required of the action of sodium phosphate on the sugar molecule as well as of the catalyst itself. It may be stated, however, that separate experiments on the effect of disodium phosphate and sodium pyrophosphate on hexose sugars now in progress show that these phosphates exert a strong enolizing influence. There are two distinct reactions produced by disodium phosphate on the glucose molecule. The first of these is quite analogous to the mutual conversions discovered by Lobry de Bruyn and van Ekenstein with stronger alkalies in which *d*-glucose is converted into a mixture of *d*-glucose, *d*-mannose, *d*-fructose, *pseudo*-fructose and α- and β-*d*-glutose.[8] That this is not a true equilibrium (at least with disodium phosphate) is indicated by the occurrence of the second of the reactions. This constitutes a splitting of the glucose molecule, probably at the double bonds of the endiols. In a solution of *d*-glucose and disodium phosphate there is considerable tar formation with an appreciable decrease in the reducing power of the mixture, though no saccharinic acids are formed. We are of the opinion that this tar formation arises from the condensation or polymerization of the products formed by the splitting of the hexose endiols. In the presence of an oxidizing or reducing agent there is no tar formation, but other substances with one, two or three carbon atoms are formed. In a mixture of *d*-glucose and disodium phosphate the reactive substances are the splitting products of the endiols. In the presence of the catalyst and atmospheric oxygen some of these products are oxidized while others are not or only partially. The oxidation products of the *d*-glucose are thus largely derived from the splitting of 1,2, 2,3, and 3,4 endiols. Such splitting may yield a multiplicity of compounds among which are the acids the oxidation of which we have studied. The investigations of the action of sodium phosphate on glucose will be described in a later paper.

The experiments on the oxidation of various organic acids are summa-

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[8] *Rec. trav. chim.*, **14**, 203 (1895); **15**, 92 (1896); **16**, 257 (1897).

rized below. These were carried out under identical conditions with the oxidation of the carbohydrates, namely: to 6.7 g. of sodium pyrophosphate decahydrate ($Na_4P_2O_7.10H_2O$) dissolved in 150 cc. of water was added 1 g. of ferrous sulfate heptahydrate; when the sodium ferro-pyrophosphate thus formed was completely dissolved 15.3 g. of sodium phosphate dodecahydrate was added and finally the sodium or potassium salt of the organic acid in molecular proportion to 3 g. of *d*-glucose. The oxidations were carried out in a thermostat at 38° in the manner described in the previous paper on this subject.

Formic Acid.—A portion of this acid (sodium salt) was immediately oxidized, but the formation of carbon dioxide soon ceased. The oxidation of glucose is possible whether the ferro or ferri compound is initially present. With formic acid, however, as soon as the ferro compound is oxidized to the ferri form, the oxidation proceeds no further. Formic acid does not reduce the ferric compound, or only very slowly, and thus cannot regenerate the ferrous compound which is the active agent in the oxidation with air. This becomes evident from the following experiments in one of which the ferric compound was used, in the other the ferrous compound. Thus, in 24 hours ferric compound gave 0.0013 g. of carbon dioxide and ferrous compound 0.0245 g., while in 48 hours the amounts were 0.0011 g. and 0.0011 g., respectively.

During the first 24 hours most of the ferrous compound is oxidized to the ferric compound which is a very poor catalyst for this oxidation.

Sodium acetate is oxidized very slowly: 0.0063 g. of carbon dioxide was produced during the first 24 hours; thereafter, it practically ceases. Potassium glycolate, sodium oxalate, sodium lactate, sodium glycerate and sodium pyruvate showed the same behavior. Potassium malonate and sodium succinate are not oxidized. The behavior of the dicarboxylic acids was somewhat surprising. In 120 hours the following amounts of carbon dioxide were formed: potassium saccharate, 0.0336 g.; sodium trioxyglutarate, 0.0344 g.; sodium potassium tartrate, 0.2601 g.; potassium tartronate, 0.1480 g.

The Rate of Reduction of Sodium Ferri-pyrophosphate

It has already been mentioned that the oxidation of glucose with air and sodium ferro-pyrophosphate may take place in two ways: one, by the direct reaction of the ferric compound with glucose; the other, by the intermediate moloxide formed from the ferrous compound and oxygen. In order that the oxidation may proceed catalytically, that is, to have the iron compound oxidize more than the stoichiometrical amount of glucose, it is necessary to assume that the glucose reduces the ferric derivative, and that the ferrous compound thus formed combines with atmospheric oxygen with the formation of either the ferric or the moloxide compound or both. For this reason it was important to determine whether glucose would reduce sodium ferri-pyrophosphate. Continuous catalysis assumes a condition of steady state; it was therefore necessary to determine whether a steady state could be postulated in this oxidation system. Such a postulate is dependent on the fact that a steady state can be reached from either side of the reaction. As it has been shown that sodium ferro-pyrophosphate is rapidly oxidized by air, it remains to be demonstrated that the ferric compound is reduced by glucose in order to justify the assumption of a condition of steady state. That such a reduction does take place will be shown in the following investigation.

Not only was it of value to show that such a reduction takes place, but for convenience in interpreting curves already obtained, it was of value to determine the rate at which the reduction takes place. If we know the rate of reduction of the ferric compound we can predict approximately how fast the atmospheric oxidation can progress, for the oxidation is dependent on the reduction of the ferric to the ferrous form. Qualitatively, these results explain the shape of the oxidation curves obtained in the earlier work. The induction period found there, has been shown to be due to a delayed reduction rather than to the oxidation of the carbonyl to the carboxyl group. This has played a large part in clarifying the organic reactions involved. Furthermore, the slowness of the reduction of the ferric compound by glucose, and the rapidity with which the ferrous compound is oxidized by air, explain why, even though we assume a steady state to be reached, no ferrous iron could be detected qualitatively. Undoubtedly, the steady state is very near the ferric side. Still our efforts to find a promoter to hasten the rate of ferrous compound formation have not as yet met with any marked success.

These experiments may throw considerable light on the mechanism by which sugars can be oxidized with air. The great difference in rate of oxidation between glucose, fructose and mannitol are clearly demonstrated.

A solution of sodium ferri-pyrophosphate was prepared by dissolving ferric pyrophosphate in sodium pyrophosphate. An excess of the former was used and the undissolved portion was removed by filtration. The reduction experiments were carried out with the resulting clear solution, diluted as required, and to this were added solutions whose effects on the reduction of the iron were to be studied. The experiments were carried out in tubes containing 30 cc. of the mixed solutions, evacuated, sealed and placed in a thermostat. The rate of reduction was determined from the amount of ferric iron remaining. This was ascertained by breaking the tube under dil. hydrochloric acid and titrating the hot solution with stannous chloride.[9] Separate experiments showed that the method was

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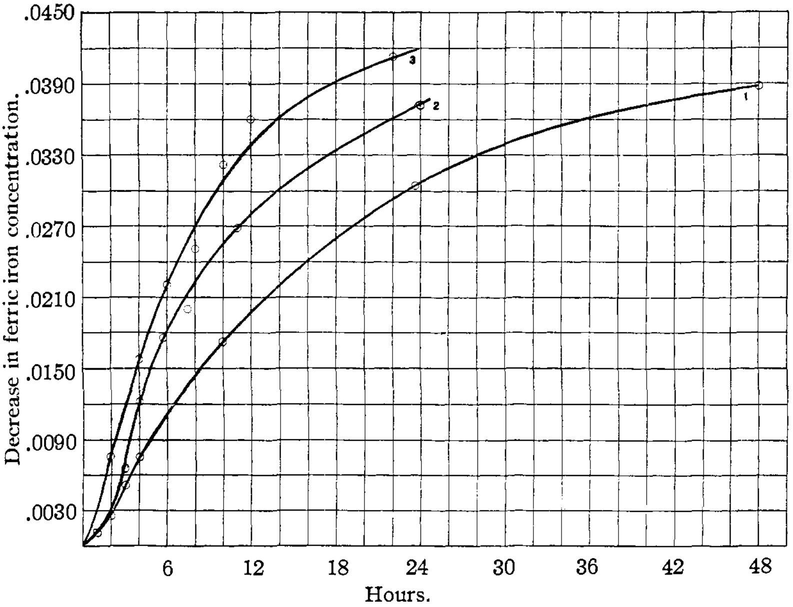
[9] Treadwell and Hall, "Analytical Chemistry. Quantitative Analysis," John Wiley and Sons, Inc., 6th edition, vol. 3, p. 594.

reliable in the presence of glucose, mannitol, formic and oxalic acids; with fructose, however, it was not entirely reliable. In many of the experiments a precipitate appeared in the sealed tubes after a few hours. It was established that there was no ferric iron in this precipitate and that in all probability it was the sodium ferro-pyrophosphate which is much more easily precipitated than the ferric compound.

In Fig. 1 is shown the influence of different concentrations of glucose on the rate of reduction. As the oxidation of glucose would make the mixture acid and acid tends to break up the iron complex, an excess of sodium pyrophosphate was used. The initial concentrations in moles per liter were as follows: iron (as sodium ferri-pyrophosphate), 0.0434; sodium pyrophosphate, 0.0300; *d*-glucose, 0.120, 0.08 and 0.04.



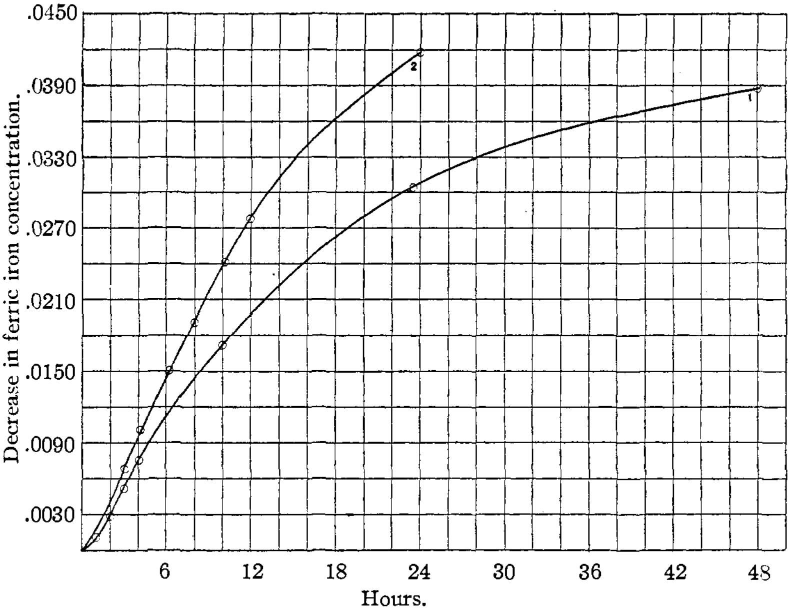
Influence of increased glucose concentration.

Fig. 1.—Rate of reduction of sodium ferri-pyrophosphate. 1, 0.04 *M;* 2, 0.08*M;* 3, 0.120 *M* glucose.

In Fig. 2 is shown the effect of different concentrations of the iron compound. In these experiments the concentrations were: sodium pyrophosphate decahydrate, 0.0300 *M;* glucose, 0.0400 *M;* and sodium ferri-pyrophosphate, 0.0434 *M* and 0.0877 *M*.

In Fig. 3 is shown the influence on the rate of reduction of different concentrations of sodium pyrophosphate; the glucose was 0.120 *M* and the iron 0.0878 *M*.

The experiments recorded in the three figures given here were carried out at 63.5°. The fact becomes evident that there is an induction period in these reduction reactions. This induction period may be due either to compound formation between the glucose and the iron compound, or to a breaking down of the glucose into reactive products or transformation into a more reactive form. That compound formation between glucose and the iron is not likely is indicated by the fact that doubling the concentration of the iron does not appreciably lower the induction period. That it is due to a change in the glucose molecule is shown by the fact that increased glucose concentration, as well as increased concentration of sodium pyrophosphate, decreases the time of the induction period. Bearing on this point are the experiments, already mentioned, which show that in a solution of sodium pyrophosphate glucose, is converted into fructose. Although it was impossible to obtain accurate data on the reducing power of fructose, on account of analytical difficulties, these are sufficient to indicate that the reduction with fructose is about 20 times as fast as with glucose and shows only a very slight induction period. This marked difference in the behavior between glucose and fructose is of considerable biological significance.[10] The results of Dakin and Dudley[11]



Influence of increased iron content.

Fig. 2.—Rate of reduction of sodium ferri-pyrophosphate. Initial concentration of ferric iron: 1, 0.0434 *M*; 2, 0.0877 *M*.

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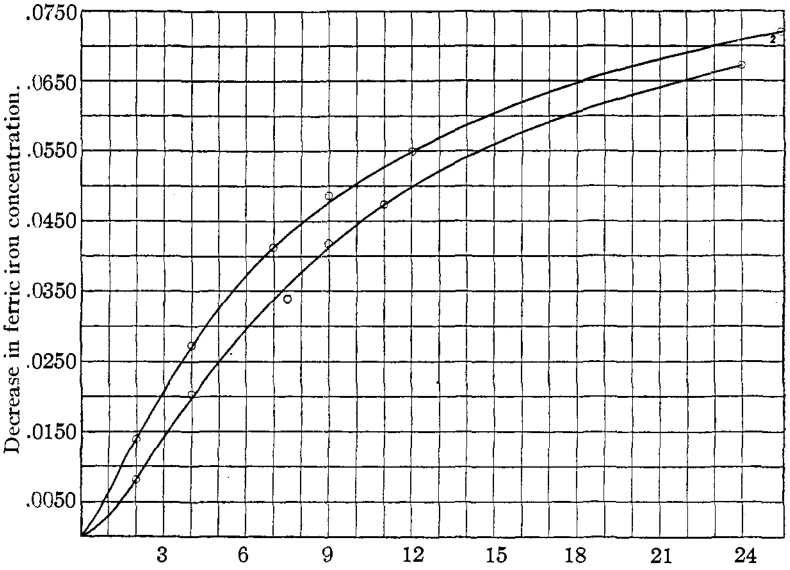
[10] Lusk and Graham, *J. Biol. Chem.*, **20**, 555 (1915). Nef, *Ann.*, **357**, 219 (1908). Warburg and Yabusoe, *Biochem. Z.*, **146**, 380 (1924).

[11] Dakin and Dudley, *J. Biol. Chem.*, **15**, 127 (1913).

and of Neuberg[12] who obtained methylglyoxal from glucose when heated with disodium phosphate are also of significance in this connection.

The rate of reduction of the ferric compound by mannitol is about $^1/_{25}$ of that by glucose. This is of great interest in view of the fact that mannitol is oxidized with air when sodium ferro-pyrophosphate is initially present and suggests that a small amount of a more strongly reducing substance (formed by the oxidation of mannitol, possibly mannose) which acts as a "primer," is essential for the relatively rapid oxidation of mannitol with air.



Influence of sodium pyrophosphate.

Fig. 3.—Rate of reduction of sodium ferri-pyrophosphate with different concentrations of sodium pyrophosphate: 1, 0.0300 *M*; 2, 0.0600 *M*.

Table II

Influence of Temperature on Reaction Rate

Glucose: 0.040 *M* $Na_4P_2O_7.10H_2O$: 0.030 *M*						Glucose: 0.120 *M* $Na_4P_2O_7.10H_2O$: 0.030 *M*			
25.25°		37°		63.5°		55°		63.5°	
Hours	Concn. Fe'''	Hours	Concn. Fe'''	Hours	Concn. Fe'''	Hours	Concn. Fe'''	Hours	Concn. Fe'''
0	0.0425	0	0.0425	0	0.0430	0	0.0873	0	0.0878
91	.0431	29	.0417	4	.0362	4.75	.0815	2	.0796
143	.0414	56	.0408	6	.0316	18.75	.0620	4	.0676
191	.0410	82	.0411	8	.0284	25	.0543	7	.0539
308	.0390	148	.0371	12	.0228	28	.0512	9	.0462
432	.0379	195	.0363	23	.0128	42.4	.0401	11	.0406
527	.0386	340	.0313	26	.0105	48	.0366	24	.0208
575	.0377	436	.0314	51	.0036	72	.0269		

We intentionally refrain from publishing temperature coefficients of the reaction at this time.

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[12] Neuberg, *Biochem. Z.*, **55**, 494 (1913); **71**, 144 (1915).

That temperature plays an exceedingly important part in the rate of reduction becomes apparent from the data in Table II, giving the decrease in the concentration of the ferric compound, expressed in moles per liter at different temperatures.

Influence of Potassium Cyanide

Warburg[13] has found that a small concentration of potassium cyanide inhibits the oxidation of amino acids at the surface of charcoal. He attributes this inhibition to the fastening of the cyanide to active points on the charcoal surface. He has also shown that the effect is more pronounced in charcoals that are rich in iron. From this he reasons that cyanide inactivates the iron present on the charcoal surface, the iron being the true catalyst for the oxidation. Since the facts seem to bear out his theory, and since such a method of inactivation should have a tremendous interest in its relationship to biological oxidation processes, it was decided to investigate the effect of cyanide on the rate of oxidation of glucose in the presence of the homogeneous catalyst, sodium ferro-pyrophosphate. Any results which accrued from these experiments might throw some light on the effect of cyanide in animal organisms.

Enough cyanide was added to the reaction mixture, described in the previous section, so that in four different experiments the ratios, CN/Fe, were 2/1, 1/1, 1/10 and 0. The results of the four oxidations are as follows: in 240 hours the total evolution of carbon dioxide at 38° with 0.468, 0.234, 0.0234 and 0.00 g. of potassium cyanide was 0.2214, 0.3828, 0.6871 and 0.6286 g., respectively.

It is evident that cyanide in the more concentrated solutions very materially lowers the rate and amount of evolution of carbon dioxide. At the ratio 1/10, however, there is a distinct stimulation of the oxidation process. In this solution the potassium cyanide is 0.0025 *M*. A duplicate experiment showed that 0.025 *M* cyanide solution was not so active, so the maxmum excitation must lie between 0.025 and 0.00025 *M* cyanide solution, probably near the concentration which we have reported, namely, 0.0025 *M*. Repetition of the experiments gave the same results.

The addition of cyanide changes the color of the reaction mixture from a bluish-green to a distinct orange. To what this color is due we have no evidence, but it is possible that the cyanide displaces the pyrophosphate from the complex and a ferricyanide results which has been shown to be inactive in catalyzing the oxidation.

Influence of Platinum Gauze

Platinum gauze was placed at the air inlet of the apparatus used to break the air streams into small bubbles. Since Wieland[14] has shown that palla-

[13] Warburg, *Biochem. Z.*, **136,** 266 (1923); **142,** 518 (1923). Meyerhoff, "Chemical Dynamics of Life Phenomena," Lippincott Co., **1924,** p. 16.

[14] Wieland, *C. A.*, **7,** 346 (1913); **8,** 715 (1914).

dium catalyzes oxidation reactions, it was thought that the platinum might be aiding in this oxidation. In order to test this matter, a glass inlet was prepared and an oxidation experiment run with sodium potassium tartrate, duplicating a like experiment using the platinum gauze. Practically no difference in the rate or total amount of carbon dioxide evolution was observed, showing that platinum does not influence this reaction.

Effect of Insulin on the Rate of Oxidation of Glucose

The determination of the strength of insulin preparations is dependent upon biological methods which are cumbersome and not very accurate. It seemed desirable to ascertain whether the oxidation of glucose, by the method described, was influenced by insulin, both because it might offer a means of assaying insulin preparations and possibly throw some light on the mechanism of the action of insulin. The experiments were carried out by Mr. Howard Estill and we are indebted to the California Institute of Technology for the insulin used. The oxidation experiments were conducted as has been previously described, and consisted of a blank containing glucose, one with 300 units of insulin and another with an equal quantity of insulin which had been inactivated by dissolving in 20 cc. of 0.01 *N* sodium hydroxide solution, heating to 97° for 15 minutes, neutralizing and passing hydrogen through the solution for 22 hours to remove any hydrogen sulfide. To each flask 0.6 cc. of xylene was added to prevent the foaming caused by the air stream. The results of the experiments were, however, negative, in that no appreciable differences in the amounts of carbon dioxide formed were obtained.

Summary

Sodium ferro-pyrophosphate, which acts as a catalyst in the oxidation of carbohydrates and hydroxy acids with air, is easily oxidized by atmospheric oxygen. One mechanism by which the oxidation may be carried on is through the reversibility of the reaction: catalyst oxidized = catalyst reduced $- e$. A study of the electronic reversibility of the catalyst indicated unit electronic change, which agrees with the formula for sodium ferro-pyrophosphate, $Na_2(FeP_2O_7)$, and for sodium ferri-pyrophosphate, $Na(FeP_2O_7)$. It was not possible to obtain pure crystalline preparations of these compounds. The catalyst is not colloidal and gives none of the characteristic tests for iron. Carbohydrates are oxidized whether the ferro or ferri compound is initially present. Formic acid is not oxidized with the ferri compound. Only such compounds as can reduce the ferri compound are capable of the catalytic oxidation. Apparently this is associated with the degree of dissociation of the hexoses brought about by the phosphates. The rate of oxidation of a number of organic acids was studied. The rates of reduction of sodium ferri-pyrophosphate by glucose, fructose and mannitol were studied. Low concentrations of potassium cyanide (.0025 *M*) accelerate the rate of oxidation. Platinum has no effect. The rate of oxidation of glucose by air in this system is not influenced by insulin.

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[CONTRIBUTION FROM THE CHEMICAL LABORATORIES OF COLUMBIA UNIVERSITY, NO. 494]

RESEARCHES ON THIAZOLES. VIII. THE CONDENSATION OF *o*-AMINOPHENYL MERCAPTAN AND *o*-AMINOPHENYL DISULFIDE WITH ACID CHLORIDES, ANHYDRIDES AND ESTERS[1]

BY MARSTON TAYLOR BOGERT AND ARTHUR STULL[2]

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Introductory

The work recorded in this paper was undertaken not alone for the purpose of contributing to our knowledge of the chemistry of the thiazoles, but also because we believe that there exist possibilities of discovering in this field some products of therapeutic value. Many of the compounds described beyond are derivatives of the *o*-aminophenyl disulfide first prepared by Hofmann,[3] and later recommended by McDonagh,[4] under the name of "Intramine," for use in the treatment of spirochetal infections. Several of these were converted into the corresponding benzothiazoles by reduction.

The disulfide and ethyl oxalate[5] interacted to form the ethyl oxanilate disulfide, $(—SC_6H_4NHCOCOOR)_2$ (I) and no oxalyl derivative, $\overline{SC_6H_4NHCOCONHC_6H_4S}$ (II).

By the action of ammonia and of aniline upon the oxanilate, the corresponding amide and anilide were obtained. Reduction of the oxanilate yielded benzothiazole-2-carboxylic acid, which has been prepared previously by other methods. Through the interaction of oxalyl chloride and zinc *o*-aminophenyl mercaptide, *bis*-benzothiazolyl (oxalamidothiophenol) was obtained in 60% yield, an interesting compound which has been synthesized before by other reactions.[6,7,8,9]

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[1] An abstract of this paper was presented at the Baltimore Meeting of the American Chemical Society, April 9, 1925.

[2] Ferguson Fellow at Columbia University for the year 1924–25.

[3] Hofmann, *Ber.*, **12**, 2360 (1879).

[4] McDonagh, *Lancet*, **190**, 238 (1916); Ger. pat. 68,697 (1892).

[5] Compare Meyer and Seeliger, *Ber.*, **29**, 2640 (1896).

[6] Hofmann, *Ber.*, **13**, 1227 (1880).

[7] Hofmann, *Ber.*, **20**, 2257 (1887).

[8] Lang, *Ber.*, **25**, 1902 (1892).

[9] Lauth, *Bull. soc. chim.*, [3] **15**, 82 (1896).