THE CATALYTIC FUNCTION OF FERROUS IRON IN OXIDATIONS WITH HYDROGEN PEROXIDE.

A Thesis submitted in accordance with
the Regulations of the University of
Glasgow for the Degree of Doctor of
Philosophy in the Faculty of Science

by

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April, 1931.

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INTRODUCTION.

INTRODUCTION.

In the period which has elapsed since the classical researches of Lavoisier on Combustion (1744-1785) chemists have paid marked attention to the phenomena subsumed under the term "Biological Oxidation" - i.e., the chemical processes whereby living organisms obtain the energy necessary for the carrying out of their vital activities.

In spite of many researches into the nature of such chemical reactions, we are still far removed from a scheme which would include and describe all the known facts and which would be of service in the elucidation of many still obscure problems.

But one point is, by now, firmly established and that is the remarkable ease with which many substances, subject to degradation "in vitro" only with great difficulty and by the aid of powerful reagents, undergo extensive breakdown at the low energy potentials obtaining in the living cell.

It is recognised that the reactions involved in such "in vivo" decompositions are catalytic in nature, and it has been shown that a considerable number of substances present in plant and animal tissues are capable of acting catalytically in this manner.

E.g.,	Unsaturated fatty acids	(Warburg, 1914).
	Lecithin-like substances	(Gallagher, 1923).
	Phenols	(Onslow, 1923).
	Sulphydryl compounds	(Hopkins, 1925),

and minute quantities of inorganic elements,

e.g.,	Iron	(Warburg, 1914, 1928)
	Copper	(Battie & Smedley-Maclean, 1929).

However, before the function of such catalysts can be discussed, it is necessary to review briefly modern ideas of the nature of Biological Oxidation.

The Classical Theory of Biological Oxidation is known as the "Oxygenase Theory" and has been based on the work of Bach and Chodat (1902-1904), Manchot (1902), and Engler and Wild (1897).

It may be summarised briefly as follows:-

There exist in all living cells mechanisms whereby molecular oxygen may be "fixed" or rendered available for combination with substances normally inert towards it. The compounds effecting this belong to the category of autoxidisable substances, i.e., substances capable of being oxidised by molecular oxygen to give peroxides.

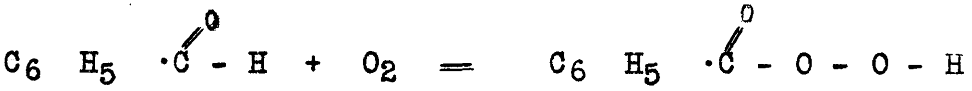
Thus:- $$\underset{\text{Autoxidisable substance.}}{A} + O_2 = \underset{\text{Peroxide.}}{AO_2}$$

Such peroxides are very powerful oxygen donators and it is believed that, as their presence in the living cell has been demonstrated, they are capable of inducing the oxidation of otherwise inert metabolites.

Thus:- $AO_2 + B = AO + BO$.

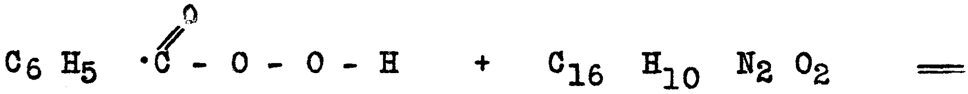
(Engler and Weissberg, 1904).

An analogy for such a process is found in the well-known reaction of Baeyer and Villiger (Baeyer and Villiger, 1900) in which indigo is oxidised by atmospheric oxygen in the presence of Benz-aldehyde.



$$C_6 H_5 \cdot \overset{O}{\overset{/\!/}{C}} - H + O_2 = C_6 H_5 \cdot \overset{O}{\overset{/\!/}{C}} - O - O - H$$

Benz-aldehyde. Benzoyl Hydrogen Peroxide.



$$C_6 H_5 \cdot \overset{O}{\overset{/\!/}{C}} - O - O - H + C_{16} H_{10} N_2 O_2 =$$

Indigo

$$2C_8 H_5 NO_2 + 2C_6 H_5 COOH$$

Isatin. Benzoic Acid.

Such a system has been termed by the workers in the field an "Oxygenase" system, and there is considerable evidence that such play an important part in the oxidations of living tissues.

The/

The fundamental idea of the theory is the "activation" of atmospheric or molecular oxygen - indeed the scheme is sometimes described as the "active oxygen" theory.

The actual "activation" of the oxygen only takes place after it has combined with the autoxidisable substance to give the peroxide, and arising from this fact it has been found that oxidase systems can be separated by suitable methods into two components:- (1) the autoxidisable substance mentioned above, and (2) the activating component or "peroxidase" which activates the oxygen of the peroxide.

The presence of such enzymic peroxidase systems has been demonstrated in plant cells (Gallagher, Warburg, Onslow), but the evidence is not so unequivocal in the case of animal tissues. This, however, would not appear to negative the probability of such systems being responsible in part for the cellular oxidations of animal organs. Before this point can be established, it is necessary to give some account of the other important Theory of Biological Oxidation - that due to Wieland, and known as the "Dehydrogenase Theory."

This originated with an idea in direct contradiction to that embodied in the Oxygenase Theory reviewed above - namely that oxidation in the living cell is due not/

not to addition of oxygen to substances but to the "activation" and removal of Hydrogen from them.

Wieland's scheme may be summarised as follows:-

The substance first forms a compound with water

(1) $X + HOH = HXOH$

from which Hydrogen is withdrawn

(2) $HXOH = XO + 2H^{\cdot}$

The Hydrogen is supposed to be "activated" by a "dehydrogenase" and taken up by a Hydrogen acceptor - i.e., a substance capable of being reduced by "active" Hydrogen.

(3) $Y + 2H^{\cdot} = YH_2$

A variety of substances has been shown to be capable of acting as Hydrogen acceptors (c.f. the work of Thunberg, Thunberg and Ahlgren. See Ahlgren "Zur Kenntnis der Tierischen Gewebsoxydation), and it is now definitely established that such systems can bring about oxidations in biological media.

It is apparent that such "indirect oxidations" could take place in the absence of oxygen, and at first sight it would appear that we have here two entirely opposed Theories of Biological Oxidation - on the one hand the direct or "active" Oxygen Theory, and on the other the indirect or Dehydrogenation Theory.

Such/

Such indeed was the view of those responsible for the introduction of the two schemes, but more recent work is influencing investigators to believe that the two may be complementary.

For example, Morgan, Stewart and Hopkins (Morgan, Stewart and Hopkins, 1922) were able to show that milk and certain animal tissues have the power of converting Hypoxanthine anaerobically into Uric Acid in the presence of Methylene Blue, the latter acting as acceptor for the activated Hydrogen as in the classic researches of Thunberg. In addition they found that oxidation could take place in the absence of Methylene Blue if Oxygen were present, the latter apparently acting as a Hydrogen acceptor in place of Methylene Blue.

This work has been carried further by Thurlow (Thurlow, 1925), who has been able to show that when Oxygen acts as a Hydrogen acceptor for "active" Hydrogen, Hydrogen Peroxide is formed which might then be available for carrying out oxidations according to the "Oxygenase" Scheme.

The failure to demonstrate the presence of peroxides in animal tissues has been shown to be due to two causes:-

(1) The presence of peroxidases which activate the oxygen of peroxides and so catalyse direct oxidations;

(2) The presence of catalases which decompose Hydrogen Peroxide giving water and molecular oxygen.

There is thus no barrier to the acceptance of the view that "Oxidase" oxidations may take place in animal tissues and it would appear that a combination of what were formerly thought to be two contradictory theories will pave the way for a better understanding of the processes involved in Biological Oxidation.

An example, based on the work of Thurlow on the oxidation of Nitrite and Hypoxanthine by milk, is given below.

A. Anaerobic oxidation of a substance (XH_2) in presence of Methylene Blue (M) and under the influence of a dehydrogenase

(1) $XH_2 = X + 2H^{\cdot}$

(2) $M + 2H^{\cdot} = MH_2$

B. Aerobic Oxidation in the absence of Methylene Blue

(1) $XH_2 = X + 2H^{\cdot}$ ($H^{\cdot}$ = "active" Hydrogen)

(2) $2H^{\cdot} + O_2 = H_2O_2$

(3) $H_2O_2 = H_2O + O^{\cdot}$ (In presence of Peroxidase $O^{\cdot}$ = "active" Oxygen).

(4) $Y + O = YO$

(5) $2H_2O_2 = 2H_2O + O_2$ (In presence of Catalase).

It will be seen from the above short review that the importance of peroxides in Biological Oxidation, on which for a time doubt was cast, has been re-established, and the study of oxidations involving peroxides is likely therefore to be of value in the elucidation of corresponding biological processes.

The simplest possible Oxygenase System would be one in which the Peroxide is Hydrogen Peroxide, and it is of interest in this connection that Dakin (Dakin 1906-1908) and others have shown that oxidations brought about by this reagent bear a close resemblance to those taking place in the living cell.

A further point of interest is that such oxidations can be catalysed; - indeed, in a reaction of this kind we have all the components of a Biological Oxygenase System,

(a) a Peroxide,
(b) a Peroxidase or catalyst,
(c) an oxidisable substance or Oxygen acceptor.

The most important of those substances which catalyse such reactions after the manner of peroxidases are the Ferrous Salts. It is therefore an important point that iron has been found to be a constituent of peroxidase preparations, and there is some evidence that the activity of these preparations depends on the iron content (Loevenhart and Kastle, 1903). (c.f. the work of Sakuma (Sakuma 1923/

1923) and of Harrison (Harrison, 1924) who have established that the rate of autoxidisation of sulphydryl preparations depends on their iron content).

It will be seen, therefore, that a study of such an ideal "oxygenase" system -- Hydrogen Peroxide -- Ferrous Salt -- Oxidisable Substances -- is likely to throw light on the more obscure systems present in the living cell.

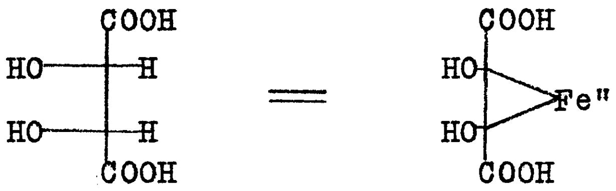
Such a study is reported in the present paper, and before proceeding with the report a short history of the previous work will be given.

Oxidation by Hydrogen Peroxide in the presence of Ferrous Salts is usually referred to as Fenton's Reaction, from the fact that it was first used by Fenton in 1896 for the oxidation of Tartaric Acid to Dihydroxy Maleic Acid.

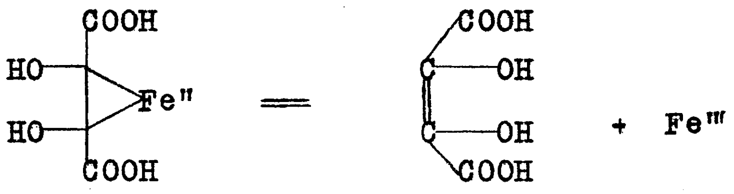
What the true function of Iron is in such processes and what intermediate reactions are involved are extremely important questions, but as yet they remain unsolved.

Fenton (Fenton, 1898, 1899) advanced the following theory.

The two non-hydroxylic hydrogen atoms of Tartaric Acid possess feebly acidic properties by virtue of the proximity of the Carboxyl groups. In the presence of a Ferrous Salt, Fenton believed that a ferrous complex was formed:-



Addition of Hydrogen Peroxide caused oxidation of the Iron to the Ferric state when the Tartaric Acid can no longer retain it. Accordingly, Ferric Iron is liberated and an unsaturated Hydroxy Acid - di-hydroxy maleic acid - is formed:-



Although plausible, the theory has no experimental evidence to support it, nor does it explain the oxidation of non-acidic substances by this method.

The second theory is that which is quoted most often in the literature, although it cannot be traced to any particular author.

This theory holds that the first action is the oxidation of the Ferrous Iron to the Ferric State

$$Fe^{++} \quad - \quad Fe^{+++}$$

The Ferric Iron compound so formed is a much more powerful oxidising agent than Hydrogen Peroxide, and in the presence/

presence of an Oxygen acceptor it is reduced, while the latter is oxidised

$$Fe^{+++} + A = Fe^{++} + AO.$$

An interesting suggestion was that of Brode (Brode, 1901) who put forward the view that an unstable compound is first formed by combination of Ferrous Salt and Hydrogen Peroxide, and that this is the actual catalyst.

Brode, however, did not elaborate his views, but we find them later incorporated in the work of Mummery (Mummery, 1913).

This worker has put forward a scheme purporting to explain the whole process, and it will be necessary to consider his ideas in some detail.

Mummery has built up his theory entirely on his observations of the interaction between Ferrous Salts and Hydrogen Peroxide, and these may be summarised as follows:-

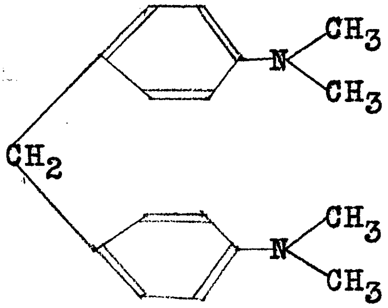
(1) When Hydrogen Peroxide is added to a solution of a ferrous salt it is found that $\frac{1}{2}$ gm. molecular proportion must be added before all the Iron is oxidised to the Ferric state. A bright yellow coloured liquid is obtained from which a yellow solid (a basic Ferric Salt) is precipitated.

(2) Large quantities of Hydrogen Peroxide may be

catalytically/

catalytically decomposed by addition to a solution of a Ferrous Salt.

(3) Trillat's reagent - Tetramethyldiaminodiphenylmethane



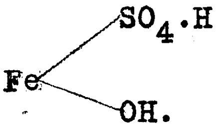
is converted into a colourless compound by Hydrogen Peroxide and by substances of the Peroxide class. With the Lead Dioxide type of Super-oxide, however, the reagent gives a carbinol compound of which the weak acid salts (e.g., with citric acid) are coloured blue.

When Hydrogen Peroxide is added to a solution of Ferrous Sulphate containing a small quantity of Trillat's reagent dissolved in citric acid a deep blue colour results. If Trillat's reagent be added after the Peroxide, no colour is given. Ferrous Salts, Ferric Salts, and Hydrogen Peroxide alone do not colour the reagent, nor do Ferric Salts in the presence of Hydrogen Peroxide.

Mummery has 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. From this he elaborates/

elaborates his theory of the function of the Iron as a catalyst.

The Isomorphous Sulphates of the Ferrous Sulphate class may be regarded as existing in solution as Hemisulphate Hemihydrols

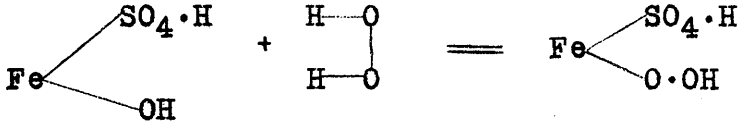


By analogy with the Hemiperhydrol of Barium which is known

Barium Hydroxide (Dihydrol).

Barium Hemiperhydrol

Mummery believes that when Hydrogen Peroxide is added to a solution of a Ferrous Salt a Perhydrol compound is formed

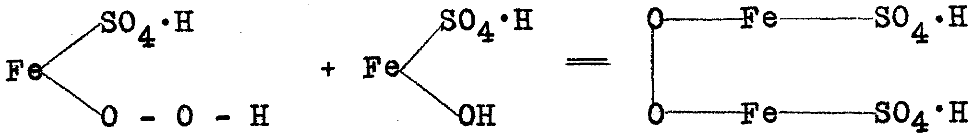


Ferrous Sulphate, Hydrol.

Ferrous Sulphate, Perhydrol.

In the absence of any other oxidisable substance this would react with Ferrous Sulphate giving a Basic Ferric/

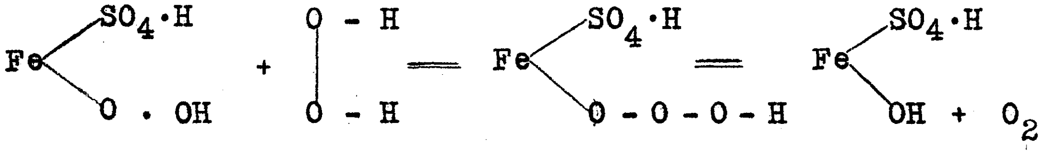
Ferric Salt



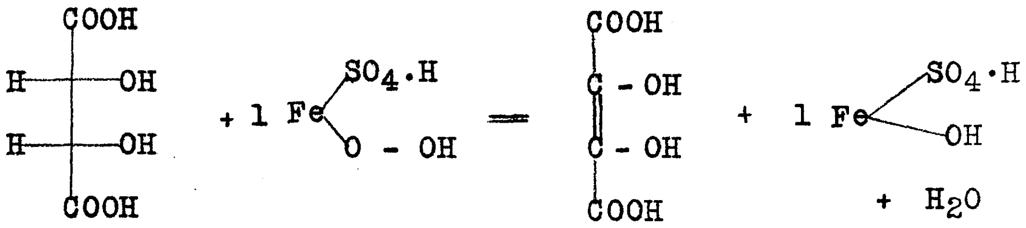
On Hydrolysis

A Basic Ferric Salt.

The oxidation of excess Hydrogen Peroxide is represented as follows:-



and that of an oxidisable substance by



While Mummery's ideas represent an interesting attempt to formulate the reactions involved in such catalytic oxidations, his scheme is decidedly fanciful, and moreover it is entirely unsupported by any observations on systems in which oxidisable substances are present.

While this work was in progress an interesting paper appeared by Küchlin and Boëseken (Küchlin and Boëseken, 1929).

Basing their conclusions on Hydrogen Peroxide concentration curves obtained in a study of the oxidation of Fructose and Glycerol these workers hold that Polyhydroxy Alcohol complexes with Iron play a big part in such oxidations.

They advance the following as a typical Reaction Scheme:-

(1) Fe^{2} + Fructose $=$ Fe^{2} Fructose Complex.

(2) Fe^{2} Fructose Complex + nH_2O_2 $=$ Fe^{2}. nH_2O_2. Fructose
(a Ternary Complex).

(3) $Fe^{2}.nH_2O_2$ Fructose $=$ Fructosone + Fe^{3} + nH_2O.

It will be seen from a consideration of the foregoing that there exists, as far as the rôle of Iron in such oxidations is concerned, considerable confusion not only of theory, but also of fact, and it was in order to clear up anomalies observed while using Fenton's Reagent that this work was undertaken.

In the present paper are reported observations on the system:-

Hydrogen Peroxide - Ferrous Sulphate - Oxidisable Substance

The experiments are qualitative and quantitative in nature, and the bearing of these results on the intermediate reactions involved is discussed.

PART I.

Qualitative Observations on the System:-

Hydrogen Peroxide - Ferrous Salt - Oxidisable Substance.

PART I.

Qualitative Observations on the System

Hydrogen Peroxide - Ferrous Salt - Oxidisable Substance.

In the first part of the work it was desired to find out, as far as possible, by means of qualitative tests, the fate of the members of the system:-

Hydrogen Peroxide - Ferrous Salt - Oxidisable Substance.

The behaviour of such systems was examined as to

(1) Fate of Hydrogen Peroxide.

(2) Fate of Ferrous Salt.

(3) Specific Properties of Individual Systems.

The results of the examination of several systems will be given in detail, but before doing so it is necessary to describe the method of experimentation.

Procedure.

The oxidisable substance was used in the form of a stock solution of known high concentration.

A measured quantity of solution was taken and to this was added the ferrous salt dissolved in a known volume/

volume of distilled water. Sufficient distilled water was then added so that after addition of peroxide (this was added last) the total volume of the solution gave the desired concentration of the reagents.

After addition of peroxide the qualitative tests for (1) Hydrogen Peroxide, (2) Ferrous Iron, (3) Ferric Iron were carried out.

Reagents.

(1) Hydrogen Peroxide. This was used in approximately 10 vol. or 3 per cent. solution. It was made up fresh before each experiment by dilution of Merck's Perhydrol Reagent (100 volume) and was neutralised to phenolphthalein by means of N/10 caustic soda solution and was standardised by means of Potassium Permanganate solution just before use.

(2) Ferrous Salt. It was found in practice that either Ferrous Sulphate or Ferrous Ammonium Sulphate could be used. In every case fresh solutions were made up just before use from B.D.H. A.R. reagents in glass distilled CO_2 free water. In this way autoxidation of the salt was prevented.

(3) Potassium Thiocyanate. 2N. Solution of B.D.H. A.R. reagent.

(4) Potassium Ferricyanide. $\frac{N}{2}$ solution of B.D.H. A.R. Reagent.

Tests.

(1) For Hydrogen Peroxide. By means of the Chromic Acid Reagent and Ether - Potassium Dichromate (A.R.) in dilute sulphuric acid.

To some of the solution to be tested is added the reagent and the liquid is then shaken with ether. A deep blue colour in the ethereal layer denotes the presence of Hydrogen Peroxide in the original liquid. The reaction is extremely delicate and specific.

(2) For Ferrous Iron. By means of Potassium Ferricyanide solution.

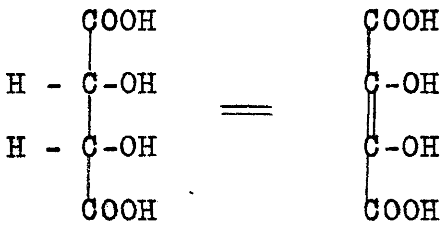
(3) For Ferric Iron. This was tested for by means of Potassium Thiocyanate solution.

It should be noted that Potassium Ferricyanide gives with Ferric Salts in the presence of Hydrogen Peroxide a greenish blue colouration. It is faint, however, and as it develops a few seconds after mixing it may be differentiated from the deep blue colour given by ferrous salts.

The System

Hydrogen Peroxide - Ferrous Salt - Tartaric Acid.

When Hydrogen Peroxide is added to a solution of Tartaric Acid containing Ferrous Salt Dihydroxy Maleic Acid is formed



$$HOOC-CH(OH)-CH(OH)-COOH = HOOC-C(OH)=C(OH)-COOH$$

Tartaric Acid. Dihydroxy Maleic Acid.

The following solutions were examined:-

(1) ·1 M Solution Tartaric Acid containing ·1 gm. molecular proportion Ferrous Salt and ·1 gm. molecular proportion Hydrogen Peroxide.

(2) ·1 M Solution Tartaric Acid containing ·1 gm. molecular proportion Ferrous Salt and 1 gm. molecular proportion Hydrogen Peroxide.

Controls.

(3) ·01 gm. molecular solution Ferrous Salt and 1 gm. molecular proportion Hydrogen Peroxide.

(4) ·01 gm. molecular solution Ferrous Salt and 10 gm. molecular proportions Hydrogen Peroxide.

Tests/

Tests were carried out immediately after addition of Peroxide.

(1) Hydrogen Peroxide. In 1, 2 and 3 negative, in 4 positive.

(2) Ferrous Iron. In 1 and 2 positive, in 3 and 4 negative.

In solutions 3 and 4 addition of peroxide gave a strong orange yellow colour - the Thiocyanate Test for ferric iron being strongly positive. In 1 and 2 after addition of peroxide a greenish yellow colour was formed, but this faded very rapidly, giving a water clear solution with a negative Thiocyanate Test. It was found that while the greenish yellow colour persisted in the solution a positive Thiocyanate Test was given.

To a solution of Tartaric Acid containing Ferrous Salt a few drops of Trillat's reagent (Tetramethyldiaminodiphenylmethane dissolved in citric acid solution) was added. On the addition of Hydrogen Peroxide a deep blue colour was developed which, however, soon faded, leaving a water clear solution.

A solution of Tartaric Acid containing Ferrous Salt was divided into two parts (a) and (b).

To/

To (a) was added a few drops of Trillat's Reagent.

To both was added Hydrogen Peroxide.

It was observed that both colours faded rapidly - the blue of (a) and the greenish yellow of (b).

Both solutions became colourless practically simultaneously.

It was found that no colouration was given when Trillat's reagent was added after the addition of Hydrogen Peroxide provided that the reagent was added to the water clear solution. If Trillat's reagent is added before the greenish yellow colour has disappeared from the solution then a blue colour is produced.

In most oxidations, using Fenton's Reagent, it is found that after addition of Hydrogen Peroxide there is a development of colour which then fades. It is found, moreover, that the time taken for this colour to fade becomes much less on the addition of a second quantity of peroxide, and still less with a third and so on.

This was demonstrated in the case of Tartaric Acid as follows:-

A ·1 M solution of Tartaric Acid was taken and ·1 gm. molecular proportion of Ferrous Sulphate was added.

·1 gm. molecular proportion Hydrogen Peroxide was now added and the time which elapsed between the addition and the decolourisation of the solution 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.

Below are given the results of two such experiments:-

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

An attempt was made to correlate the rate of disappearance of the colour produced by adding Hydrogen Peroxide to solutions of Tartaric Acid and Ferrous Salt with the concentration of the reagents, but this proved abortive.

To a solution of a ferrous salt a large quantity of Hydrogen Peroxide was added. Immediately the solution became brown in colour and effervescence took place.

A solution of Tartaric Acid was now added. Immediately the deep brown colour faded to a greenish yellow which continued in intensity for a time and finally faded to a water clear solution.

There are several points of interest to be noted in the above observations:-

(1)/

(1) The reaction appears to be an extremely rapid one for in the short time which elapsed between addition of peroxide and testing all the peroxide had disappeared even when ten gm. molecular proportions of Hydrogen Peroxide per gm. mol. Ferrous Salt had been added.

(2) Ferric Iron appears to be produced even in the presence of an Oxygen acceptor when Hydrogen Peroxide is added.

(3) Oxidation of Tartaric Acid appears to take place when it is added to a solution of a Ferrous Salt to which a large excess of Hydrogen Peroxide had been added. In this case no Ferrous Salt could be present in the solution previous to the addition of Tartaric Acid.

It should be noted that an excess of Peroxide must be present. No immediate production of Ferrous Salt can be noted when Tartaric Acid is added to a solution containing only Ferric Salt produced by the oxidation of Ferrous Salt by the requisite quantity ($\frac{1}{2}$ gm. molecular proportion) of Hydrogen Peroxide.

(4) Whatever may be the real significance of the reaction with Trillat's Reagent it is evident that the compound which gives it has a somewhat longer existence in the presence of an Oxygen acceptor than in the absence of such.

(5) It is difficult to correlate the quickening of the decolourisation process noted with further additions of peroxide with the idea of an interaction between Tartaric Acid and an iron containing compound.

The reactions taking place in this system would be expected to conform to the Mass Action Law, and since, after the first addition of Peroxide, the concentration of Tartaric Acid is decreased, any reaction depending on this concentration would be slowed down.

However, decrease in concentration of Tartaric Acid is accompanied by an increase in concentration of Dihydroxy-maleic Acid which is a much more active reducing agent.

The increase in rate of decolourisation is understandable when it is ascribed to a secondary reaction with Dihydroxy-maleic acid.

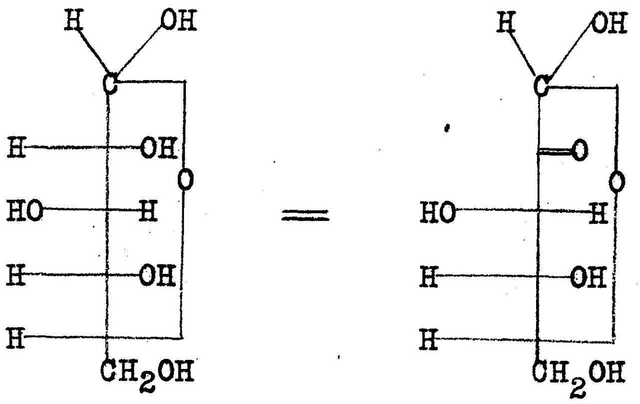
This point will be dealt with more fully later. First must be given the results of observations on systems containing the same oxidising agents and other oxidisable substances.

The System:-

d-Glucose - Ferrous Salt - Hydrogen Peroxide.

Although d-Glucose is not very readily attacked in neutral solution by Hydrogen Peroxide, Morrell and Crofts (Morrell and Crofts, 1899) have shown that in the presence of/

of Ferrous Iron it is easily oxidised to d-Glucosone.



d-Glucose d-Glucosone.

This system was studied in a fashion similar to that in the case of Tartaric Acid. The results are given below.

When Hydrogen Peroxide is added to a solution of d-Glucose containing Ferrous Salt there is immediately developed a yellowish-brown colouration. This, as shown by a positive Thiocyanate reaction, is due to the production of Ferric Iron.

This colour (and consequent positive test for Ferric Iron) persists for a long time, but it slowly disappears and there is finally obtained a solution which is coloured only slightly yellow. The Thiocyanate test for Ferric Iron is now negative, but the Ferricyanide test for Ferrous Iron strongly positive.

It was found that by adding sufficient peroxide

the/

the presence of this reagent could be detected in the solution for some time after addition. The peroxide was found, however, to disappear quite rapidly from the solution,- always in a few minutes - the time depending on the amount added.

The disappearance of the peroxide did not, however, coincide with the disappearance of the Ferric Iron. Indeed a very much longer time was required for the solution to react negatively to Thiocyanate.

(In one experiment a solution was made up which was ·1 M with respect to Glucose, ·01 M with respect to Ferrous Sulphate, and ·1 M with respect to Hydrogen Peroxide, the oxidising agent being added last. It was found that all Peroxide had disappeared from the solution after 12 minutes from the time of addition, while the Ferric Iron did not disappear until 24 hours had elapsed.

TABLE 1.

No. of tube	1	2	3	4	5	6	7	8	9	10
Molarity d-Glucose	.1	.1	.1	.1	.1	.1	.1	.1	.1	.1
Molarity Ferrous Sulphate	.01	.01	.01	.01	.01	.01	.01	.01	.01	.01
Molarity Hydrogen Peroxide	.01	.02	.03	.04	.05	.06	.07	.08	.09	.1
Thiocyanate Test	+	+	+	+	+	+	+	+	+	+
Ferricyanide Test	+	+	+	+	-	-	-	-	-	-
Chronic Acid Test	-	-	-	-	+	+	+	+	+	+

Above is given, in tabular form, the results of a typical system containing d-Glucose.

It will be seen that in every case addition of Peroxide was followed by the production of Ferric Iron.

In the first four tubes (i.e., up to four gm. molecular proportions of Hydrogen Peroxide per gm. mol. of Ferrous Iron) the Ferric Iron co-existed in the solution with Ferrous Iron though the test for the former increased in intensity as the latter decreased.

The presence of Hydrogen Peroxide was observed only in those tubes from which all the Ferrous Iron had disappeared.

The solutions in tubes 5-10 were of a uniform yellowish colour. All showed the presence of excess Peroxide and absence of Ferrous Iron.

The Hydrogen Peroxide disappeared from the solutions, the time taken being successively longer from 5 to 10.

The following points have also to be noted:-

(1) In no case did evolution of gas take place indicating absence of catalytic decomposition of Hydrogen Peroxide according to the equation

$$2H_2O_2 = 2H_2O + O_2$$

(2) In tubes 1 and 2 on standing for a short time a cloudiness appeared - precipitation of a light yellow coloured/

coloured solid. This did not take place in tubes 3-10.

(3) Shortly after the disappearance of Peroxide from tubes 5-10, and while the Thiocyanate test was still positive, the Ferricyanide test showed the regeneration of Ferrous Iron.

It will be seen that the conditions obtaining in this system are similar to those in the case of Tartaric Acid. Here, however, the reaction, as shown by the longer time taken for the disappearance of Peroxide, is not so fast. The colour produced by the addition of Peroxide is much more intense than is the case in the oxidation of Tartaric Acid and takes a much longer time to disappear.

Here again, however, is to be noticed the disparity of times taken for the disappearance of the Hydrogen Peroxide from the system and that for the reduction of all the Ferric Iron to the Ferrous state.

A further point to be noticed is that, in the example reported above, four gm. molecular proportions of Hydrogen Peroxide per gm. mol. Iron must be added before all the Iron has been oxidised to the Ferric state. In the absence of an Oxygen acceptor $\frac{1}{2}$ gm. molecular proportion only is required.

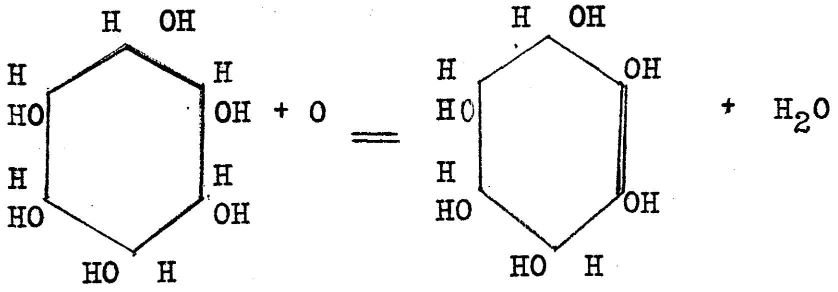
So far no information has been obtained as to the fate of/

of the third member of the system - the Oxygen acceptor. A method of studying this is reported in the following section.

The System:-
i-Inositol - Ferrous Salt - Hydrogen Peroxide.

In the course of a study of the oxidation of i-Inositol I have found that when Hydrogen Peroxide is added to a solution of i-Inositol (Hexahydroxycyclohexane) containing Ferrous Salt a deep blue colour is produced.

From such oxidations there have been obtained solutions of a substance (free from Iron) which give a deep blue colour with ferric salts. The evidence leads one to the conclusion that this substance is probably the first oxidation product of i-Inositol according to the equation:-



i-Inositol.

When this substance is produced from i-Inositol

there/

there is Ferric Salt in solution, and accordingly the blue colour is produced.

This system is therefore self-indicating as far as the oxidation of the third member (oxidisable substance) is concerned, and as such it is of interest here.

When Hydrogen Peroxide is added to a solution of i-Inositol containing Ferrous Salt there is initially developed a yellowish brown colour and the solution reacts positively to Thiocyanate.

In a few minutes the yellowish colour merges into green and gradually into a deep blue. The deep blue colour persists for a long time, but gradually fades to a water clear solution, giving no reaction for Ferric Iron.

Below is given a typical series of experiments.

TABLE 2.

No. of Tube.	1	2	3	4	5	6	7	8	9	10
Molarity i-Inositol.	.1	.1	.1	.1	.1	.1	.1	.1	.1	.1
Molarity Ferrous Sulphate	.01	.01	.01	.01	.01	.01	.01	.01	.01	.01
Molarity Hydrogen Peroxide	.01	.02	.03	.04	.05	.06	.07	.08	.09	.1
Thiocyanate Test	+	+	+	+	+	+	+	+	+	+
Ferricyanide Test	+	+	+	+	-	-	-	-	-	-
Chromic Acid Test	-	-	-	-	+	+	+	+	+	+
Development of blue colour in solution.	-	-	-	-	+	+	+	+	+	+

Tubes 1 and 2 gave, on standing a short time, a bright yellow precipitate.

Tubes 3 and 4 remained yellow in colour.

Tubes 5-10 changed colour shortly after addition of peroxide - a greenish yellow in tube 5, deepening to a deep blue in tube 10.

After appearance of blue colour in tubes 5-10 the chromic acid test for peroxide was negative.

The tubes were allowed to stand stoppered and gradually the blue colour disappeared first in tube 5 and last in tube 10. Water clear solutions were given in each case with negative Thiocyanate test and positive Ferricyanide test.

The yellow colour of tubes 3 and 4 did not disappear on standing.

From these observations it would appear that no oxidation of the oxygen acceptor takes place until all the Iron has been converted into the ferric state.

It must be noted that this finding is anomalous in view of some work to be recorded later, and it might be thought that this is due to the low concentration of the chromagenic substance.

There was, however, a very distinct change of colour between tubes 4 and 5.

The products of oxidation in the three cases so far examined/

examined - Dihydroxymaleic acid from Tartaric Acid, d-Glucosone from d-Glucose, and the Tetrahydro-tetrahydroxy o-diphenol compound from i-Inositol are all active reducing agents, being capable of much further oxidation.

It is desirable, therefore, to examine the behaviour with an oxidisable substance, the oxidation products of which are not capable of further oxidation.

For this purpose was chosen Formic Acid.

The System:-

Formic Acid - Ferrous Salt - Hydrogen Peroxide.

When Formic Acid is oxidised by Hydrogen Peroxide in the presence of Ferrous Iron it gives rise to Carbon Dioxide and water

$$2H\cdot COOH + O_2 = 2CO_2 + 2H_2O$$

The products being incapable of further oxidation and of reacting with other members of the system, Formic Acid fulfils the requirements noted at the end of the last section.

The results of a series of experiments on this system are given below:-

TABLE 3.

No. of Tube.	1	2	3	4	5	6	7	8	9	10
Molarity Formic Acid	.1	.1	.1	.1	.1	.1	.1	.1	.1	.1
Molarity Ferrous Sulphate	.01	.01	.01	.01	.01	.01	.01	.01	.01	.01
Molarity Hydrogen Peroxide	.01	.02	.03	.04	.05	.06	.07	.08	.09	.1
Thiocyanate Test	+	+	+	+	+	+	+	+	+	+
Ferricyanide Test	+	+	+	+	-	-	-	-	-	-
Chromic Acid Test	-	-	-	-	+	+	+	+	+	+

It will be seen from the above table that this system conforms to those already studied.

There are several points of dissimilarity, however.

In the first place no regeneration of ferrous salt was observed, and this confirms the statement that the reduction of the ferric iron to the ferrous state is a secondary reaction.

In the case of Formic Acid the oxidation products are not further oxidisable and accordingly no reduction to Ferrous Iron takes place.

A further observation, that on allowing the deep brown coloured solution to stand for a short time a brownish precipitate settles out soluble in mineral acids. This gave a positive list for the presence of Ferric Iron.

In addition to those reported, a variety of oxidisable substances were examined with results so similar to those given that they need not be given.

In the next section the results reported are summarised and they are discussed with regard to their bearing on the problem.

A scrutiny of the results of qualitative tests on the system

Oxidisable Substance - Ferrous Salt - Hydrogen Peroxide

reveals the fact that always there is initially produced Ferric Iron.

There is, of course, the possibility that this oxidation of Iron has nothing to do with the actual catalysis of the oxidation of the third member of the system and is the result of a side reaction between Hydrogen Peroxide and Ferrous Iron.

Secondly, it may be that, after the oxidation of the oxidisable substance, Iron is liberated from the oxidising agent in the Ferric form.

And lastly, there is the possibility that before oxidation can take place there must be Ferric Iron in the system. The actual catalyst would therefore be a compound/

compound of Ferric Iron.

The evidence certainly favours the third of these.

(1) It has been shown that when Tartaric Acid is added to a solution of a Ferrous Salt to which an excess of Hydrogen Peroxide has been added (Ferrous Salt has been therefore all oxidised) a reaction takes place similar to that obtaining when Hydrogen Peroxide is added to a solution of Tartaric Acid containing Ferrous Salt.

(2) In the case of i-Inositol it has been shown that oxidation takes place when sufficient Hydrogen Peroxide is added to a solution of the oxidisable substance and Ferrous Salt to ensure the complete oxidation of the latter.

It would appear, therefore, that the first reaction in the system is the oxidation of Ferrous Iron to the Ferric State.

This has no point of contact with the theory put forward by Mummery in which only compounds containing Ferrous Iron play a part. Küchlin and Boëseken also suggest that the first reaction is the formation of a Ferrous Iron-Hydrogen Peroxide complex.

We must now examine the other idea that oxidation is brought about by the interaction between Ferric Iron and the oxidisable substance.

This theory requires that at the end of the oxidation all/

all the Iron shall be in the Ferrous state.

In the oxidation of Formic Acid by this method this is certainly not the case. There is no reduction of the initially formed.

This suggests that reduction of the Ferric Iron is a secondary process depending on the production of oxidation products which are themselves capable of further oxidation. Evidence for this has been obtained.

It has been found that rate of disappearance of Hydrogen Peroxide from such systems (and therefore the rate of oxidation) varies very considerably with the nature of the oxidisable substance. In the cases examined it was found that with equal concentrations of reagents it varied from instantaneously in the case of Tartaric Acid to periods up to half-an-hour in length with polyhydroxy alcohols of the Mannitol class.

The time for the disappearance of the Ferric Iron also varied enormously and seemed to depend on the reducing power of the oxidation product. It was shortest in the case of Tartaric Acid (giving Dihydroxymaleic Acid) and longest in the case of the polyhydroxy alcohols (giving aldoses). In the case of Formic Acid from which system Hydrogen Peroxide disappears very rapidly Ferric Iron is not reduced at all.

Further evidence that the reduction of the Ferric

Iron/

Iron is brought about by the oxidation products is found in the case of Tartaric Acid where the rate of disappearance of the Ferric Salt depends on the concentration of Dihydroxy Maleic Acid in the solution.

The reduction of the Ferric Iron would therefore seem to have no connection with the initial oxidation process.

The evidence so far favours such a scheme as the following as representing the various reactions taking place:-

(1) Ferrous Salt + Hydrogen Peroxide = Ferric Complex.

(2) Ferric Complex + Oxidisable Substance = Oxidation Products (1) + Ferric Salt.

And where the oxidation products are themselves capable of further oxidation we have the following reaction:-

(3) Oxidation Products (1) + Ferric Salt = Oxidation Products (2) + Ferrous Salt.

It was with this provisional scheme that the quantitative part of the work was begun.

The bearing of the results obtained in this part on the scheme set forth above will be discussed later.

PART II.

Quantitative Observations on the System:-

Formic Acid - Ferrous Salt - Hydrogen Peroxide.

PART II.

Quantitative Observations on the System

Formic Acid - Ferrous Salt - Hydrogen Peroxide.

The Interaction between Ferrous Salt and Hydrogen Peroxide.

In this section of the work it was necessary to make use in calculations of the molecular relationship between Iron and Hydrogen Peroxide in the oxidation from the Ferrous to the Ferric state by means of this reagent.

Mummery has found, by means of qualitative tests, that in the presence of Sulphuric Acid, one gm. atom of Ferrous Iron requires for its oxidation to the Ferric state one half gm. molecule Hydrogen Peroxide.

The reaction is therefore:-

$$2FeSO_4 + H_2O_2 + H_2SO_4 = Fe_2(SO_4)_3 + 2H_2O.$$

In the present study Ferrous Iron is oxidised by Hydrogen Peroxide and it was desirable to find what quantitative relationships exist between these two under such conditions.

The method adopted was as follows:-

Ten tubes were made up each containing a known quantity of Ferrous Sulphate solution. Known quantities of/

of Hydrogen Peroxide - the amounts increasing from tube 1 to tube 10 - were then added. Immediately after addition of Peroxide, the solutions were acidified with Sulphuric Acid and titrated with Potassium Permanganate solution.

Below are given the results of two such experiments; it was found that change in concentration had no effect on the figure obtained in these.

TABLE 4.

Tube No.	1	2	3	4	5	6	7
Gm.Mols. Ferrous Salt	.001	.001	.001	.001	.001	.001	.001
Gm.Mols. Peroxide/ Gm.Mols. Fe" Salt.	-	.1	.2	.3	.4	.5	.6
ccs. ·04868 N $KMnO_4$	19.8 19.8	15.8 15.9	12.0 12.0	8.9 8.9	5.1 5.3	2.5 2.3	.3 .2

It will be seen that the permanganate titre is minimal in Tube 7 (after this it increases owing to the presence of excess peroxide) and we may conclude that ·6 gm. mol. Hydrogen Peroxide is required for the complete oxidation of 1 gm. mol. Ferrous Salt in the absence of Sulphuric Acid.

N.B. It has never been found possible to obtain the figure ·5 in the absence of Sulphuric Acid. The reason for this is not far to seek. Sulphuric Acid inhibits the catalytic/

catalytic decomposition of Hydrogen Peroxide and the slightly high figure obtained in its absence is to be attributed to decomposition of Peroxide.

It is to be concluded that in the absence of Sulphuric Acid 1 gm. mol. Hydrogen Peroxide oxidises 2 gm. mols. Ferrous Salt to the Ferric state.

A basic Ferric Salt will be formed, but regarding how this action should be formulated or the structure of the end product there is no evidence.

The System:-
Hydrogen Peroxide - Ferrous Salt - Formic Acid.

In the search for a system in which quantitative data would throw light on the reactions taking place, it was realised after the results of the qualitative work that secondary reactions would complicate the problem. This was confirmed by preliminary experiments of a quantitative nature.

It was found finally that the only oxidisable substance, the oxidation of which lent itself to anything like exact quantitative determination, was Formic Acid.

As has been noted in Part I, this system is not complicated/

complicated by secondary oxidations.

Further, a system which would meet the following requirements was sought:-

(1) The amount of the oxidisable substance undergoing oxidation must lend itself to determination.

(2) The amount of iron undergoing oxidation must be determined.

As regards the first of these, this is a simple matter in the case of Formic Acid for one of the oxidation products - Carbon Dioxide - is very easily estimated.

And, since at ordinary temperature Formic Acid is not attacked to any appreciable extent by Potassium Permanganate, Iron may be easily determined in the presence of the acid by this reagent.

Accordingly, Formic Acid was chosen for the purposes of quantitative study.

The Influence of the Ratio Formic Acid/Ferrous Salt on the amount of Iron oxidised.

It has been found in the qualitative study of the Formic Acid System that in the course of the oxidation of the Formic Acid there was a simultaneous oxidation of the Ferrous Iron.

It was desirable, therefore, to see if all the Hydrogen Peroxide in the system could be accounted for by the oxidation of Formic Acid and Iron.

At the outset, however, difficulties were encountered.

If one refers to Table III, page 34, it will be seen that before all the Iron is converted into the ferric state there had to be initially in the system about five gm. molecules of Peroxide per gm. atom. Iron.

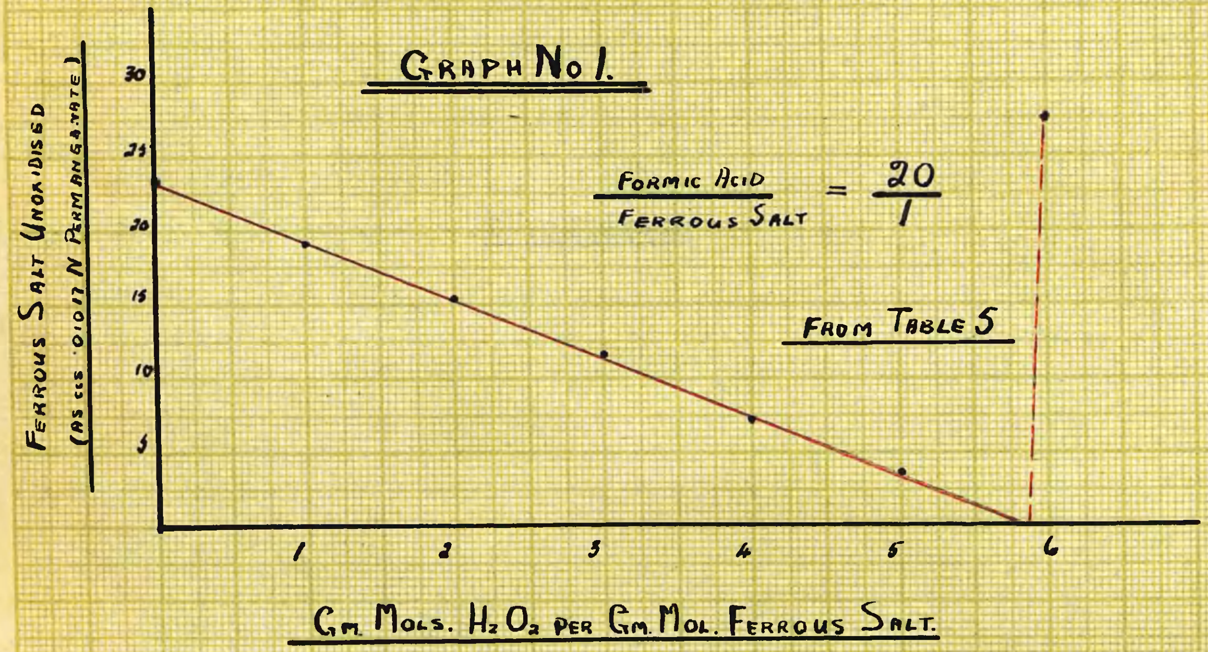
It was found in practice, however, that this figure was by no means constant - and indeed it depends on the Ratio Concentration Formic Acid/Contration Ferrous Salt.

In order, therefore, to study the fate of the Iron in such systems it became necessary to examine the influence of the above ratio on the oxidation.

The procedure was as follows:-

Throughout all the experiments the total volume of solution was kept constant and the ratio was varied by varying the concentration of Formic Acid.

Graph No. 1.



GRAPH No 1.

FORMIC ACID / FERROUS SALT = 20 / 1
FROM TABLE 5
FERROUS SALT UNOXIDISED (AS CCS ·01017 N PERMANGANATE)
30
25
20
15
10
5
1
2
3
4
5
6
GM. MOLS. H_2O_2 PER GM. MOL. FERROUS SALT.

In each experiment (i.e., for each ratio) ten tubes were made up each containing the same amount of Ferrous Sulphate solution and the same amount of Formic Acid Solution. Distilled water was then added so that after addition of Hydrogen Peroxide each tube would contain the same volume of liquid. The Hydrogen Peroxide solution was added last and in increasing amount from Tube 1 to Tube 10.

After addition of Peroxide, Sulphuric Acid was added and the amount of Ferrous Iron determined by titrating with standard Potassium Permanganate solution.

The results of these experiments are given in the following tables and they are also represented graphically.

TABLE 5.

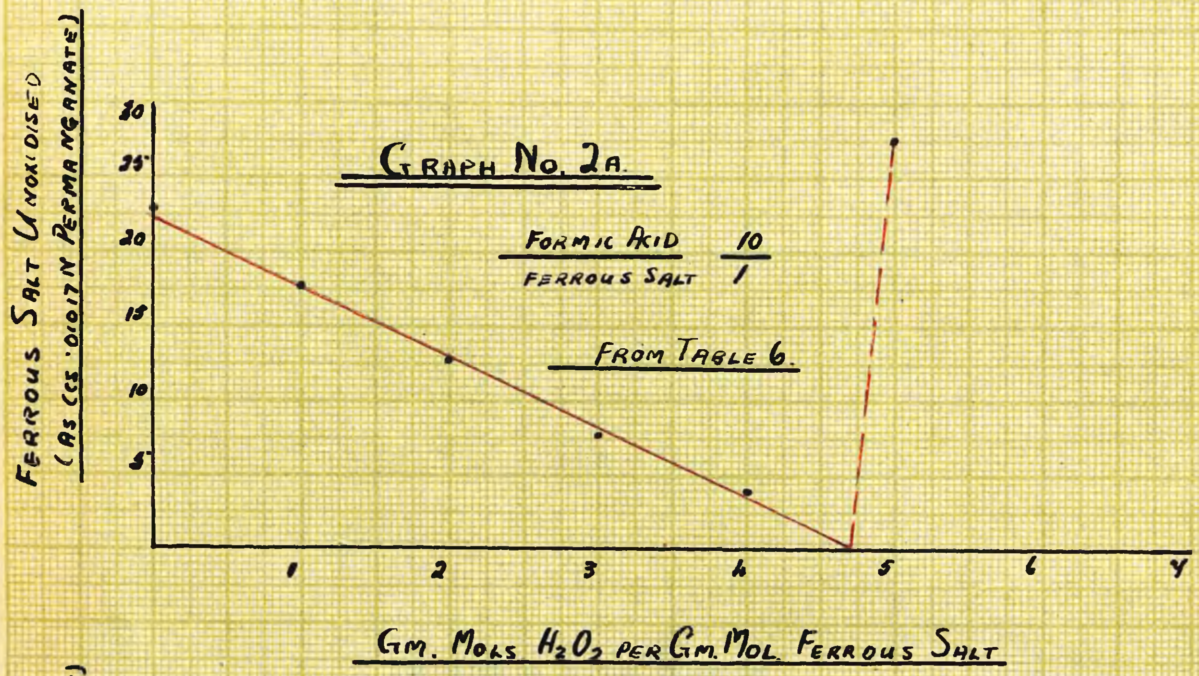
I. Ratio $\frac{\text{Formic Acid}}{\text{Ferrous Salt}} = \frac{20}{1}$ Molarity Ferrous Salt =.01

Molarity Formic Acid	.2	.2	.2	.2	.2	.2	.2
Molarity Ferrous Salt	.01	.01	.01	.01	.01	.01	.01
Molarity Hydrogen Peroxide.	.00	.01	.02	.03	.04	.05	.06
Ratio H_2O_2/Fe'' Salt	0	1	2	3	4	5	6
ccs. ·01017N $KMnO_4$ solution.	23.9	19.1	15.3	11.1	7.5	3.7	27.2

See also Graph No.I.

(The titres of seven tubes only have been given. The high titres from Tube 6 onwards are due to excess Peroxide).

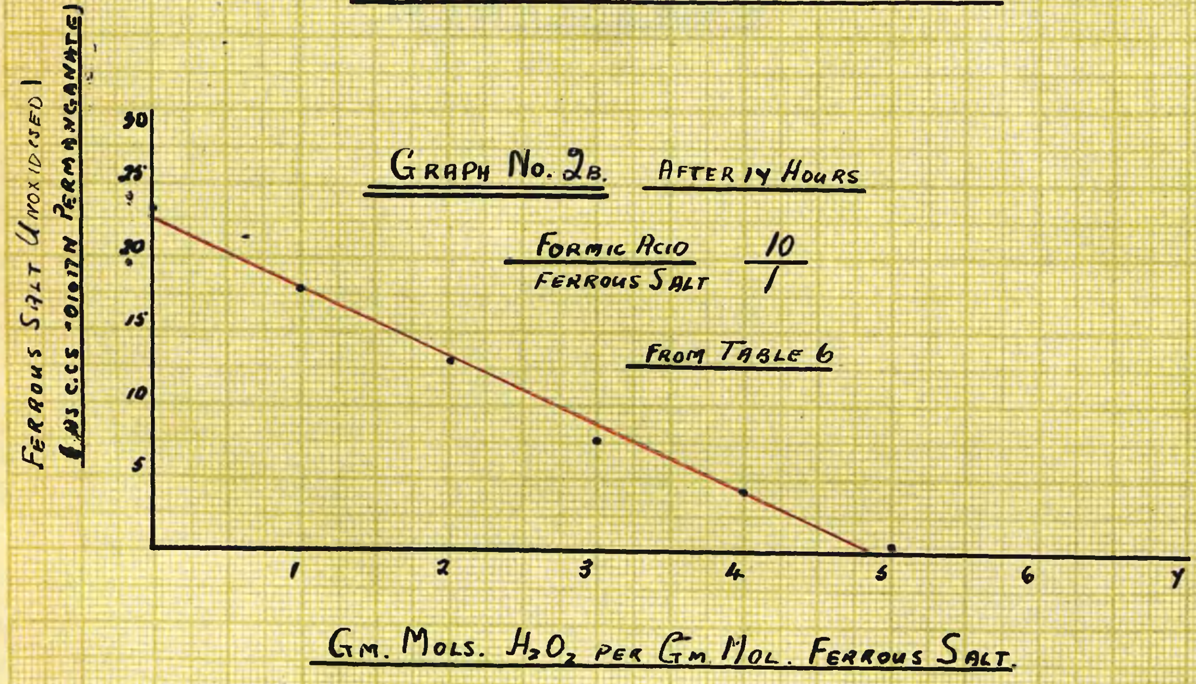
Graphs No.2 A and B.



GRAPH NO. 2A.

FORMIC ACID / FERROUS SALT 10/1

FROM TABLE 6.



GRAPH NO. 2B. AFTER 17 HOURS

FORMIC ACID / FERROUS SALT 10/1

FROM TABLE 6

TABLE 6.

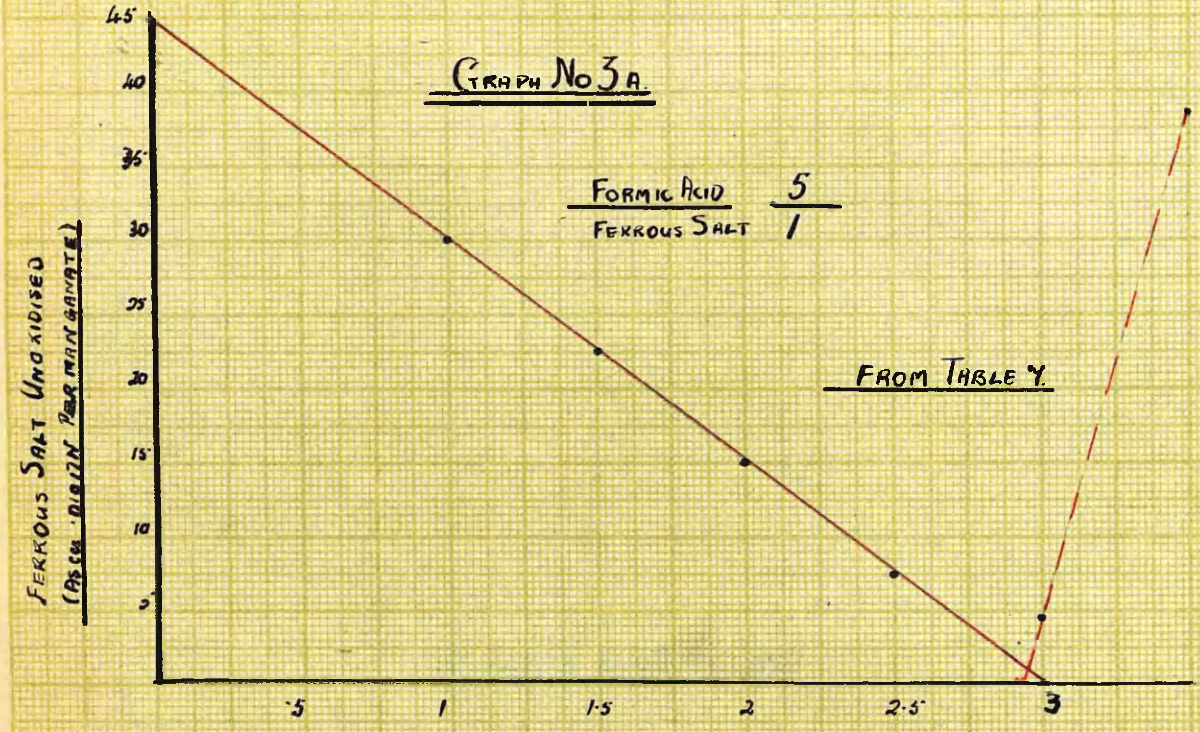
2. Ratio $\frac{\text{Formic Acid}}{\text{Ferrous Salt}} = \frac{10}{1}$ Molarity Ferrous Salt = .01

Molarity Formic Acid	.1	.1	.1	.1	.1	.1	.1
Molarity Ferrous Salt	.01	.01	.01	.01	.01	.01	.01
Molarity Hydrogen Peroxide	.00	.01	.02	.03	.04	.05	.06
Ratio H_2O_2/Fe" Salt	0	1	2	3	4	5	6
ccs. .01017N $KMnO_4$	23.2	17.4	13.0	7.6	4.2	28.1	48.1
* " " " (after 17 hrs.)	23.5	17.6	13.5	7.15	4.1	.8	.25

See also Graphs No. 2 A and B.

* In this experiment the solutions were not acidified with Sulphuric Acid immediately after addition of Peroxide, but were allowed to stand closely stoppered for 17 hours, by which time a brown precipitate had settled out. They were then acidified and titrated with Permanganate solution.

Graphs No.3 A and B.


Graph No 3A.
Formic Acid 5
Ferrous Salt 1
From Table Y.
Ferrous Salt Unoxidised
(As ccs ·01012N Permanganate)
45
40
35
30
25
20
15
10
5
·5
1
1·5
2
2·5
3
Gm Mols H_2O_2 per Gm. Mol. Ferrous Salt

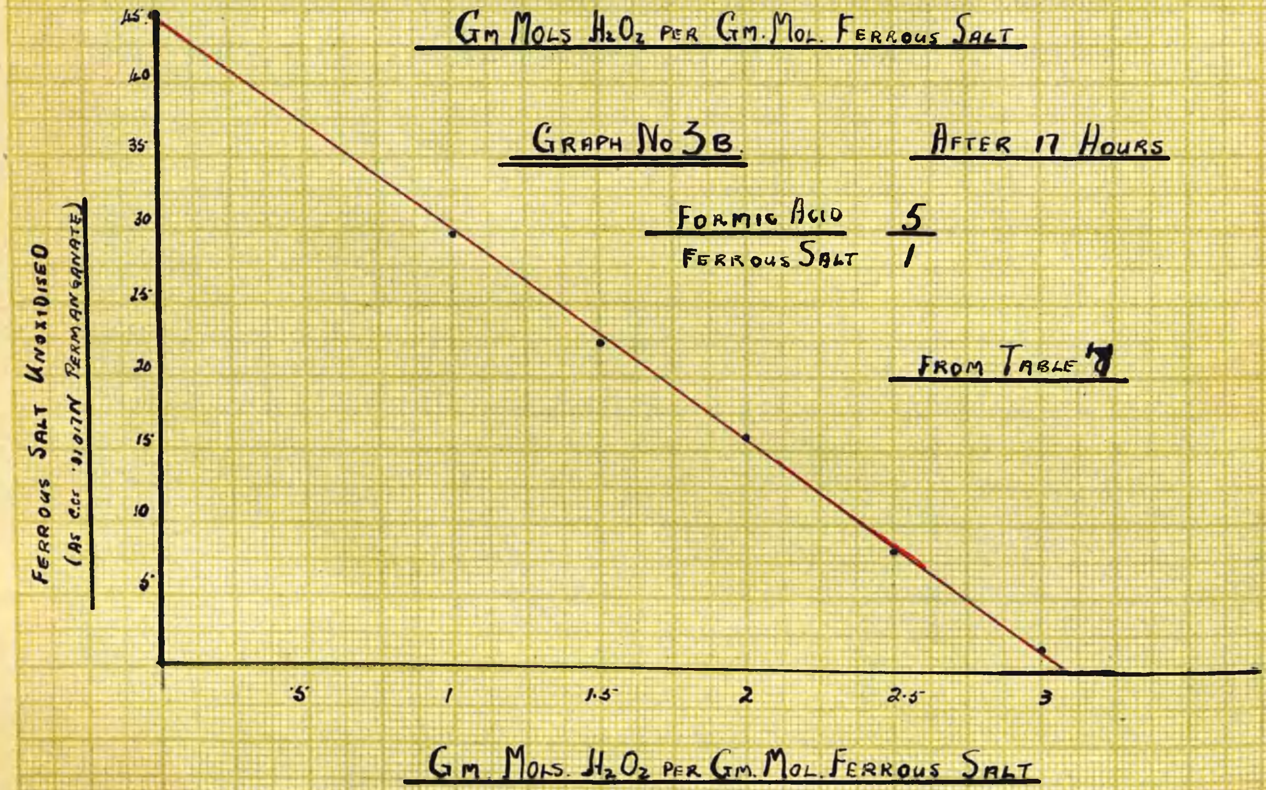

Graph No 3B.
After 17 Hours
Formic Acid 5
Ferrous Salt 1
From Table 'Y'
Ferrous Salt Unoxidised
(As c.cs ·01017N Permanganate)
45
40
35
30
25
20
15
10
5
·5
1
1·5
2
2·5
3
Gm. Mols. H_2O_2 per Gm. Mol. Ferrous Salt

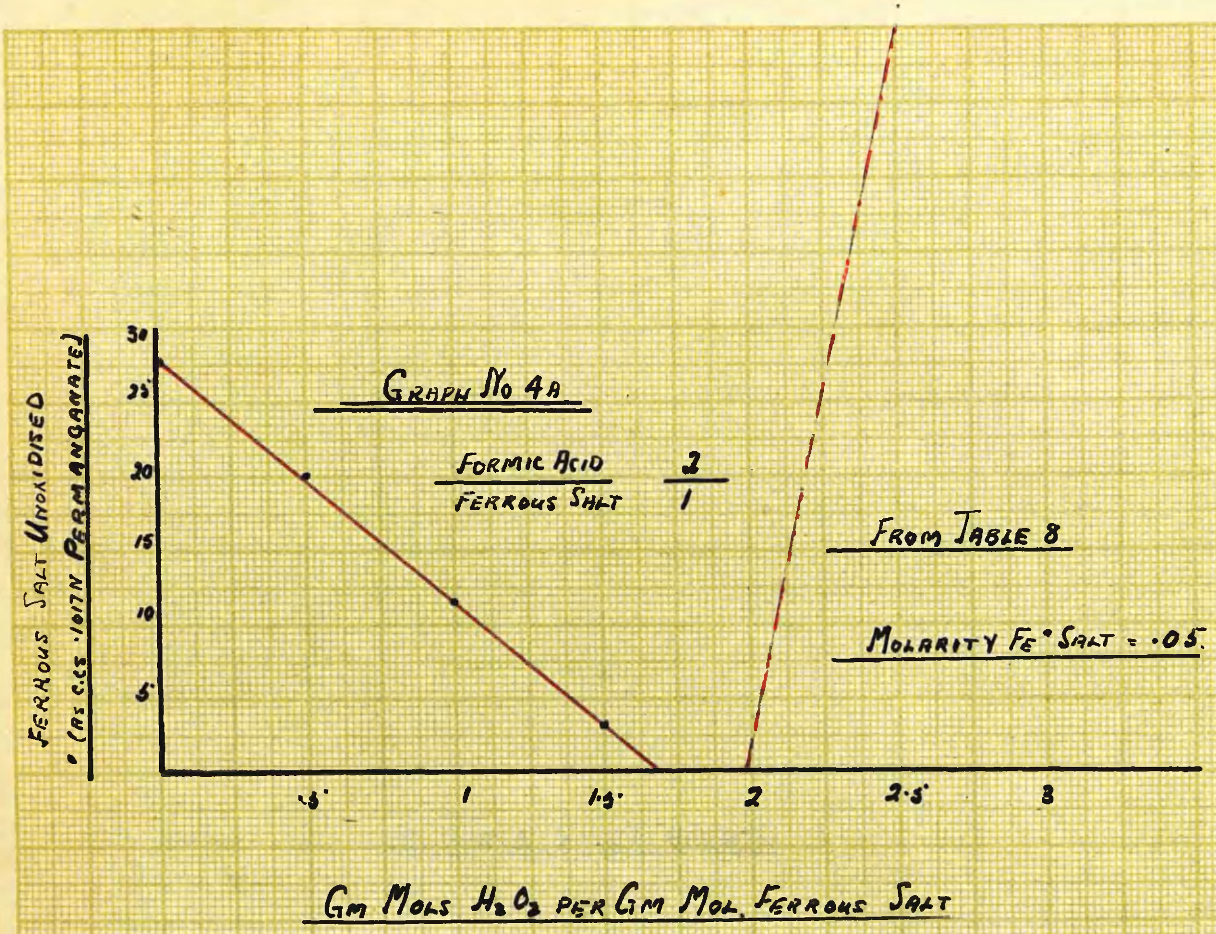
TABLE 7.

3. Ratio $\frac{\text{Formic Acid}}{\text{Ferrous Salt}} = \frac{5}{1}$ Molarity Ferrous Salt = .02

Molarity Formic Acid	.1	.1	.1	.1	.1	.1	.1
Molarity Ferrous Salt	.02	.02	.02	.02	.02	.02	.02
Molarity Hydrogen Peroxide	.00	.02	.03	.04	.05	.06	.07
Ratio Hydrogen Peroxide/Fe" Salt.	0	1	1.5	2	2.5	3	3.5
ccs. .01017 N Permanganate.	44.9	30.2	22.8	14.6	7.5	4.8	38.4
" " " (after 17 hrs.)	44.9	29.7	22.3	15.1	8.1	1.6	.3

See also <u>Graphs No. 3 A and B</u>.

Graphs No. 4 A and B.


Graph No 4A
Formic Acid / Ferrous Salt 2/1
From Table 8
Molarity Fe" Salt = ·05
Ferrous Salt Unoxidised
(as c.cs ·1017N Permanganate)
30
25
20
15
10
5
·5
1
1·5
2
2·5
3
Gm Mols H2O2 per Gm Mol. Ferrous Salt

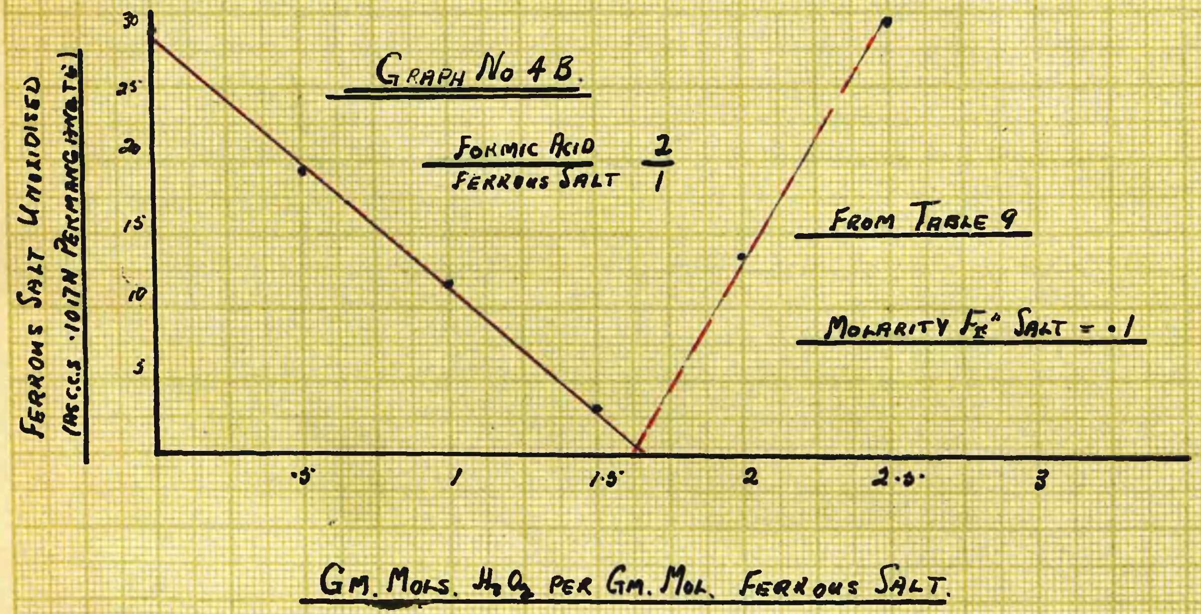

Graph No 4B.
Formic Acid / Ferrous Salt 2/1
From Table 9
Molarity Fe" Salt = ·1
Ferrous Salt Unoxidised
(as c.c.s ·1017N Permanganate)
30
25
20
15
10
5
·5
1
1·5
2
2·5
3
Gm. Mols. H2O2 per Gm. Mol. Ferrous Salt.

TABLE 8.

4. Ratio $\frac{\text{Formic Acid}}{\text{Ferrous Salt}} = \frac{2}{1}$

A. Molarity Ferrous Salt = .05

Molarity Formic Acid	.1	.1	.1	.1	.1	.1	.1
Molarity Ferrous Salt	.05	.05	.05	.05	.05	.05	.05
Molarity Hydrogen Peroxide	.00	.025	.05	.075	.1	.15	.175
Ratio Hydrogen Peroxide/Fe" Salt.	0	.5	1	1.5	2	3	3.5
ccs. ·1017 N Permanganate.	11.1	8.0	4.6	1.5	.5	20.5	42.4

See also Graph No. 4 A.

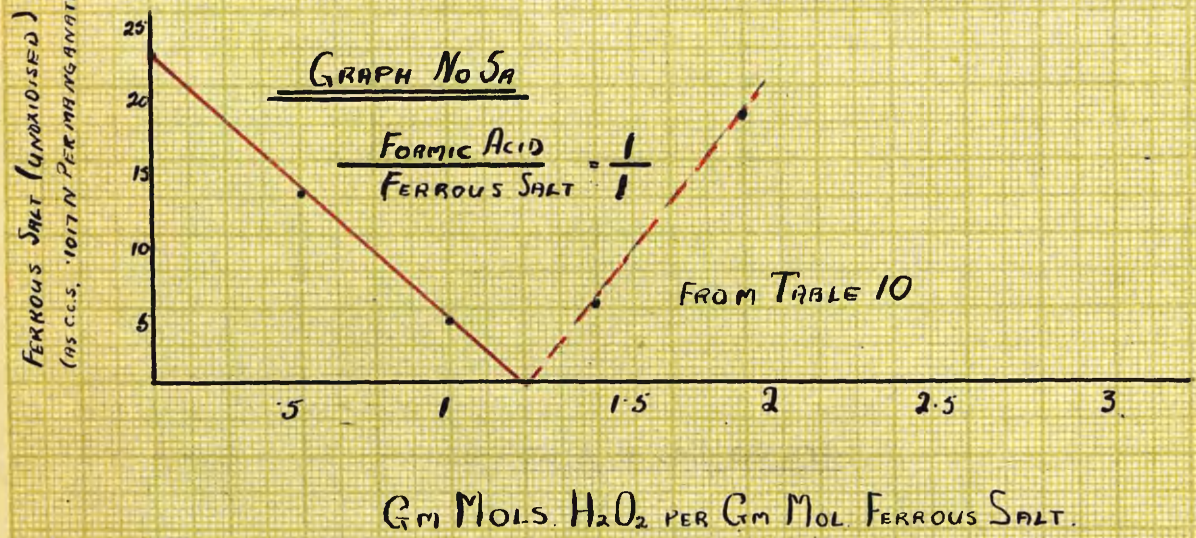
TABLE 9.

B. Molarity Ferrous Salt = .1

Molarity Formic Acid	.2	.2	.2	.2	.2	.2
Molarity Ferrous Salt	.1	.1	.1	.1	.1	.1
Molarity Hydrogen Peroxide	.00	.05	.1	.15	.2	.25
Ratio Hydrogen Peroxide/Fe" Salt.	0	.5	1	1.5	2	2.5
ccs. ·1017 N Permanganate.	23	15.4	9.2	2.7	10.8	24

See also Graph No. 4 B.

Graphs No. 5 A and B.


Graph No 5A
Formic Acid / Ferrous Salt = 1/1
From Table 10
Ferrous Salt (Unoxidised)
(as c.c.s. ·1017 N Permanganate
25
20
15
10
5
·5
1
1·5
2
2·5
3
Gm Mols. H2O2 per Gm Mol. Ferrous Salt.

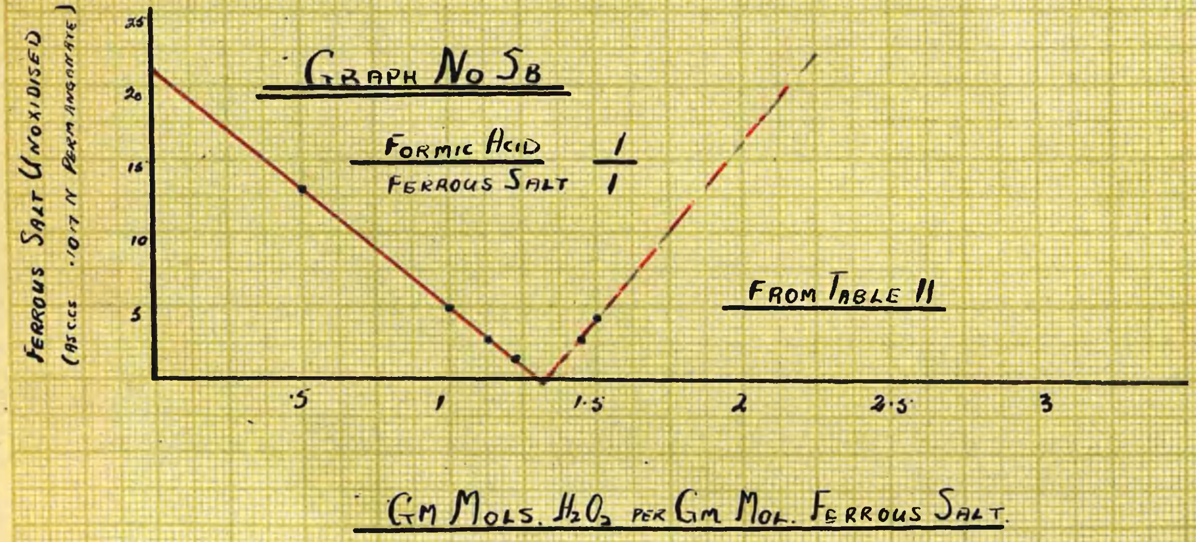

Graph No 5B
Formic Acid / Ferrous Salt 1/1
From Table 11
Ferrous Salt Unoxidised
(as c.c.s ·1017 N Permanganate)
25
20
15
10
5
·5
1
1·5
2
2·5
3
Gm Mols. H2O2 per Gm Mol. Ferrous Salt.

TABLE 10.

5. Ratio $\frac{\text{Formic Acid}}{\text{Ferrous Salt}} = \frac{1}{1}$ Molarity Ferrous Salt = .1

A.

Molarity Formic Acid	.1	.1	.1	.1	.1	.1
Molarity Ferrous Salt	.1	.1	.1	.1	.1	.1
Molarity Hydrogen Peroxide	.00	.05	.1	.15	.2	.25
Ratio Hydrogen Peroxide/Fe" Salt.	0	.5	1	1.5	2	2.5
ccs. ·1017 N Permanganate.	22	12.9	4.3	5.3	18.9	53.5

See also Graph No. 5 A.

TABLE 11.

B.

Molarity Formic Acid	.1	.1	.1	.1	.1	.1	.1	.1
Molarity Ferrous Salt	.1	.1	.1	.1	.1	.1	.1	.1
Molarity Hydrogen Peroxide.	.00	.05	.1	.11	.12	.13	.14	.15
Ratio Hydrogen Peroxide/Fe" Salt.	0	.5	1	1.1	1.2	1.3	1.4	1.5
ccs. Permanganate solution	20.8	12.6	4.1	2.6	1.1	.2	2.6	4.3
" " (after 17 hrs.)	20.7	12.3	4.5	2.6	1.0	.6	.1	.1

See also Graph No. 5 B.

From a scrutiny of these results it will be seen that Ferric Iron appears in the system long before all the Formic Acid is 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 latter has been oxidised.

Moreover, the greater the amount of peroxide actually in the system at the beginning, then the greater the amount of Iron oxidised.

Further, it will be seen that it is possible to have all the iron oxidised to the Ferric state before sufficient Peroxide has been added which would bring about the complete oxidation of all the Formic Acid.

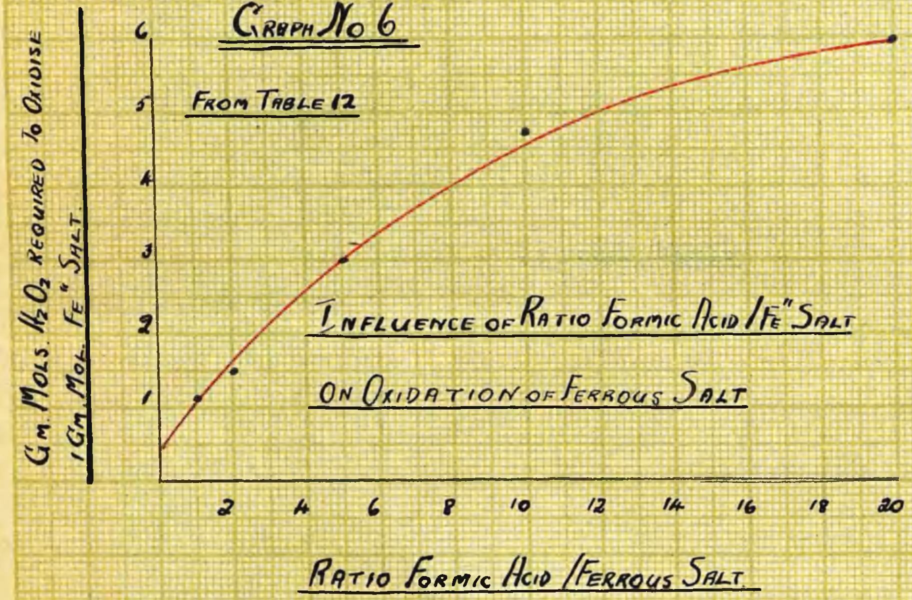
A very interesting point emerges from these results.

It will be seen that the amount of Hydrogen Peroxide which must be added initially before all the Ferrous Salt is oxidised varies greatly.

In Experiment 4 there are two parts. The absolute concentrations of the members of the system have been changed, though the ratio Formic Acid/Ferrous Salt has not. It will be seen that the amount of Hydrogen Peroxide which must be added initially (i.e., Ratio Hydrogen Peroxide/Ferrous Salt) before all the Ferrous Salt is oxidised is the same in each.

This/

Graph No. 6.



GRAPH No 6

FROM TABLE 12

INFLUENCE OF RATIO FORMIC ACID / FE" SALT ON OXIDATION OF FERROUS SALT

This amount, however, varies from experiment to experiment, and it seems to vary with the relative proportions of Formic Acid and Ferrous Salt present.

With a high ratio Formic Acid/Ferrous Salt a larger amount of Hydrogen Peroxide must be added to cause disappearance of the Ferrous Salt than with a low one.

This is best seen from a table summarising the results given in Tables 5 to 11.

(The figures given are not intended to be taken as accurate, but they show the influence of the Formic Acid/Ferrous Salt ratio).

TABLE 12.

Ratio Formic Acid/Fe" Salt	20	10	5	2	1
Gm.mols. H_2O_2 which must be added initially to oxidise 1 gm. atom Fe"	5.85	4.8	3.0	1.6	1.4

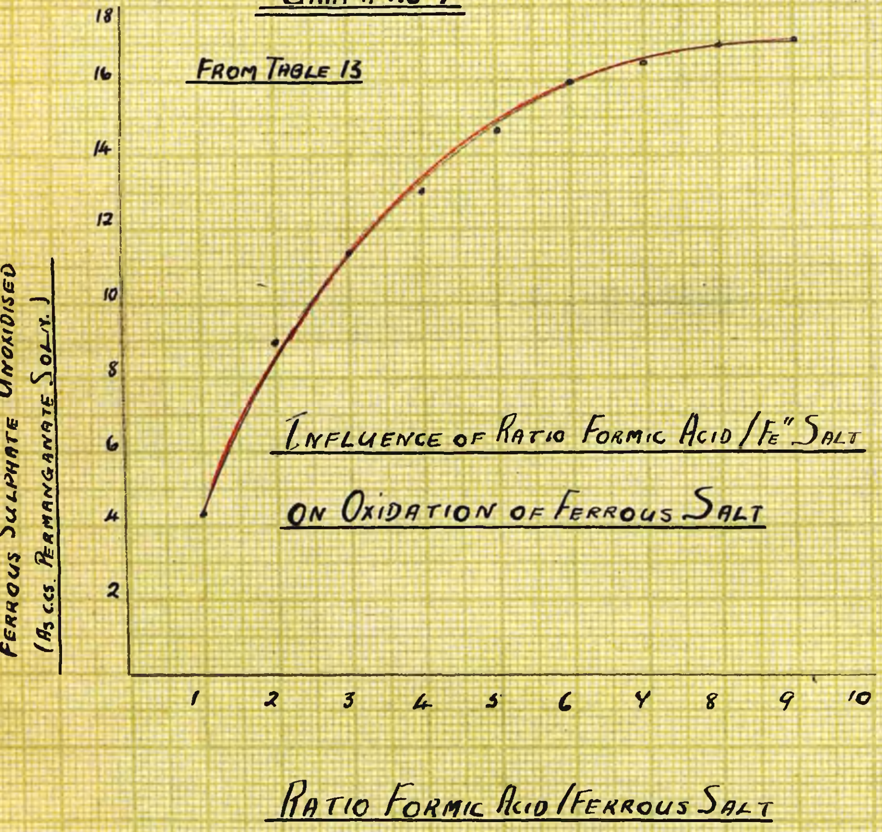
See also Graph No. 6.

In the above experiments the concentration of Ferrous Sulphate and Formic Acid is kept unchanged in each experiment, and it was found in every case that all the Ferrous Iron could be oxidised.

This point being established, the influence of the Ratio Formic Acid/Ferrous Salt may also be demonstrated as follows:-

Graph No. 7.

GRAPH No 7



FROM TABLE 13

INFLUENCE OF RATIO FORMIC ACID / Fe'' SALT ON OXIDATION OF FERROUS SALT

The concentration of Ferrous Salt and Hydrogen Peroxide is kept unchanged in ten tubes, while the concentration of Formic Acid is varied.

Method. Ten tubes were taken, each containing a known amount of Formic Acid solution - the amount increasing from Tube 1 to Tube 10. Into each was put Ferrous Sulphate solution - the same amount being put in each followed by distilled water so that the volume of liquid in each tube was the same.

Finally was added Hydrogen Peroxide - the same amount to each tube. After addition of Peroxide, Sulphuric Acid was added, and the amount of Ferrous Salt estimated by titrating with Permanganate solution.

Results of such an experiment are given below.

TABLE 13.

Ratio Formic Acid/Fe" Salt	1	2	3	4	5	6	7	8	9	10
Gm.Mols.Ferrous Salt.	$\frac{1}{1047}$	$\frac{1}{1047}$	$\frac{1}{1047}$	$\frac{1}{1047}$	$\frac{1}{1047}$	$\frac{1}{1047}$	$\frac{1}{1047}$	$\frac{1}{1047}$	$\frac{1}{1047}$	$\frac{1}{1047}$
Gm. Mols. Hydrogen Peroxide.	$\frac{1}{1047}$	$\frac{1}{1047}$	$\frac{1}{1047}$	$\frac{1}{1047}$	$\frac{1}{1047}$	$\frac{1}{1047}$	$\frac{1}{1047}$	$\frac{1}{1047}$	$\frac{1}{1047}$	$\frac{1}{1047}$
Gm. Mols. Formic Acid.	$\frac{1}{1047}$	$\frac{2}{1047}$	$\frac{3}{1047}$	$\frac{4}{1047}$	$\frac{5}{1047}$	$\frac{6}{1047}$	$\frac{7}{1047}$	$\frac{8}{1047}$	$\frac{9}{1047}$	$\frac{10}{1047}$
ccs.Permanganate Soln.	4.4	9.1	11.5	13.1	14.8	15.7	16	16.4	16.8	17.1

See also Graph No. 7.

Before the significance of the influence of the Ratio Formic Acid/Ferrous Salt can be discussed, it will be necessary to correlate these findings with the oxidation of Formic Acid.

In the next section of the work are reported results of determinations of the amount of Formic Acid oxidised with different conditions of concentration, &c.

The Oxidation of Formic Acid.

In order to examine the fate of Formic Acid when a member of the system - Hydrogen Peroxide - Ferrous Salt - Formic Acid, it was necessary to have a method of estimating the Carbon Dioxide evolved.

This was done very simply by the use of Standard Baryta and Oxalic Acid solutions.

The solution containing the Formic Acid and Ferrous Salt was placed in a flask fitted with a three-holed stopper carrying (1) a reflux condenser attached to a Baryta absorption apparatus, (2) a burette, and (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/

Hydrogen Peroxide was then introduced by means of the burette, the apparatus shaken for a short time, and after the introduction of concentrated Sulphuric Acid the contents of the flask were boiled while a stream of CO_2 free air was aspirated through the solution.

When all the Carbon Dioxide had been carried over the amount evolved was determined by titrating the excess Baryta with standard Oxalic Acid solution.

Some preliminary observations have to be recorded.

(1) It was found that Carbon Dioxide was evolved in detectable amounts when very small quantities of Peroxide were added.

(2) It was found that oxidation was inhibited by the previous addition of strong mineral acid.

(3) It was found that when a normal ferric salt was substituted for the ferrous one no oxidation took place.

(4) If Formic Acid be added to a solution containing a Ferric Salt obtained by the oxidation of Ferrous Salt by the calculated amount of Peroxide, followed by the addition of Hydrogen Peroxide, then evolution of Carbon Dioxide takes place.

Below are given results of the examination of a typical system.

TABLE 14.

Ratio $\frac{\text{Formic Acid}}{\text{Ferrous Salt}} = 1.$

Ratio Hydrogen Peroxide/H.COOH	.5	1	1.5	2	2.5
Gm.Mols.Formic Acid present.	.004	.004	.004	.004	.004
Gm.Mols.Ferrous Salt present.	.004	.004	.004	.004	.004
Gm.Mols.Hydrogen Peroxide present	.002	.004	.006	.008	.01
ccs. ·3922 N Baryta required	5.08 5.18	9.87 10.07	15.21 15.16	18.84 18.44	20.00 19.68
Gm.mols. CO_2 evolved.	.0009963 .001016	.001974 .001936	.002982 .002973	.003694 .003616	.003920 .003860
Gm.mols.Formic Acid oxidised.	.0009963 .001016	.001974 .001936	.002982 .002973	.003694 .003616	.003920 .003860
Mean	.0010061	.001953	.002978	.003655	.003890

It will be seen that the amount of Formic Acid oxidised is very far from being equivalent to the amount of Peroxide added. As has been noted, some of the peroxide is utilised in the (in this case) irreversible oxidation of Iron. How far this amount and the amount utilised in the oxidation of the Formic Acid account for the Hydrogen Peroxide initially in the system will be considered later.

The above figures for the oxidation of Formic Acid were obtained by adding Hydrogen Peroxide to a solution containing Formic Acid and Ferrous Salt.

Qualitative observations lead to the conclusion that oxidation can also take place when the Ferrous Salt has been previously oxidised by means of the equivalent quantity of Hydrogen Peroxide.

This point has been examined quantitatively and the results are given in the next section.

The Oxidation of Formic Acid in the Presence of previously oxidised Ferrous Salt.

This was examined in two ways:-

(1) To a solution of a ferrous salt was added the necessary quantity of Hydrogen Peroxide (·6 gm. molecular proportion) to oxidise the divalent Iron completely. The Peroxide was added quickly and with shaking. Immediately was added Formic Acid and further Hydrogen Peroxide and the amount of Carbon Dioxide evolved measured in the usual way.

For purpose of comparison control experiments were also made (by adding the same total amount of Hydrogen Peroxide to a solution with the same amount of Formic Acid and Ferrous Salt).

Typical results of such experiments are given in the table below:-

TABLE 15.

Gm.Mols Formic Acid present	Gm. Mols Hydrogen Peroxide present.	Gm.Mols Ferrous Salt present	ccs. ·3922N Baryta required to absorb CO_2.		Gm.Mols. Formic Acid Oxidised.	
					Fe" previously oxidised.	Fe" not previously oxidised.
.004	.0064	.004	12.39	15.92	.002429	.003121
			12.89	16.62	.002528	.003258
Mean					.002479	.003190
.004	.0084	.004	15.02	18.76	.002946	.003680
			14.55	19.61	.002853	.003845
Mean					.002899	.003763

The results reported above are sufficient to show that even when oxidation of the Ferrous Salt has been initially carried out by means of the equivalent quantity of Peroxide the oxidation of Formic Acid can still be catalysed.

The discrepancy in the results of the two procedures was always found. At first it was thought that this might be due to the fact that oxidation of the Formic Acid when the Iron had previously been oxidised might be a much slower process. This, however, was rendered unlikely when it was found that Hydrogen Peroxide disappeared from systems formed in the first way as from systems formed in the second way.

Further, it was found that the amount of Carbon Dioxide produced could not be increased by longer aeration and it was concluded that less Formic Acid was actually being oxidised.

It is believed that this is merely due to increased catalytic decomposition of Hydrogen Peroxide when the Ferrous Salt has been previously oxidised. This point will be referred to again later.

(2) In the second method Formic Acid was added to a solution which had initially contained Ferrous Salt and to which a large excess of Hydrogen Peroxide had been added.

Thus when the Formic Acid had been introduced, Hydrogen Peroxide/

Peroxide was itself undergoing catalytic decomposition, but provided a sufficiently large excess of the oxidising agent was used complete oxidation of the acid could be obtained.

TABLE 16.

Gm. Mols. Formic Acid present.	Gm. Mols. Ferrous Salt present.	Gm. Mols H_2O_2 present.	ccs. ·3922N Baryto required.	Gm. Mols. Formic Acid oxidised.
.004	.004	.04	19.56 19.77	.003836 .003877
.004	.004	.08	19.55 20.02	.003834 .003917

In all these experiments so far reported in which the amount of Formic Acid undergoing oxidation has been determined, the Ratio Formic Acid/Ferrous Salt has been the same, - namely 1:1.

It has been previously noted that with the system Formic Acid : Hydrogen Peroxide : Ferrous Salt, the amount of iron undergoing oxidation on the addition of peroxide depended on the Ratio Formic Acid : Ferrous Salt.

With a ratio of unity the amount is large, and as the ratio increases the amount becomes increasingly smaller.

It is a matter of interest, therefore, to find that

as/

as the ratio increases the amount of formic acid oxidised also increases.

In the following table results are given illustrating this point.

The determinations were carried out exactly as before, Hydrogen Peroxide being added to Formic Acid solutions containing Ferrous Sulphate.

The total volume of solution and amount of Ferrous Salt present were constant throughout the experiments. - The ratio was varied by varying the amount of Formic Acid present.

TABLE 17.

Ratio Formic Acid/ Ferrous Salt.	1	2	5	10	20
Gm.Mols. Ferrous Salt present.	.004	.004	.004	.004	.004
Gm.Mols. Hydrogen Peroxide present.	.004	.004	.004	.004	.004
Gm.Mols. Formic Acid present.	.004	.008	.02	.04	.08
Gm.Mols. Formic Acid oxidised.	.001974 .001936	.0021 .0027	.003 .002928	.0033 .00324	.00343 .00353
Mean	.001955	.0024	.002964	.00327	.00348

Before the significance of the quantitative results in the bearing on the rôle of Iron can be discussed an attempt must be made to correlate them with each other.

This is done in the following section.

TABLE 18. Ratio Formic Acid : Ferrous Salt. = 1

		*				
Gm.Mols. Formic Acid present.	1	.004	.004	.004	.004	.004
Gm.Mols. Ferrous Salt present.	2	.004	.004	.004	.004	.004
Gm.Mols. Hydrogen Peroxide present.	3	.002	.004	.006	.008	.01
Gm.Mols. Formic Acid oxidised.	4	.001	.002	.003	.0036	.0039
Gm.Mols. Ferrous Salt oxidised.	5	.001654	.0032	.004	.004	.004
Gm.Mols. H_2O_2 to oxidise 1 gm.atom Fe''	6	.61	.62	.75	1.1	1.5

The figures in column 4 were obtained by direct determination of the amount of CO_2 evolved and are taken from Table 14.

The figures in column 5 were obtained by titrating the unoxidised Ferrous Salt with Permanganate, and are taken from Table 10.

The figures in column 6 are obtained by deducting the amount of Hydrogen Peroxide equivalent to the amount of Formic Acid oxidised from the total amount initially in the system. It is then assumed that this unaccounted for Hydrogen Peroxide has been utilised in the oxidation of Ferrous Iron. The excess peroxide over that utilised by the Formic Acid is expressed in column 6 as the amount necessary to oxidise 1 gm. atom Ferrous Iron under these conditions. (~~See also Graph~~).

A specimen calculation is given below to illustrate this.

Column marked x in Table 18.

Gm. Mols. Formic Acid oxidised = .001

Gm. Mols. Hydrogen Peroxide required for this = .001

Total Amt. Hydrogen Peroxide in System (Gm.Mols) = .002

Gm. Mols. Hydrogen Peroxide not accounted for by oxidation of Formic Acid = .001

ccs. Permanganate required for unoxidised Ferrous Salt (Table 10) = 12.9

(·004 gm.Mols. Ferrous Salt requires 22 ccs. $KMnO_4$ solution)

∴ Gm. Mols. Ferrous Salt unoxidised = .002346

" " " " oxidised = .001654

Assuming that the Peroxide unaccounted for is utilised in the oxidation of Ferrous Salt

.001 gm. Mol. Hydrogen Peroxide oxidises .001654 gm. Mol. Ferrous Salt.

∴ 1 gm. Mol. Ferrous Salt requires ·61 gm. Mol. Hydrogen Peroxide.

From a scrutiny of the Table it will be seen that in this case, where the amounts of Formic Acid and Hydrogen Peroxide are molecularly equivalent oxidation of the salt takes place to a greater extent than does that of the acid.

From Table 4 it will be seen that 1 gm. Mol. of Ferrous Salt, in the absence of any other oxidisable substance, requires for its complete oxidation to the ferric state approximately ·6 gm. Mol. Hydrogen Peroxide.

From Table 18 it will be seen that, assuming that the Hydrogen Peroxide not accounted for by the oxidation of the Formic Acid is utilised in the oxidation of Ferrous Iron, this figure is obtained in experiments 1 and 2.

The figure obtained by this method of calculation is very much higher in experiments 3, 4, and 5, and we have to consider why this should be.

It will be seen that the high figures are found in the cases in which all the ferrous salt has been oxidised to the Ferric state.

In such cases, as has been shown by the qualitative tests in Table 3, there is for a short time in the solution free Hydrogen Peroxide. It is probable that, since Hydrogen Peroxide is itself catalytically decomposed by Ferrous Salts, the high figures of experiments 3, 4, and 5 are to be accounted for in this way.

Support is also lent to this idea by a consideration of the results reported in Table 16.

In these experiments Hydrogen Peroxide has been added immediately after Formic Acid to a solution in which the Ferrous Salt has been oxidised by the calculated quantity of Peroxide.

The/

The amount of Formic Acid oxidised is not, as was expected, equivalent to the amount of Peroxide added.

This also may be accounted for by the partial catalytic decomposition of the added peroxide, for here the oxidising agent is introduced in excess.

A similar treatment to that in the above table may be adopted in the case of other ratios of Formic Acid/Ferrous Salt. This has been done with the results recorded in Table 17, and are shown below.

TABLE 19.

Ratio Formic Acid/Ferrous Salt.	1	1	2	5	10	20
Gm.Mols. Formic Acid present.	2	.004	.008	.02	.04	.08
Gm.Mols. Ferrous Salt present.	3	.004	.004	.004	.004	.004
Gm.Mols. Hydrogen Peroxide present.	4	.004	.004	.004	.004	.004
Gm.Mols. Formic Acid oxidised.	5	.001955	.0024	.00296	.00327	.0035
Gm.Mols. Ferrous Salt oxidised.	6	a .0032	b .0023	c .0014	d .001	e .0008
Gm.Mols. H_2O_2 to oxidise 1 gm. atom Fe"	7	.63	.69	.76	.673	.62

The figures in column 5 are taken from Table 17.

The figures in column 6 are calculated from the Permanganate titrations reported earlier in the paper. Above each/

each figure is given the table from which it has been taken.

a Table 10: b Table 8: c Table 7: d Table 6
e Table 5.

It will be seen that here, where the Ferrous Salt has not been all oxidised in any of the experiments, quite good agreement is got between the total amount of Hydrogen Peroxide initially in the solution and the amount equivalent to the Formic Acid and Ferrous Salt oxidised.

A further point is that the amount of Formic Acid oxidised also depends on the Ratio:- Formic Acid/Ferrous Salt.

In the next section the catalytic phenomenon is discussed in the light of the results reported in this paper.

* * *

DISCUSSION.

DISCUSSION.

In any discussion of these results the first point that must be noticed is that in all cases of oxidation examined there is also oxidation of Iron from the Ferrous to the Ferric state.

The possibility that this is an accidental process having no connection with the main reaction is negatived by the evidence offered in the previous pages.

This evidence may be summarised as follows:-

(1) When Hydrogen Peroxide is added to a solution of an oxidisable substance and a Ferrous Salt in such amount that the latter is completely oxidised, catalysis of the reaction between the Peroxide and the oxidisable substance still takes place.

(2) If the Ferrous Salt is, previous to the addition of the oxidisable substance, oxidised to the ferric state by the calculated quantity of Peroxide, then the ferric compound so formed still acts catalytically.

One is therefore led to the conclusion that the actual catalyst is a ferric compound formed by the interaction between Ferrous Salt and Hydrogen Peroxide.

The objection that normal Ferric Salts do not act catalytically is easily met.

It/

It has to be remembered that in the absence of Sulphuric Acid the action between Hydrogen Peroxide on Ferrous Sulphate is to produce a basic Ferric Salt. This differs from normal Ferric Sulphate in being much more easily hydrolysed, and it is probable that its behaviour towards Hydrogen Peroxide is also different.

It has been noted that catalytic oxidation is inhibited by the presence in the system of Sulphuric Acid, and this is readily understandable on the basis of the above.

In the presence of Sulphuric Acid Hydrogen Peroxide oxidises Ferrous Sulphate according to the equation

$$2\,FeSO_4 + H_2SO_4 + H_2O_2 = Fe_2(SO_4)_3 + 2H_2O.$$

A normal salt is formed, and as we have seen such cannot act as catalysts.

A further observation bearing on this point is this, that when a large excess of Hydrogen Peroxide is added to a solution of a Ferrous Salt catalytic decomposition of the former is brought about. If now sulphuric acid be added the decomposition stops. It is difficult to find any other explanation for this than that the catalyst has been destroyed. The action of sulphuric acid is to form a normal Ferric Sulphate.

The question now arises - What is the mode of action of this Basic Ferric Salt?

There/

There are two possibilities:-

(1) That this Basic Ferric Salt is itself the oxidising agent, reacting with the oxidisable substance.

Such would require the simultaneous reduction of the Basic Ferric Salt, and it has to be noted that such has been observed.

In the summary appended to the qualitative section of this work it has been shown that the evidence is all against this observed reduction being connected in any way with the initial oxidation.

It appears that this reduction is a secondary process depending on the production of highly oxidisable substances in the initial oxidation. Where the products of oxidation are not further oxidisable (as in the case of Formic Acid) it does not occur.

The second possibility is therefore more likely.

(2) That the Basic Ferric Salt initially formed reacts with more Hydrogen Peroxide to give a highly oxygenated Iron compound. This is the actual oxidising agent and after reacting with the oxidisable substance it gives back the Basic Ferric Salt initially formed.

The evidence for this, of course, must be indirect.

First of all, as seen from Tables 18 and 19, 1 gm. Molecule Ferrous Salt requires for its complete oxidation

the/

the same amount of Hydrogen Peroxide whether oxidisable substance is present or not. (The figure found was about ·6 gm. mol. Hydrogen Peroxide, but this should undoubtedly be ·5 gm. mol., the higher figure being due to catalytic decomposition of the Peroxide.

But the Basic Ferric salt so formed can not itself act as an oxidising agent (e.g., if Formic Acid be added to such a compound, oxidation of the former does not take place).

But that it may catalyse the reaction between Hydrogen Peroxide is easily shown by first of all oxidising Ferrous Salt with the calculated quantity of Hydrogen Peroxide then adding Formic Acid followed by Hydrogen Peroxide. Oxidation of the acid now takes place. (It is to be understood, of course, that these processes must be carried out swiftly).

We have to believe, therefore, that the actual catalyst is a Basic Ferric Salt and that it acts by forming with Hydrogen Peroxide a highly active and unstable oxidising agent.

As far as the actual structure and graphical representation of the compounds and reactions involved are concerned, it would be idle to speculate, for there is no evidence to be offered.

It is however possible to put forward the following as a scheme incorporating all the phenomena observed:-

(1) In absence of Oxidisable Substance or excess Hydrogen Peroxide

$2\,FeSO_4 + H_2O_2 =$ Soluble Basic Ferric Salt

Soluble Basic Ferric Salt + H_2O =

Insoluble Basic Ferric Salt.

(2) In presence of excess Hydrogen Peroxide

Soluble Basic Ferric Salt + nH_2O_2

= Oxidising Agent.

Oxidising Agent + H_2O_2 = Soluble Basic Ferric Salt.

+ $H_2O + O_2$

Soluble Basic Ferric Salt + H_2O

= Insoluble Basic Ferric Salt.

(3) In presence of Hydrogen Peroxide and Oxidisable Substance (Substance A).

Soluble Basic Ferric Salt + nH_2O_2 = Oxidising Agent

Oxidising Agent + A = AO + Basic Ferric Salt.

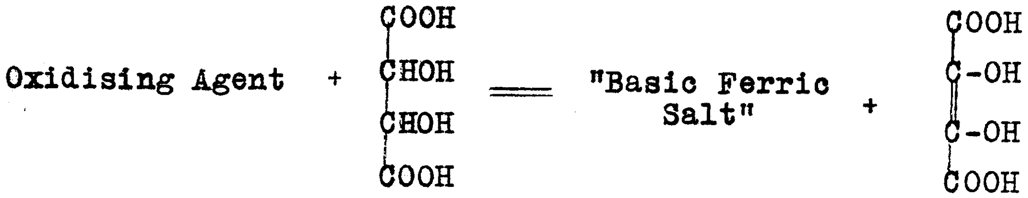
And where AO is capable of further oxidation we have

AO + Basic Ferric Salt = A·O·O + Ferrous Salt.

Thus the oxidation of Tartaric Acid might be represented:-

$$2\ FeSO_4 + H_2O_2 = \text{"Basic Ferric Salt"}$$

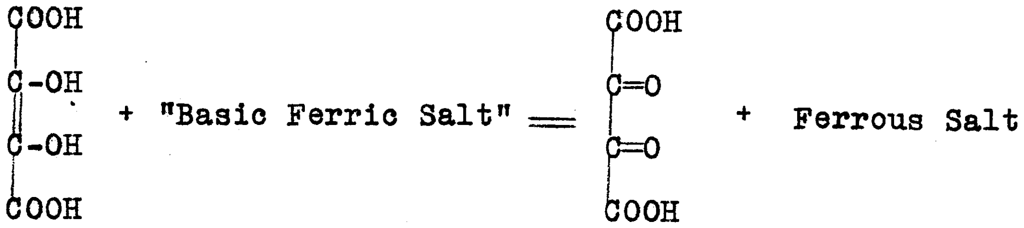
$$\text{"Basic Ferric Salt"} + nH_2O_2 = \text{Oxidising Agent.}$$



$$\text{Oxidising Agent} + \begin{array}{l} COOH \\ | \\ CHOH \\ | \\ CHOH \\ | \\ COOH \end{array} = \text{"Basic Ferric Salt"} + \begin{array}{l} COOH \\ | \\ C{-}OH \\ \| \\ C{-}OH \\ | \\ COOH \end{array}$$

Tartaric Acid.

Dihydroxy Maleic Acid.



$$\begin{array}{l} COOH \\ | \\ C{-}OH \\ \| \\ C{-}OH \\ | \\ COOH \end{array} + \text{"Basic Ferric Salt"} = \begin{array}{l} COOH \\ | \\ C{=}O \\ | \\ C{=}O \\ | \\ COOH \end{array} + \text{Ferrous Salt}$$

Such a scheme is in harmony with all the observed facts and a survey of the literature has not brought to light any observations antagonistic to it.

We have now to examine, in the light of this scheme, the partition of Hydrogen Peroxide between Oxidisable Substance and Iron and its observed dependence on the Ratio:- Oxidisable Substance/Ferrous Iron.

We must first of all refer to Table 13.

From this it will be seen that, as the concentration of oxidisable substance initially in the system increases, the amount of ferrous salt finally in the system also increases.

It/

It has also been shown in the later work that the amount of oxidisable substance undergoing oxidation correspondingly increases.

It has also been shown (see Tables 5-11) that, no matter what may be the value of the ratio, Oxidisable Substance/Ferrous Salt, all the iron may be oxidised to the Ferric State. The amount of Hydrogen Peroxide necessary to do this, however, varies with the above ratio, increasing as it increases.

What possible explanation can be advanced for this in the light of the scheme which has been offered here?

It has been suggested that the first reaction which takes place in such a system results in the formation of a Basic Ferric Salt which, through the agency of further Hydrogen Peroxide, gives a highly oxygenated and unstable Iron compound - the actual oxidising agent. As soon as any of this latter compound is formed in the system it would react with the oxidisable substance and would be reduced, giving back "Basic Ferric Salt." We have now two sources of "Basic Ferric Salt" - (a) from the interaction of Ferrous Salt and Hydrogen Peroxide, and (b) from the reaction between oxidising agent and oxidisible substance.

The amount of "Basic Ferric Salt" formed will depend on the amount of Ferrous Salt entering into reaction with Hydrogen Peroxide, and this again will depend upon the relative/

relative rates of reactions (a) and (b) noted above.

As soon as any of the actual oxidising agent is in the system it will depend on whether the rate of reaction of this with the oxidisable substance is greater than the rate of reaction of Ferrous Salt with Hydrogen Peroxide whether Basic Ferric Salt is formed by reaction (1) or reaction (2).

Now accordingly any factor which would increase the rate of reaction (2) while leaving that of reaction (1) unchanged would favour the production of "Basic Ferric Salt" from the oxidising agent. This "Basic Ferric Salt" would then be available for further reaction with Hydrogen Peroxide.

Such a factor would therefore tend to decrease the amount of Ferrous Sulphate oxidised.

By the Mass Action Law the rate of a reaction in any one direction is directly proportional to the concentration of the reactants.

It is probable, therefore, that increasing the concentration of oxidisable substance in our system would have the effect of speeding up the reaction between it and the oxidising agent, and, according to the foregoing reasoning, this would be expected to decrease the amount of Ferrous Salt undergoing oxidation.

This agrees well with the known facts. It has been found/

found that the greater the amount of oxidisable substance in the system the smaller the amount of Ferrous Salt undergoing oxidation. Not only so, but it has also been found that there is a corresponding increase in the amount of Formic Acid oxidised. It was to be expected that, since a smaller quantity of Ferrous Salt is undergoing oxidation, a greater amount of Hydrogen Peroxide would be available for the oxidation of Formic Acid.

From the above discussion it is apparent that the theory that oxidation in such systems is effected by the simultaneous reduction of Ferric Iron to the Ferrous State is untenable. Equally so is that of Mummery who has advanced the view that the oxidising agent is a Ferrous compound.

The scheme put forward by Küchlin and Boëseken also supposes that the oxidising agent is a Ferrous Salt. For example, they explain the oxidation of Fructose by this method in the following way:-

(1) Fe'' + Fructose $=$ Fe'' - Fructose Complex.

(2) Fe'' Fructose Complex + nH_2O_2 $=$ Fe'' nH_2O_2-Fructose Complex.

(3) Fe'' nH_2O_2 Fructose Complex $=$ Fe''' + Fructosone + nH_2O

(4) Fe''' + Fructosone $=$ Fe'' + Ketonic Acid.

(5) Fe'' + Osone $=$ Fe'' Osone Complex.

It will be seen that this scheme requires the Iron of the/

the actual oxidising agent to be in the Ferrous State. The observations recorded here favour the idea that the catalyst is a Ferric compound, and that the actual oxidising agent is a highly oxygenated Ferric complex.

Regarding the formation of complexes of Iron with substrate, some observations in the foregoing pages have a bearing on this point.

It is found that the soluble "Basic Ferric Salt" formed by the action of Hydrogen Peroxide on a Ferrous Salt is, in the absence of an oxidisable substance, very liable to hydrolytic attack, and that shortly after the addition of Peroxide the solution becomes turbid through the precipitation of an insoluble Basic Ferric Salt.

With oxidisable substances, the oxidation products of which still contain hydroxyl groups, this precipitation does not occur. As has been shown, secondary reactions involving the reduction of the Basic Salt take place in such cases, and this might be thought to account for the absence of hydrolytic action.

These secondary reductions are generally, however, very slow reactions compared with the hydrolytic one that where the latter does not take place shielding of the "Basic Ferric Compound" is likely. It is obvious that the formation of complexes with Hydroxyl groups in the substrate would be likely to perform this function.

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It is significant that a different behaviour obtains in systems in which the oxidisable substance is Formic Acid.

With Formic Acid/Ferrous Salt ratios approaching unity oxidation of the acid is followed immediately by deposition of insoluble basic Ferric Salt. With high ratios the solutions remain clear for a very long time after oxidation.

This might be explained by the assumption that the acid hydroxyl group of the Formic Acid has also some shielding action of the Basic Ferric Salt. After oxidation of the Formic Acid the concentration of the latter is diminished and the shielding effect is lessened.

Hydrolysis takes place and an insoluble Basic Ferric Salt is precipitated out of the system.

In conclusion, some practical considerations arise from the observations.

It has been seen that the oxidation of the substrate is by no means quantitative, for two reasons:-

(1) Simultaneous oxidation of Iron takes place;

(2) There is also some catalytic decomposition of Hydrogen Peroxide.

These losses, as has been seen, may be lessened by keeping the ratio

$$\frac{\text{Concentration of Substrate}}{\text{Concentration of Catalyst}}$$

as high as possible.

A further source of loss is to be found in the fact that when secondary reduction actions take place there is oxidation of the initial product with reduction of iron and consequent using up of peroxide in the oxidation of this.

The method in vogue - as used by Fenton, Morrell and Crofts, and others - depends on the theory that the actual oxidation is brought about by the reduction of Iron from the Ferric to the Ferrous state.

Hydrogen Peroxide is added to a solution of the oxidisable substance and ferrous iron. The solution is allowed to stand until all the iron has been reduced back to the Ferrous state. Further addition of Peroxide is then made and the process repeated until the total amount of Peroxide has been added.

Oxidation is much more favourably carried out by merely adding the requisite quantity of Peroxide to a solution of the substrate containing a trace of Ferrous Salt.

The solution is allowed to stand until it gives a negative test for Peroxide. In this way it is found that very little of the starting material remains unoxidised and that the production of secondary oxidation products is minimal.

SUMMARY.

SUMMARY.

The catalytic oxidation of substances by Hydrogen Peroxide in the presence of Ferrous Salts has been studied.

It has been found that experimental evidence supports strongly the contention that the actual catalyst contains Iron in the Ferric state - probably of the nature of a Basic Ferric Salt.

In this way is explained the failure of Normal Ferric Salts to catalyse such oxidations and the inhibiting action of low P.H's brought about by the presence of mineral acids.

The view has been advanced that catalysis is effected by the production of a highly oxygenated complex compound of Iron by the interaction of the "Basic Ferric Salt" noted above and Hydrogen Peroxide.

The scheme suggested differs essentially from that which holds that oxidation is brought about by the reduction of Ferric Iron to the Ferrous state.

It also negatives the views of Mummery who believes that only compounds containing Ferrous Iron are involved. In this respect also it differs from that of Küchlin and Boëseken though not antagonistic to their idea that complexes with the substrate may play a part in such oxidations.

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The scheme advanced purports also to explain the reactions involved, though no information can be given as to the structure of the compounds taking part in the reactions.

From these observations information has been gained as to the best method for the practical use of such systems.

In conclusion, I have to thank the Senate of the University of Glasgow for the Barbour Research Scholarship held by me, during the tenure of which this work was carried out.

It is a great pleasure to be able to record my indebtedness to Professor E. P. Cathcart, LL.D., F.R.S., Director of the Institute of Physiology, for the interest he has at all times taken in the work and for the advice which he has given.

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