CXLV. THE CATALYSIS OF PEROXIDE OXIDATIONS BY FERROUS IONS.

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THE importance of "oxygenase" systems in biological oxidations has been abundantly shown by the work of Engler and Wild [1897], Bach and Chodat [1902-1904], Manchot [1902], Gallagher [1923], Warburg [1921-1928], and Thurlow [1925]. The "oxygenase" theory may be briefly summarised as follows.

There exist in living cells certain autoxidisable substances capable of uniting with molecular oxygen and so giving rise to highly oxygenated peroxide-like compounds which act as powerful oxidising agents. If A represents such an autoxidisable substance and B a substance capable of acting as an oxygen acceptor, then the oxidation of B is represented by the following scheme:

A model for this reaction is to be found in the well-known induced oxidation of indigo to isatin in the presence of benzaldehyde, first described by Baeyer and Villiger [1900].

The transference of oxygen from the "peroxide" (AO_2) to the oxidisable substance B is made possible by the intervention of enzymic systems known as peroxidases. How these exert their specific influence is still a matter of conjecture, but it is thought that metals such as iron, copper and manganese may play a part. Warburg [1925], indeed, ascribes to iron an extremely important rôle in tissue oxidations and postulates a complete "oxygenase" system in which the iron activates molecular oxygen and brings about its transference to organic substances. Closely related is the *in vitro* function of iron as a catalyst in reactions between organic substances and oxygen which is already in an activated form. As such the function of iron is that of a peroxidase. This catalytic function of ferrous iron was first observed by Fenton [1898] who demonstrated the oxidation of tartaric acid to dihydroxymaleic acid by hydrogen peroxide in the presence of ferrous salts. Brode [1901] put forward the view that an unstable compound is first formed by combination of the ferrous salt and hydrogen peroxide and that this is the actual catalyst. Mummery [1913], investigating the reaction between ferrous salts and hydrogen peroxide, found that each gram-atom of ferrous iron required for oxidation to the ferric state one half-gram-molecule of hydrogen peroxide, and further, that hydrogen peroxide may be catalytically decomposed by addition to a solution of a ferrous salt. Mummery made use of Trillat's reagent (tetramethyldiaminodiphenylmethane in citric acid solution) which gives a blue colour with the lead dioxide class of superoxides but remains unchanged in the presence of true peroxides. He found that when hydrogen peroxide is added to a solution of a ferrous salt containing Trillat's

reagent a deep blue colour results. No colour is given when Trillat's reagent is added to a solution of a ferrous salt after the addition of hydrogen peroxide. The reagent is not coloured by ferrous salts, ferric salts or hydrogen peroxide alone or by ferric salts in the presence of hydrogen peroxide.

Mummary concluded from these findings that in the oxidation of ferrous salts by hydrogen peroxide there is an intermediate stage with the production of an unstable and extremely active oxidising agent. He suggested that this might be the actual oxidising agent in Fenton's reaction and assigned to it a perhydrol structure $\text{HO}\cdot\text{O}-\text{Fe}-\text{SO}_4\text{H}$, ferrous sulphate perhydrol.

More recently Küchlin and Boëseken [1929], studying the oxidation of fructose and glycerol, have suggested that in such oxidation systems the first reaction is the formation of a binary complex of ferrous salt and the oxidisable substance. This may then be followed by an addition of hydrogen peroxide giving a ternary complex which is disrupted by the act of oxidation, liberating ferric iron and the oxidation product.

It is evident from the foregoing résumé that the rôle of iron in catalysed oxidations is far from clear. It is still uncertain whether the iron undergoes a change of valency while acting as a catalyst and how such a change of valency, if it does take place, is related to other reactions occurring between members of the system. The present study has been directed on both qualitative and quantitative lines towards the elucidation of the reactions involved in the catalysis of peroxide oxidations by means of ferrous iron. The qualitative observations are reported in Part I and the quantitative aspects in Part II.

I. QUALITATIVE ASPECTS.

Several systems of the form hydrogen peroxide—ferrous iron—oxidisable substance have been examined. Qualitative tests have been carried out to determine the fate of each member of the system, while any special features of each system having a bearing on the points at issue have been noted.

Ferrous iron was detected by means of the ferricyanide reaction and ferric iron by the colour given with thiocyanates. Hydrogen peroxide was detected by means of the blue ether-extractable colour given with chromic acid. Oxidisable substances have been used in the form of stock solutions of known strength. Dilutions were made from these as required. Ferrous salts have been used in the form of freshly prepared solutions of known strength in glass-distilled CO_2 -free water. Hydrogen peroxide was used in the form of neutral dilutions from Merck's perhydrol. Each solution was standardised against KMnO_4 immediately before use.

The method of carrying out the examinations was as follows:

A measured quantity of the stock solution of the oxidisable substance was added to a known volume of the ferrous salt solution. This was followed by distilled water of such volume that after addition of the hydrogen peroxide solution (which was added last) the required concentrations of the reagents were attained.

After addition of the hydrogen peroxide the qualitative tests were carried out.

The oxidation of tartaric acid.

The following solutions were examined:

- (1) 0.1 *M* tartaric acid which was 0.01 *M* with respect to ferrous salt and 0.01 *M* with respect to hydrogen peroxide.

(2) 0.1 *M* tartaric acid which was 0.01 *M* with respect to ferrous salt and 0.1 *M* with respect to hydrogen peroxide.

(3) and (4) as (1) and (2) but without tartaric acid.

Controls:	1	2	3	4
Hydrogen peroxide	-	-	-	+
Ferrous iron	+	+	-	-

In solutions 3 and 4 addition of peroxide gave rise to a strong orange-yellow colour which persisted—the thiocyanate test being strongly positive.

Addition of hydrogen peroxide to solutions 1 and 2 gave a greenish yellow colour which faded rapidly giving a water-clear solution free from ferric iron. A positive thiocyanate reaction was only given while the greenish yellow colour persisted in the solution.

It was noted that the time required for the disappearance of the colour produced by addition of hydrogen peroxide to a solution of tartaric acid containing ferrous iron became less with successive additions.

A typical example may be given.

0.1 *M* tartaric acid containing 0.01 *M* ferrous sulphate was taken.

0.01 *M* hydrogen peroxide was added and the time taken for the colour so produced to fade was noted by means of a stop-watch. A second proportion of peroxide was now added and the time again noted, the process being repeated until, in all, ten additions of peroxide had been made.

No. of additions of peroxide	1	2	3	4	5	6	7	8	9	10
Time in seconds	194	130	83	81	79	75	76	85	74	74
	198	128	81	81	78	76	74	72	73	72

It will be seen that the time required for the disappearance of the ferric iron initially formed decreases as the concentration of the tartaric acid decreases—the reduction therefore cannot be brought about by a reaction in which tartaric acid plays a part.

To a solution of tartaric acid containing ferrous iron there were added a few drops of Trillat's reagent (1 % tetramethyldiaminodiphenylmethane in 1 % citric acid). On addition of hydrogen peroxide a deep blue colour was formed. This, however, quickly faded giving a water-clear solution.

A solution of tartaric acid containing ferrous iron was divided into two portions *a* and *b*. To *a* a few drops of Trillat's reagent were added. Hydrogen peroxide was then added to both solutions. It was observed that the colours initially formed faded rapidly, the blue of *a* and the greenish yellow of *b*, and that the solutions became colourless simultaneously. No colour was given on the addition of Trillat's reagent to solution *b* after the fading of the greenish yellow colour. Addition of Trillat's reagent while the greenish yellow colour persisted gave the blue colour.

The above observations lead to the conclusion that the same substance is responsible for giving the ferric iron test, the greenish yellow colour of the solution and the reaction with Trillat's reagent. It appears to have but a temporary existence, and the rate of its disappearance does not appear to bear any relationship to the amount of tartaric acid present in the solution.

A further experiment bearing on the production of this labile substance may be reported.

To a solution of a ferrous salt an amount of hydrogen peroxide more than

sufficient to oxidise all the iron to the ferric state was added. Immediately the solution became brown in colour and effervescence took place. If allowed to stand the effervescence stopped and a brown precipitate formed. No ferrous iron could be detected in the solution.

If, however, a solution of tartaric acid were added while effervescence was taking place the deep brown colour of the solution faded immediately to greenish yellow. This latter colour persisted for some time and finally faded to leave a water-clear solution. Only ferrous iron could now be detected in the solution. The production of the labile substance mentioned above does not seem therefore to be conditional on the presence of ferrous iron in the solution.

The discussion of the facts reported in this section is postponed until the examination of other oxidation systems has been described. The reactions taking place during the oxidation of tartaric acid appear to proceed very rapidly and it was felt that it would be advantageous to examine systems requiring a longer time for the completion of the various phases of reaction.

The oxidation of d-glucose.

d-Glucose was shown to be oxidised to glucosone by Fenton's reaction by Morrell and Crofts [1899].

Preliminary study revealed that with *d*-glucose the reaction appeared to proceed at a much slower rate than with tartaric acid, and it has accordingly been chosen to illustrate certain points.

When hydrogen peroxide is added to a solution of *d*-glucose containing ferrous iron there is initially developed a yellowish brown colour. This, as shown by a positive thiocyanate test, is due to the presence of ferric iron. The colour persists for a long time but slowly disappears until finally a solution is obtained which is only slightly yellow in colour. This solution is now free from ferric iron but contains ferrous iron.

It was found that on adding sufficient hydrogen peroxide the presence of this reagent could be detected in solution. The peroxide was found to disappear quite rapidly from the solution, the time required depending on the amount of peroxide added. The disappearance of the ferric iron did not, however, coincide with the disappearance of the peroxide. Indeed a very much longer time was required for the solution to react negatively to thiocyanate. In one experiment a solution was made 0.1 *M* with respect to *d*-glucose, 0.01 *M* with respect to ferrous sulphate and 0.1 *M* with respect to hydrogen peroxide, the oxidising agent being added last. It was found that all the peroxide had disappeared from the solution in 12 minutes, while the ferric iron did not disappear until after 24 hours had elapsed.

Table I.

d-Glucose 0.1 *M* and FeSO₄ 0.01 *M* throughout.

No. of tube	1	2	3	4	5	6	7	8	9	10
Molarity hydrogen peroxide	0.01	0.02	0.03	0.04	0.05	0.06	0.07	0.08	0.09	0.10
Ferric iron	+	+	+	+	+	+	+	+	+	+
Ferrous iron	+	+	+	+	-	-	-	-	-	-
Hydrogen peroxide	-	-	-	-	+	+	+	+	+	+

In Table I are given the results of observations on a typical glucose system, the tests being carried out immediately after the addition of hydrogen peroxide.

It will be seen that in every case, with additions of peroxide up to concentrations representing 4 g.-mol. proportions of hydrogen peroxide per g. atom of iron, ferric and ferrous iron were found in the solution. The intensity of the thiocyanate test increased as that of the ferricyanide test decreased. The presence of hydrogen peroxide could be detected only in those tubes from which all the ferrous iron had disappeared. Solutions 5–10 were of a uniform yellow colour; all showed the presence of hydrogen peroxide and absence of ferrous iron.

The difference in times taken for the disappearance of ferric iron and hydrogen peroxide strengthens the impression obtained in the study of tartaric acid oxidations that the reappearance of ferrous iron is not associated with the primary oxidation taking place in such systems. The amount of hydrogen peroxide required to be added before all the ferrous iron disappears should be contrasted with the 0.5 g.-mol. proportion required in the absence of an oxidisable substance. This point is dealt with more fully in Part II.

The oxidation of i-inositol.

So far no information has been obtained as to the behaviour of the oxidisable substance in such systems. In order to provide this, some observations may be reported on the properties of peroxide-iron systems containing *i*-inositol.

In the course of a study of the oxidation of *i*-inositol it was observed that addition of hydrogen peroxide to solutions of *i*-inositol containing ferrous iron gave rise to the production of a deep blue colour. This colour is the result of a reaction between ferric ions and the first oxidation product of *i*-inositol—an example of the general reaction between ferric ions and substances containing phenolic groups.

i-Inositol

First oxidation product
(Gives blue colour with ferric ions)

It will be readily appreciated that such systems are self-indicating so far as the presence of oxidation product is concerned.

When hydrogen peroxide is added to a solution of *i*-inositol containing ferrous iron there is initially developed a yellowish brown colour and the thiocyanate test is positive. In a short time the yellowish colour merges into green and finally into a deep blue. This colour persists for a considerable time but gradually fades leaving a water-clear solution giving no reaction for ferric iron.

In Table II is given a summary of a typical experiment with *i*-inositol.

In tubes 1–4 there was no development of blue colour, showing that no oxidation of the *i*-inositol had taken place. In these tubes the ferric iron did not disappear on standing.

In tubes 5–10 the development of the blue colour was followed by a gradual fading to a water-clear solution free from ferric iron.

Table II.

i-Inositol 0.1 *M* and FeSO₄ 0.01 *M* throughout.

No. of tube	1	2	3	4	5	6	7	8	9	10
Molarity hydrogen peroxide	0.01	0.02	0.03	0.04	0.05	0.06	0.07	0.08	0.09	0.10
Ferric iron	+	+	+	+	+	+	+	+	+	+
Ferrous iron	+	+	+	+	—	—	—	—	—	—
Hydrogen peroxide	—	—	—	—	+	+	+	+	+	+
Development of blue colour	—	—	—	—	+	+	+	+	+	+

There would not appear to be any oxidation of the *i*-inositol until all the iron is converted into the ferric state.

The oxidation of formic acid.

The substances examined so far, tartaric acid, *d*-glucose and *i*-inositol, yield on oxidation products which are themselves capable of further oxidation, thus introducing secondary effects. In order to rule out such secondary effects it was decided to study the behaviour of formic acid. This substance gives on oxidation carbon dioxide and water which, being incapable of further degradation, do not introduce secondary effects.

Table III summarises a series of experiments on the oxidation of formic acid.

Table III.

Formic acid 0.1 *M* and FeSO₄ 0.01 *M* throughout.

Tube No.	1	2	3	4	5	6	7	8	9	10
Molarity hydrogen peroxide	0.01	0.02	0.03	0.04	0.05	0.06	0.07	0.08	0.09	0.10
Ferric iron	+	+	+	+	+	+	+	+	+	+
Ferrous iron	+	+	+	+	—	—	—	—	—	—
Hydrogen peroxide	—	—	—	—	+	+	+	+	+	+

This system corresponds exactly with those already reported with the exception that although the hydrogen peroxide disappears rapidly there is no regeneration of ferrous iron. Instead, on standing, the solutions deposit a brownish yellow precipitate which is soluble in mineral acids and gives a positive test for ferric iron.

II. QUANTITATIVE ASPECTS.

Mummery [1913] has found by means of qualitative tests that in the presence of sulphuric acid, 1 g.-mol. of ferrous iron requires for its oxidation to the ferric state 0.5 g.-mol. of hydrogen peroxide.

In the present study ferrous iron is oxidised by hydrogen peroxide and it was desirable to find the quantitative relationships existing between these two substances under the conditions employed.

Procedure. Tubes were made up, each containing a known quantity of ferrous sulphate solution. Known quantities of hydrogen peroxide in increasing amounts throughout the series were then added. The solutions were immediately acidified with sulphuric acid and titrated with potassium permanganate solution. The results of two such experiments are shown in Table IV.

It will be seen that the permanganate titre is least in tube 7 and it may be concluded that in the absence of sulphuric acid 1 g.-mol. of hydrogen peroxide

Table IV.

Tube No.	1	2	3	4	5	6	7
g.-mol. ferrous salt	0.001	0.001	0.001	0.001	0.001	0.001	0.001
g.-mol. peroxide/ferrous salt	—	0.1	0.2	0.3	0.4	0.5	0.6
ml. 0.04868 <i>N</i> KMnO ₄	19.8	15.8	12.0	8.9	5.1	2.5	0.3
	19.8	15.9	12.0	8.9	5.3	2.3	0.2

oxidises 2 g.-mol. of ferrous salt to the ferric state. The somewhat higher figure obtained in the absence of sulphuric acid is due to catalytic decomposition of some hydrogen peroxide.

The results of Part I already reported indicated that secondary reactions in the system chosen for quantitative study would give rise to complications, and in fact it was found that the only oxidisable substance whose oxidation could be followed simply and quantitatively was formic acid.

*The influence of the ratio formic acid/ferrous salt on
the amount of iron oxidised.*

In the qualitative study of the oxidation of formic acid it was found that there was a simultaneous oxidation of ferrous iron. Reference to Table III shows that in the conditions there represented before all the iron is converted into the ferric state there must be in the system an initial concentration of 5 g.-mol. of hydrogen peroxide per g.-atom of iron. This figure was by no means constant—appearing to depend on the ratio formic acid/ferrous salt, as is shown in the following experimental series.

For each ratio ten tubes were made up, each containing the same amounts of formic acid solution and ferrous sulphate solution. Distilled water was then added so that after addition of hydrogen peroxide each tube would contain the same volume of liquid. Hydrogen peroxide solution was added finally and in increasing amount from tube 1 to tube 10. The contents of the tubes were immediately acidified with sulphuric acid, and the amount of ferrous iron remaining in each was determined by permanganate titration. Figs. 1 to 5 show the results in graphical form. In Exps. 2 and 3 (Figs. 2 and 3), solutions of a duplicate series were not acidified with sulphuric acid immediately after the addition of hydrogen peroxide, but were allowed to stand closely stoppered for 17 hours, in which time a brown precipitate settled out. They were then acidified and titrated with permanganate solution.

It will be seen that ferric iron appears in the system long before all the formic acid can have been oxidised. The oxidation of iron and formic acid is therefore simultaneous, or at least the oxidation of the former is not brought about by excess peroxide after all the formic acid has been oxidised. Moreover, the greater the amount of hydrogen peroxide in the system, the greater the amount of iron oxidised. The sharp angle of the curves after meeting the abscissa is due to the presence of excess peroxide in those solutions which were titrated immediately. It will also be seen that it is possible to have all the iron oxidised to the ferric state before sufficient hydrogen peroxide has been added to bring about the complete oxidation of all the formic acid. Although the amount of hydrogen peroxide which must be added initially before all the ferrous salt is oxidised varies greatly with the ratio formic acid/ferrous salt, Fig. 4 shows that this amount is not dependent on the absolute concentrations of the members of the system. With a high ratio formic acid/ferrous salt a larger amount of hydrogen

Fig. 1 $\frac{\text{Formic acid}}{\text{Ferrous salt}} = \frac{20}{1}$.

Fig. 2 $\frac{\text{Formic acid}}{\text{Ferrous salt}} = \frac{10}{1}$.

• Acidified immediately; ■ acidified after 17 hrs.

Fig. 3 $\frac{\text{Formic acid}}{\text{Ferrous salt}} = \frac{5}{1}$.

Fig. 4 $\frac{\text{Formic acid}}{\text{Ferrous salt}} = \frac{2}{1}$.

Fig. 5 $\frac{\text{Formic acid}}{\text{Ferrous salt}} = \frac{1}{1}$.

peroxide must be added to bring about oxidation of ferrous iron than with a low one. These results are roughly summarised in Table V (Fig. 6).

Table V.

Ratio formic acid/ferrous salt	20	10	5	2	1
g.-mol. H_2O_2 which must be added initially to oxidise 1 g.-atom ferrous iron	5.85	4.8	3.0	1.6	1.4

Fig. 6.

Fig. 7.

In the first series of experiments the concentrations of ferrous sulphate and formic acid were unchanged and in every case all the ferrous iron could be oxidised. The influence of the ratio formic acid/ferrous salt is also seen in the following series where the concentrations of hydrogen peroxide and ferrous salt are kept unchanged in 10 tubes while the concentration of formic acid is varied.

Table VI. (Fig. 7.)

FeSO_4 and H_2O_2 $9 \times 10^{-4} M$ throughout.

Ratio formic acid/ferrous salt	1	2	3	4	5	6	7	8	9	10
ml. permanganate solution	4.4	9.1	11.5	13.1	14.8	15.7	16	16.4	16.8	17.1

The further significance of the influence of this ratio will be considered later. Turning now to the correlation of these results with the oxidation of formic acid, the following experiments report the results of determinations of the amount of formic acid oxidised under differing conditions.

The oxidation was followed by the use of a standard baryta apparatus for the absorption of the carbon dioxide evolved. The solution containing the formic acid and ferrous salt was placed in a flask fitted with a three-holed rubber stopper carrying (1) a reflux condenser attached to a baryta absorption apparatus, (2) a burette for the introduction of hydrogen peroxide, (3) an inlet tube for CO_2 -free air. The apparatus was made air-tight and free from carbon dioxide and the absorption apparatus connected. Hydrogen peroxide was introduced and the apparatus shaken for a short time. Concentrated sulphuric acid was then added, and the contents of the flask were boiled while a stream of CO_2 -free air was aspirated through the solution. The amount of carbon dioxide evolved was determined by titrating the excess of baryta with standard oxalic acid solution.

It was found that (1) carbon dioxide was evolved in detectable amounts when very small quantities of hydrogen peroxide were added, (2) oxidation was inhibited by the previous addition of strong mineral acid, (3) when the ferrous salt was replaced by a normal ferric salt no oxidation took place on addition of H_2O_2 , (4) if formic acid was added to a solution of a ferric salt obtained by the oxidation of a ferrous salt by the calculated amount of hydrogen peroxide, evolution of carbon dioxide followed a further addition of hydrogen peroxide.

The following table gives the results of the examination of a typical system.

Table VII.

Ratio formic acid/ferrous salt = 1. Molarity of $\text{FeSO}_4 = 0.004$.

Ratio hydrogen peroxide/ H.COOH	0.5	1	1.5	2	2.5
g.-mol. hydrogen peroxide present	0.002	0.004	0.006	0.008	0.01
ml. 0.3922 <i>N</i> baryta required	5.08 5.18	9.87 10.07	15.21 15.16	18.84 18.44	20.00 19.68
g.-mol. formic acid oxidised	0.00100 0.00102	0.00197 0.00194	0.00298 0.00297	0.00369 0.00362	0.00392 0.00386
Mean	0.00101	0.00195	0.00298	0.00365	0.00389

It will be seen that the amount of formic acid oxidised is very far from being equivalent to the amount of hydrogen peroxide added. Some of the peroxide can, of course, be accounted for by the oxidation of iron which is in this case irreversible. A further series of experiments is summarised in Table VIII. In

Table VIII.

g.-mol. formic acid present	g.-mol. hydrogen peroxide present	g.-mol. ferrous salt present	ml. 0.3922 <i>N</i> baryta required to absorb CO_2	g.-mol. formic acid oxidised	
				Ferrous previously oxidised	Ferrous not previously oxidised
0.004	0.0064	0.004	12.39	0.00243	0.00312
			15.92		
			12.89 16.62	0.00253	0.00326
			Mean	0.00248	0.00319
0.004	0.0084	0.004	15.02	0.00295	0.00368
			18.76		
			14.55 19.61	0.00285	0.00385
			Mean	0.00290	0.00376

these the oxidation of formic acid was carried out in the presence of iron previously oxidised by the requisite amount of hydrogen peroxide (0.6 g.-mol. proportion). These experiments were controlled by others in which the iron was not oxidised before the addition of formic acid.

It appears that the oxidation of formic acid by hydrogen peroxide can be catalysed by the previously oxidised iron salt. The discrepancy between the two methods was however always found. Since hydrogen peroxide disappeared from both types of system in the same way and since the amount of carbon dioxide evolved could not be increased in the first system by a longer period of aeration, the differences observed are not due to differing rates of reaction but to a smaller amount of the formic acid present actually undergoing oxidation in the first system. This is believed to be accounted for by the increased catalytic decomposition of hydrogen peroxide in the presence of the previously oxidised iron salt.

If formic acid was introduced into a solution containing initially a ferrous salt and a large excess of hydrogen peroxide, *i.e.* a solution in which the peroxide itself was undergoing catalytic decomposition, complete oxidation of the acid could be obtained provided the excess of peroxide was sufficiently large.

Table IX.

g.-mol. formic acid present	g.-mol. ferrous salt present	g.-mol. H_2O_2 present	ml. 0.3922 <i>N</i> baryta required	g.-mol. formic acid oxidised
0.004	0.004	0.04	19.56 19.77	0.00384 0.00388
0.004	0.004	0.08	19.55 20.02	0.00393 0.00392

In all the experiments reported the formic acid/ferrous salt ratio has been unity. It has been shown that with this ratio the amount of iron undergoing oxidation is large and that as the ratio increases, the amount becomes progressively smaller. The ratio was now varied, keeping the total volume of the solution and the amount of ferrous salt present constant and the oxidation followed as before. The results of Table X show that as the ratio increases the amount of formic acid oxidised also increases.

Table X.

Molarity of FeSO_4 and $\text{H}_2\text{O}_2 = 0.004$.

Ratio formic acid/ferrous salt	1	2	5	10	20
g.-mol. formic acid present	0.004	0.008	0.02	0.04	0.08
g.-mol. formic acid oxidised	0.00197 0.00194	0.0021 0.0027	0.003 0.00293	0.0033 0.00324	0.00343 0.00353
Mean	0.00196	0.0024	0.00296	0.00327	0.00348
g.-mol. ferrous salt oxidised	0.0032 (<i>a</i>)	0.0023 (<i>b</i>)	0.0014 (<i>c</i>)	0.001 (<i>d</i>)	0.0008 (<i>e</i>)
g.-mol. H_2O_2 to oxidise 1 g.-atom ferrous iron	0.63	0.69	0.76	0.673	0.62

In Table XI the quantitative results obtained are correlated.

Table XI.

Ratio formic acid: ferrous salt = 1. Molarity of $\text{FeSO}_4 = 0.004$.

g.-mol. hydrogen peroxide present	0.002	0.004	0.006	0.008	0.01
g.-mol. formic acid oxidised	0.001	0.002	0.003	0.0036	0.0039
g.-mol. ferrous salt oxidised	0.00165	0.0032	0.004	0.004	0.004
g.-mol. H_2O_2 to oxidise 1 g.-atom ferrous iron	0.61	0.62	0.75	1.1	1.5

The excess hydrogen peroxide over that utilised by the formic acid is expressed in line 4, Table XI, as the amount necessary to oxidise 1 g.-atom of ferrous iron under these conditions. From a scrutiny of the table it will be seen that in this case, where the amounts of hydrogen peroxide and formic acid are molar equivalents, oxidation of the salt takes place to a greater extent than does that of the acid. The figure 0.6 g.-mol. of hydrogen peroxide for the oxidation of 1 g.-mol. of ferrous salt is obtained in Exps. 1 and 2 but not in the others. In these experiments, as the qualitative results of Table III show, there is for a short time in the solution free hydrogen peroxide. The high figures of Exps. 3, 4 and 5 can then be accounted for by catalytic decomposition of hydrogen peroxide by iron salts. The data of Table IX also support this view.

Lines 5 and 6, Table X, are compiled from the data for other values of the ratio formic acid/ferrous salt.

The figures in line 5, Table X, are calculated from the permanganate titrations reported earlier in the paper. Beside each number is given the figure from which it has been taken: (a) Fig. 5; (b) Fig. 4; (c) Fig. 3; (d) Fig. 2; (e) Fig. 1.

It will be seen that where all the ferrous salt has not been oxidised there is good agreement between the amount of hydrogen peroxide added to the solution and the amount equivalent to the formic acid and ferrous salt oxidised. The amount of formic acid oxidised is also seen to depend on the ratio formic acid/ferrous salt.

DISCUSSION.

Each oxidation system which has been examined is characterised by the appearance of ferric iron. This may result in one of three ways:

(1) The change of valency of the iron may be in no way related to the catalysis.

(2) The actual catalyst may be a compound of bivalent iron, but the metal may be liberated from the catalytic reaction in the ferric form.

(3) The production of ferric iron may be a necessary preliminary to catalysis, and the catalyst may be actually a derivative of ferric iron.

The evidence is definitely in favour of the third of these possibilities.

(1) It has been shown that when tartaric acid is added to a solution of a ferrous salt containing hydrogen peroxide in amount more than sufficient for the complete oxidation of the iron, the same reaction takes place as when hydrogen peroxide is added to a solution of tartaric acid containing ferrous iron.

(2) It has been shown that when hydrogen peroxide in amount more than sufficient to oxidise all the iron is added to a solution of *i*-inositol containing ferrous iron, oxidation of the *i*-inositol takes place.

It is difficult to reconcile these two facts with the idea either that the catalyst is a ferrous iron compound liberating ferric iron after oxidation of the organic substance has taken place, or that oxidation and the appearance of ferric iron are unrelated phenomena.

It would appear that change of valency of the iron must be regarded as a necessary preliminary to the oxidation of organic substances by hydrogen peroxide when ferrous salts act in a catalytic capacity. In such systems it seems therefore that the first reaction is between the ferrous iron and hydrogen peroxide.

We have now to consider what further reactions are entered into by the ferric iron derivative, evidence for the formation of which has been reviewed above. At first sight it might seem as if we must assume the intervention of a reaction which Warburg has postulated to explain iron catalysis in the living cell, namely

where A is an organic oxidisable substance.

Such a theory would require that at the end of the catalytic reaction all the iron should be in the ferrous state, for we can only conceive of the various members of the system reacting together in stoichiometrical proportions. Accordingly when the last equivalent of hydrogen peroxide in the system has raised the iron to the ferric state the reaction between the ferric compound so formed and the oxidisable substance would bring about complete reduction of the iron.

It has been observed, however, that regeneration of ferrous iron, does not take place in the case of the oxidation of formic acid, that is, with a substance

which on oxidation gives rise to products which are not further oxidisable (see Figs. 2 and 3 after 17 hours). The reduction to the ferrous state does not seem to be necessary for oxidation to take place. This conclusion is supported by further evidence. With tartaric acid systems it was shown that with subsequent additions of peroxide the rate of disappearance of ferric salt is greatly increased, apparently reaching a maximum. This cannot be explained on the basis of a reaction between ferric iron and tartaric acid, for with each subsequent addition of hydrogen peroxide the concentration of tartaric acid becomes less. If the reaction were between ferric iron and tartaric acid, according to the law of mass action, the rate of disappearance of ferric iron should decrease with each addition of hydrogen peroxide. The regeneration of ferrous iron must therefore be due to a reaction between ferric iron and a component of the system, the concentration of which increases with each addition of hydrogen peroxide. The reduction of the ferric iron must be brought about by reaction with dihydroxy-maleic acid, the oxidation product of tartaric acid.

That the reduction of the ferric iron is a secondary reaction is also shown by the fact that in the oxidation of *i*-inositol and glucose the disappearance of the ferric iron from the system is a very slow process as compared with the disappearance of hydrogen peroxide.

We are forced therefore to conclude that the ferric compound initially formed by the interaction of ferrous iron and hydrogen peroxide possesses the power of donating oxygen to oxidisable substances and that in this process there is no reversal of the initial change of valency of the iron.

Discussing now the partition of hydrogen peroxide between oxidisable substance and iron, reference to Table VI shows that as the concentration of oxidisable substance initially present in the system is increased the amount of ferrous salt finally in the system also increases. The amount of oxidisable substance undergoing oxidation correspondingly increases. It has also been shown that irrespective of the value of the ratio formic acid/ferrous salt all the iron in the system may be oxidised to the ferric state—the amount of hydrogen peroxide required varying directly with the ratio. If the theory of the catalytic function of iron presented is correct it should be possible to explain these facts in terms of it.

If the first reaction in such a system gives rise to a basic ferric salt (reaction *a*) and this salt unites with hydrogen peroxide to give an unstable iron compound which is the actual oxidising agent, then in the reaction between oxidising agent and oxidisable substance the basic ferric salt would again be produced (reaction *b*). There are then two sources of the basic ferric salt and the amount formed will depend on the amount of ferrous salt reacting with hydrogen peroxide and on the relative velocities of the reactions *a* and *b*. Any factor which would increase the rate of reaction *b* while leaving *a* unchanged would favour the production of basic ferric salt from the oxidising agent. This would then be available for further reaction with hydrogen peroxide. Such a factor would therefore tend to decrease the amount of ferrous sulphate oxidised. According to the law of mass action it is probable, therefore, that an increase in the concentration of oxidisable substance by hastening its reaction with the oxidising agent, would decrease the amount of ferrous salt undergoing oxidation.

This agrees with the known facts. It has been shown experimentally that the greater the amount of oxidisable substance in the system, the smaller the amount of ferrous salt undergoing oxidation and the greater the amount of hydrogen peroxide available for the oxidation of formic acid. It should be noted that this theory depends on a difference of velocity between the oxida-

tions of Fe^{++} salt and of formic acid which should be capable of experimental demonstration.

There is evidence that association between oxidisable substance and catalyst takes place in the systems examined. It has been noted that when hydrogen peroxide is added to solutions of ferrous sulphate there is precipitation of a brown solid which is doubtless a basic ferric sulphate produced by hydrolytic decomposition of the first product of the reaction between ferrous iron and hydrogen peroxide. This precipitation was also observed during the oxidation of formic acid. In the oxidation of tartaric acid, *d*-glucose and *i*-inositol no such precipitation was observed. Instead there was noted the reduction of the iron to the ferrous state, a process which in the case of *d*-glucose and *i*-inositol was found to be very slow. When we remember the susceptibility of the ferric derivative to hydrolytic change when no oxidisable substance is present, we can only assume that protection after the oxidation of organic substances is afforded by association with the substrate. Such association would in all probability be achieved through the free hydroxyl groups of the oxidisable substance, an assumption which might explain the precipitation observed in the oxidation of formic acid. The observations reported here as evidence of association between catalyst and substrate would be in line with the suggestion of Kchlin and Boseken that the formation of ternary complexes is an accompaniment of the oxidation of organic substances by hydrogen peroxide in the presence of ferrous salts. It should be noted that, if such complexes are formed, the observations reported in this paper require that they should be capable of ionisation, for the thiocyanate reaction is not given by iron in a non-ionised association.

In conclusion it may be noted that the oxidation of the substrate in these systems is by no means quantitative with respect to the H_2O_2 since simultaneous oxidation of iron takes place and there is also some catalytic decomposition of hydrogen peroxide. These losses may be diminished by maintaining a high ratio substrate/catalyst.

A further source of loss is found in the reduction of iron in secondary reactions with the initial products necessitating a subsequent oxidation to the ferric state by further hydrogen peroxide. The method as used by Fenton, Morrell and Crofts, and others, depending on their own theory of the mode of the reaction, does not give the maximum amount of oxidation products. Oxidation is most favourably carried out by adding the requisite quantity of hydrogen peroxide to a solution of the substrate containing a trace of ferrous salt. When the solution gives a negative test for peroxide very little of the starting material remains unoxidised and the amount of secondary oxidation products is minimum.

SUMMARY.

In the oxidation of organic substances by hydrogen peroxide in the presence of ferrous iron the first reaction is between the iron and the peroxide.

A derivative of ferric iron is produced and this acts as an oxygen donator.

In the reaction between this ferric derivative and the oxidisable substance there is no reduction of the iron.

The reduction of the iron is a secondary process resulting from a reaction between oxidation products and the ferric compound liberated from the reaction between oxygen donator and oxidisable substance.

There is evidence that in the catalytic oxidations considered in this paper there is formation of complexes of oxygen donator *plus* oxidisable substance.

This work was carried out by the late Dr Stirling during the tenure of the Barbour Research Scholarship in the Institute of Physiology of the University of Glasgow, and was the subject of a Thesis for the degree of Doctor of Philosophy. It was preliminary to a thorough study of the oxidation products of *i*-inositol, and it was Dr Stirling's intention to pursue this study in the quantitative manner of Part II. The quantitative studies were, however, incomplete at the time of his death on February 12th, 1933.

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