6. Fibrils deposited from preheated aqueous solution have a shrunken, curled-up and twisted appearance.

7. The structures of the tropomyosin units, fibrils and crystals are discussed in the light of the above findings and data reported in the accompanying communications. It is suggested that tropomyosin is a typical 'monomer' of the *k-m-e-f* group, the long fibres of which are built up by a process of 'polymerization' involving linkages of a more permanent character than those which operate in the formation of the fibrils found in aqueous solution.

The latter, however, would appear to be of the nature of impermanent prototypes of the fibres formed *in vivo*.

8. Available diffusion and viscosity data lend reasonable support to the hypothesis that the tropomyosin unit consists of a pair of chains, or a looped single chain, in the α -configuration.

It is clear that the investigation described in this paper could not have been carried out without Dr K. Bailey's close co-operation, and we wish gratefully to acknowledge this and the help of many discussions.

REFERENCES

- Astbury, W. T. (1942). *J. chem. Soc.* p. 337.
 Astbury, W. T. (1945). *Nature, Lond.*, **155**, 501.
 Astbury, W. T. (1947a). *J. Chim. phys.* **44**, 3.
 Astbury, W. T. (1947b). *Proc. 6th Int. Congr. exp. Cytol., Stockholm* (in the Press).
 Astbury, W. T. (1947c). *Nature, Lond.*, **160**, 388.
 Astbury, W. T. & Bell, F. O. (1941). *Nature, Lond.*, **147**, 696.
 Astbury, W. T. & Dickinson, S. (1940). *Proc. Roy. Soc. B*, **129**, 307.
 Astbury, W. T., Dickinson, S. & Bailey, K. (1935). *Biochem. J.* **29**, 2351.
 Astbury, W. T., Perry, S. V., Reed, R. & Spark, L. C. (1947). *Biochim. Biophys. Acta*, **1**, 379.
 Astbury, W. T. & Sisson, W. A. (1935). *Proc. Roy. Soc. A*, **150**, 533.
 Astbury, W. T. & Woods, H. J. (1933). *Philos. Trans. A*, **232**, 333.
 Bailey, K. (1948). *Biochem. J.* **43**, 271.
 Bailey, K., Astbury, W. T. & Rudall, K. M. (1943). *Nature, Lond.*, **151**, 716.
 Bailey, K., Gutfreund, H. & Ogston, A. G. (1948). *Biochem. J.* **43**, 279.
 Cohn, E. J. & Edsall, J. T. (1943). *Proteins, Amino Acids and Peptides*, p. 406. New York: Reinhold Publishing Corporation.
 Hall, C. E. (1947). *Amer. Annu. Photogr.* **61**, 19.
 Jakus, M. A. & Hall, C. E. (1947). *J. biol. Chem.* **167**, 705.
 Mehl, J. W., Oncley, J. L. & Simha, R. (1940). *Science*, **92**, 132.
 Perrin, F. (1936). *J. Phys. Radium*, (7), **7**, 1.
 Simha, R. (1940). *J. phys. Chem.* **44**, 25.
 Straub, F. B. (1942). *Stud. Inst. med. Chem. Univ. Szeged*, **2**, 3.
 Straub, F. B. (1943). *Stud. Inst. med. Chem. Univ. Szeged*, **3**, 23.
 Svedberg, T. & Pedersen, K. O. (1940). *The Ultracentrifuge*, p. 41. Oxford: The Clarendon Press.
 Szent-Györgyi, A. (1942). *Acta Physiol. Scand.* **9**, Suppl. 25.
 Szent-Györgyi, A. (1947). *Chemistry of Muscular Contraction*. New York: Academic Press.
 Waugh, D. F. (1946). *J. Amer. chem. Soc.* **68**, 247.

A Comparison of the Decomposition of Hydrogen Peroxide by Catalase, Ferrous and Ferric Ions, Haemin and Ferrous Phthalocyanine

By P. GEORGE, *Molteno Institute, University of Cambridge*

(Received 22 January 1948)

The catalytic decomposition of hydrogen peroxide into water and molecular oxygen has been shown to be a very complicated reaction. In the earlier work up to 1923 it was established that the reaction with many different catalysts is quasi-unimolecular: for instance, there are the typical experiments of Bredig & Berneck (1899), Duclaux (1922) and Bertalan (1920) on the decomposition catalyzed by colloidal metals and ferrous and ferric salts. From 1920 to 1932 more detailed kinetic analyses were carried out on the bromine-bromide ion and iodine-iodide ion

systems, and the first general kinetic mechanism for the decomposition was devised by Abel (1920) and Bray & Livingston (1923). This is the principle of compensating reactions in which both the oxidized and reduced forms of the catalyst react with the peroxide, giving a dynamic equilibrium between these two states and a resultant catalytic decomposition of the peroxide. For the heavy metal catalysis the chemical mechanism was represented by more or less cumbersome stoichiometric equations.

The catalytic decomposition of the peroxide is a problem similar to that of the induced reactions studied extensively since the classical experiments of Manchot in 1901. In these reactions there are three components, *A* the actor, *B* the inductor and *C* the acceptor, all oxidizing or reducing agents. In the typical induced reaction *A* can react directly with *B* but not with *C*, also *B* does not react directly with *C*: however, *C* does react with *A* if it is present with the reacting system *A* and *B*. The actor and inductor in many of the systems investigated have been oxygen or hydrogen peroxide and ferrous iron, and to explain the reactions higher oxides of iron, FeO_2 , Fe_2O_5 and FeO_3 , or iron-peroxide complexes have been suggested (Hale, 1929).

In 1931 Haber & Willstätter suggested that free radicals are intermediates in many chemical reactions in solution, and in 1934 Haber & Weiss published a kinetic study of the decomposition by ferrous and ferric ions and proposed a chain mechanism for the reaction involving $\text{OH}^\cdot$ and $\text{O}_2\text{H}^\cdot$ radicals, which under certain experimental conditions could become a more simple sequence of radical reactions.

The principle of compensating reactions has been widely accepted for the catalysis of the bromine-bromide ion type, and the Haber & Weiss mechanism has proved an extremely useful working hypothesis for the catalysis by metal ions. It has recently been developed very successfully by Baxendale, Evans & Park (1946) to account for the initiation of the polymerization of certain olefines by ferrous sulphate and hydrogen peroxide in acid solution.

In this and following papers a detailed kinetic analysis of the reaction with various catalysts will be described. There are three features of the decomposition as yet uncorrelated which have directed the experimental work. They derive in turn from the three lines of development of the subject outlined above.

(1) In many of the catalytic systems there is initially a rapid evolution of oxygen, followed by a much slower evolution. This is known as α - and β -activity. It suggests that some steady state is reached in agreement with the principle of compensating reactions.

(2) During this transition from α - to β -activity the equivalent of peroxide destroyed may be in excess of the equivalents of catalyst present. Haber & Weiss (1934) found this for the decomposition with ferrous iron, and it is their chief evidence for the chain character of the reaction. Many oxidase reactions show the same behaviour: Stern has stressed this point in a recent review (1942). The same behaviour occurs in most of the induced reactions. The participation of higher oxides of iron or iron-peroxide complexes was put forward to explain the oxidation of two or three molecules of

acceptor whilst the ferrous iron is converted to ferric iron, and side reactions of the higher oxide or complex with hydrogen peroxide, regenerating ferrous iron, can account for greater turnover numbers.

(3) Leaving aside the question of the kinetic mechanism of the reaction, the accepted idea of single electron transfers in this type of oxidation-reduction system makes it necessary to assume the participation of free-radical intermediates in the several unit reactions by which the overall decomposition of the peroxide occurs.

Experiments on the initial evolution of oxygen in the reaction between hydrogen peroxide and catalase, ferrous and ferric ions, haemin and ferrous phthalocyanine are described in this paper, and possible explanations for the transition from α - to β -activity are critically examined.

METHOD

The rate of O_2 evolution has been investigated for reaction mixtures of H_2O_2 and the following catalysts: haemin, ferrous phthalocyanine, ferrous ammonium sulphate, ferric sulphate and liver catalase. The liver catalase was a highly purified preparation obtained from horse liver by the method described by Keilin & Hartree (1945). The kinetic measurements were carried out in two different types of apparatus, Barcroft manometers and a special pressure-gauge apparatus constructed for following the very rapid evolution of O_2 which occurs in the initial stages of the reaction with certain catalysts. Tests were made to ensure that the rate of O_2 evolution measured was a true chemical rate and not conditioned by any physical factors such as diffusion.

Fig. 1. Diffusion test with Barcroft manometers. Temp. 20° ; pH 5.85; oxygen evolved from H_2O_2 + catalase. Shaking speed in cyc./min.

Diffusion test with Barcroft manometers. In these experiments the left-hand flask contained 3.30 ml. H_2O and the right-hand flask 0.30 ml. 0.125 M-phosphate buffer, pH 5.85, 0.10 ml. 2% gelatin solution, 1.10 ml. water and 1.70 ml. H_2O_2 (about 20 vol.) to bring the solution to 1.0M, together

with the dangling tube holding 0.10 ml. dilute catalase solution. The effect on the reaction rate of keeping the manometers stationary and of varying the shaking speed was determined. The results are given in Fig. 1: from curve *A* it is apparent that physical conditions limit the rate measured when the manometer is stationary, but that on recommencing rapid shaking the true chemical rate is once more established. The latter part of curve *A* is an accurate extrapolation of the former part before shaking was stopped and so the chemical reaction proceeds at the same rate whether the flask is shaken or not. Curve *B* gives similar experimental results on varying the speed of shaking. At shaking speeds above 80 cyc./min. the true chemical rate can be measured: a speed of 150 cyc./min. was used throughout this work.

Diffusion tests with the pressure-gauge apparatus. This apparatus is a simple modification of that described by Meldrum & Roughton (1934) as the 'boat technique'. In

Fig. 2. Diffusion test with pressure-gauge apparatus. Temp. 0°; *a* and *b*, test experiments with $\text{H}_2\text{O}_2 + \text{KMnO}_4$; *c*, typical experiment with $\text{Fe}^{3+} + \text{H}_2\text{O}_2$.

place of the oil manometer for measuring the evolution of gas a pressure gauge was used. This consists of a flat brass cylinder (12 cm. diam., 2 cm. deep) soldered at the bottom to a heavy brass plate. A thin brass diaphragm is fitted over the top and held in place by a screwed flange, which enables the tension on the diaphragm to be altered. A fine steel pin (1 cm. high) is fixed to the centre of the diaphragm: the pin is in contact with one end of a small duralumin lever, pivoted about its centre, which carries a galvanometer mirror at the other end. By means of a galvanometer lamp and scale the slight movements of the diaphragm caused by pressure changes in the apparatus are very much magnified. The apparatus is very sensitive to rapid changes of pressure; the lag time is much less than 0.1 sec. The tension on the diaphragm was adjusted, and a test with a subsidiary oil manometer in the system showed that over the range 0–120 scale divisions the deflexion was directly proportional to the pressure change (1 scale division $\equiv 0.336$ mm. paraffin). The scale can be read to half a division. A set of six boats was calibrated for the apparatus by both the chemical method (acidified KMnO_4 and H_2O_2) and the direct method described by Münzer & Neumann (1917). One scale division is approximately equal to 9.2 μl. of O_2 . In experiments with this apparatus the scale reading was recorded every 5 sec.

Biochem. 1948, 43

Diffusion tests were carried out at 0° with the same solutions employed in the chemical calibration. The two sides of the boat were filled:

- (i) 1.5 ml. 0.0875 $\text{N-H}_2\text{O}_2 + 0.5$ ml. H_2O .
- (ii) 2.0 ml. 0.195 N-KMnO_4 in 0.5 $\text{N-H}_2\text{SO}_4$.

The evolution of O_2 from this mixture (1480 μl.) is given in Fig. 2. Curves *a* and *b* show the effect of medium and very fast shaking. The points on curve *b* were independent of any further increase in the shaking speed, so this curve actually represents the chemical rate of the $\text{KMnO}_4\text{-H}_2\text{O}_2$ reaction. The apparatus is clearly capable of measuring true chemical rates of at least 200 μl./sec. The very fast shaking speed was always employed. The results of a typical experiment are shown in curve *c* for the evolution of O_2 from acidified ferrous ammonium sulphate and H_2O_2 . The experimental details are:

Boat filled: (i) 0.5 ml. $\text{FeSO}_4(\text{NH}_4)_2\text{SO}_4 \cdot 6\text{H}_2\text{O}$ solution (12 g./100 ml.) and 1.5 ml. very dilute H_2SO_4 .

- (ii) 0.37 ml. $\text{H}_2\text{O}_2 + 1.63$ ml. water.

Final concentrations: 0.2 $\text{M-H}_2\text{O}_2$, acid $\equiv 0.142$ ml. $\text{N-H}_2\text{SO}_4$.

RESULTS

With some catalysts there is a very rapid evolution of O_2 at the beginning of the reaction which quickly subsides leaving the decomposition proceeding at a much slower rate. The experiments that follow are designed to show whether this effect is to be attributed to the chemical destruction of the catalyst, or whether it is a general kinetic feature of the decomposition arising from the interplay of chemical reactions.

In all the experiments the total volume of O_2 evolved is very small compared with the H_2O_2 concentration present, and so it can be assumed for the kinetic analysis that the peroxide concentration remains constant throughout each experiment. Control experiments with no catalyst present showed the 'blank' reaction to be negligible.

Fig. 3. Haemin. Oxygen evolution with time. Temp. 0°; pH 5.85; identical haemin concentrations. H_2O_2 : 1.0, 0.5 and 0.1 M .

Oxygen evolved with haemin. Since haemin is very rapidly destroyed by H_2O_2 at room temperature, these experiments were carried out at 0° with the

pressure-gauge apparatus. The O_2 evolved during the first 4 min. at three different H_2O_2 concentrations is shown in Fig. 3.

The boats were filled:

(i) 0.5 ml. haemin solution (0.117 g. dissolved in a little NaOH and made up to 50 ml.); 0.5 ml. 0.125M-phosphate buffer, pH 5.85, + 1.0 ml. water.

(ii) 2 ml. H_2O_2 + H_2O to give required final molarity.

Oxygen evolved with ferrous phthalocyanine. These experiments were carried out in Barcroft manometers at 20°.

The flasks were filled:

Left-hand side, 3.30 ml. H_2O .

Right-hand side, 0.5 ml. 0.125M-phosphate buffer, pH 5.85. H_2O_2 + water to 3.2 ml. giving required molarity. Dangling tube 0.1 ml. iron phthalocyanine suspension containing 0.2 mg. dry weight/ml.

Fig. 4 shows the O_2 evolution at three H_2O_2 concentrations during the first 5 min.

Fig. 4. Ferrous phthalocyanine. Oxygen evolution with time. Temp. 20°; pH 5.85; identical ferrous phthalocyanine concentrations. H_2O_2 : 2.0, 1.0 and 0.1 M.

Oxygen evolved with ferrous iron. These experiments were also carried out in Barcroft manometers at 20°. No buffer solution was used: 0.1 ml. of a solution of acidified $FeSO_4(NH_4)_2SO_4 \cdot 6H_2O$ (1.66 g./7 ml.) was placed in the dangling tube, the total acid concentration present being equivalent to 0.565 ml. N- H_2SO_4 . The results for 1.0M and 0.05M- H_2O_2 are given in Fig. 5.

Oxygen evolved with ferric iron. The decomposition of H_2O_2 in the presence of ferric salts falls off very rapidly as the acidity of the solution is increased. Ferric sulphate in aqueous solution, owing to its hydrolysis, has a very acid reaction, and to obtain a measurable O_2 evolution in these experiments it was necessary to add a very small quantity of NaOH. The amount added was not enough to alter

the state of the ferric iron: the solution still had the pale yellow colour characteristic of the ferric iron in acid solution. The experiments were carried out in the pressure-gauge apparatus at 0°. 1.0 ml. of a solution of $Fe_2(SO_4)_3 \cdot 9H_2O$ (8.7 g./50 ml.) + NaOH equivalent to 0.03 ml. N-solution was used in each run, together with H_2O_2 and H_2O to give the required molarity. Typical results are shown in Fig. 6.

Fig. 5. Ferrous iron. Oxygen evolution with time, showing initial activity. Temp. 20°; acid solution; identical Fe^{++} concentrations. H_2O_2 : 1.0 and 0.05 M.

Fig. 6. Ferric iron. Oxygen evolution with time. Temp. 0°; acid solution; identical Fe^{+++} concentrations. H_2O_2 : 1.0 and 0.1 M.

Oxygen evolved with liver catalase. Barcroft manometers were used at 20°.

Into the right-hand flask were measured 0.3 ml. 0.125M-phosphate buffer, pH 5.85, + 0.1 ml. 2% gelatin solution together with H_2O_2 and water for the required molarity. A very dilute catalase solution (0.1 ml.) was put in the dangling tube. This solution was prepared as follows: stock solution A contained 34 mg. catalase/ml. One drop of this solution (0.045 g.) was added to 20 ml. water giving solution B. Solution B (0.6 ml.) was made up to 50 ml. This was kept in ice in a thermos flask, 0.1 ml. being used for each run.

Typical results for the decomposition with 1.0M- and 0.2M- H_2O_2 for the first 15 min. are given in Fig. 7. Fig. 8 shows the results of a similar experiment lasting for 1 hr. Here the catalase concentration was one sixth of its previous strength.

Fig. 7. Liver catalase. Oxygen evolution with time showing initial activity. Temp. 20°; pH 5.85; identical catalase concentrations. H_2O_2 : 1.0 and 0.2M.

Fig. 8. Catalase. Oxygen evolution with time showing a diminished activity after 15 min., attributed to destruction of the enzyme. Temp. 20°; pH 5.85; 0.5M- H_2O_2 .

DISCUSSION

The destruction of the catalyst

An examination of the experimental curves for the various iron-containing catalysts studied shows that kinetically the catalysts are of two types. Ferrous iron and catalase bring about a reaction in which there is an initial rapid evolution of O_2 which is followed by a much slower steady decomposition. Haemin, ferrous phthalocyanine and ferric iron do not give this initial 'burst' but only the steady rate. The initial evolution in the catalase reaction is extremely interesting: it has been observed by other investigators (Morgulis, Beber & Rabkin, 1926), but as yet no attempt has been made to explain it kinetically and account for it in the mechanism proposed for the reaction. It might be due to the destruction of the enzyme or to some special feature in the decomposition of the peroxide. The observed

behaviour with the other catalysts strongly supports the second explanation for the following reasons:

(1) The reaction with catalase and with ferrous iron is very similar (Figs. 5 and 7). With ferrous iron

Fig. 9. Variation of rate with time. Values plotted from Figs. 3 and 4. A, haemin + 1.0M- H_2O_2 ; B, ferrous phthalocyanine + 2.0M- H_2O_2 .

Fig. 10. Variation of rate with time. Values plotted from Figs. 5 and 7. Bottom curve: ferrous iron + 1.0M- H_2O_2 . Top curve: catalase + 0.2M- H_2O_2 .

no destruction of the catalyst can occur, and the kinetic effect here is due to the oxidation of ferrous to ferric iron by the peroxide (Haber & Weiss, 1934; Baxendale *et al.* 1946). There is no initial 'burst' with ferric iron (Fig. 6).

(2) With haemin and ferrous phthalocyanine destruction of the catalyst does occur. The solutions rapidly lose their characteristic colours. This is very marked at high peroxide concentrations (1.0M and 2.0M) when the rate falls off after the first minute. At low concentration (0.1M- H_2O_2) this is hardly noticeable. The initial rapid evolution with catalase and ferrous iron is just as marked at low as at high concentrations. This behaviour can be demonstrated by plotting 'rate' against 'time' as in Figs. 9 and 10: the shape of the curve is completely different in the two cases. With catalase and ferrous iron the rate rapidly falls to an almost constant value (Fig. 10), whereas with haemin and ferrous phthalocyanine the rate continually decreases (Fig. 9).

(3) After the initial activity is over the reaction with catalase proceeds at this almost steady rate, which above a certain peroxide concentration is less the higher the peroxide concentration (Fig. 7). This will be described in detail in a later paper. If enzyme destruction is responsible for this initial activity the rate should diminish continuously to zero. These constant rates at different peroxide concentrations cannot be explained by the enzyme destruction hypothesis.

(4) Enzyme destruction does occur with catalase, but it is only apparent after a much longer time. This is shown in Fig. 8: here the curve from 2 min. to 1 hr. resembles that for haemin from 0 to 5 min.

Thus, whilst the complete destruction of the enzyme cannot be responsible for these kinetic effects, there remains the further possibility that the initial activity results from its partial destruction, leaving a degradation product which is still extremely catalytically active. In a later paper, in which a detailed kinetic study of the reaction will be described, it will be shown that these results cannot be accounted for in this way, and so the initial activity is due to some special feature in the catalytic decomposition of the peroxide.

The following correlation can be made between the state of oxidation of the catalyst and its kinetic effect. The different kinetic behaviour of the various catalysts, the initial rapid evolution of O_2 observed with some and its absence with others, depends on the state of oxidation of the catalyst initially and the concentration of this state in the equilibrium mixture obtained in peroxide solution. In the ferrous-ferric iron system the equilibrium mixture contains predominantly ferric iron, and so if ferrous iron is added to H_2O_2 there is a rapid evolution of O_2 whilst the oxidation of Fe^{++} to Fe^{+++} proceeds. With ferric iron, haemin and ferrous phthalocyanine the initial oxidation state of the catalyst corresponds to that predominating in the equilibrium mixture, and so there is no initial rapid evolution. Catalase, although containing ferric iron, is in the same kinetic class as free ferrous iron. In H_2O_2 solution the initial rapid evolution of O_2 suggests that some type of oxidation-reduction equilibrium is being attained.

The principle of compensating reactions, which provides a qualitative explanation for this, will now be discussed and its quantitative aspects examined. It will be realized that this principle is the general kinetic mechanism for the simple valency change reactions (with the exception of the chain reaction) which have been proposed as mechanisms for the decomposition.

The principle of compensating reactions

The simple picture is that of two reactions; in one the oxidized form of the catalyst is reduced by the peroxide liberating oxygen, in the other the reduced

form of the catalyst is oxidized by the peroxide giving water. The net effect of these compensating reactions is the familiar catalytic decomposition:

This mechanism has been reviewed by Bray (1932): it has been widely used to explain the catalysis by halogens and the corresponding halide ions. These correspond to the oxidized and reduced state of the catalyst. The mechanism may be illustrated by the decomposition by Br_2 and Br^- .

If H_2O_2 is added to a solution containing Br^- at high H^+ concentration, Br_2 is rapidly formed and soon reaches a constant concentration:

If H_2O_2 is added to bromine water when either H^+ or Br^- concentrations are low, Br_2 is rapidly reduced to Br^- and oxygen is evolved:

If the temperature and the final concentrations of H^+ and Br^- are the same as in (a) an identical bromine concentration is reached which again remains constant. Under these conditions the rates of the two reactions (a) and (b) are equal: H_2O_2 is decomposed catalytically—this is the steady state. O_2 is evolved in reaction (b), but not in (a), so if H_2O_2 is added to bromine water a transition from α - to β -activity will be observed. The reaction with certain heavy metal catalysts is very similar kinetically.

However, for the catalysis by ferrous and ferric ions this simple picture is not sufficient. The obvious compensating reactions in this case are:

Now in the oxidation of Fe^{++} to Fe^{+++} by this mechanism, whilst there would be a rapid destruction of H_2O_2 in attaining the equilibrium state, yet there would be no rapid evolution of O_2 , since this arises in the reaction of ferric ion only. This shows conclusively that the reaction is more complicated. The experiments of Haber & Weiss (1934) led them to propose the chain mechanism which can account for it. They measured ΔH_2O_2 , the amount of peroxide destroyed in the oxidation of Fe^{++} to Fe^{+++} in neutral solution. Under certain experimental conditions they found that the ratio of ΔH_2O_2 to the initial equivalent concentration of Fe^{++} was greater than one, and attributed this to the length of a chain reaction, initiated by the production of $OH^\cdot$ radicals

by Fe^{++} and H_2O_2 and propagated by the reactions of the $\text{OH}^\cdot$ and $\text{O}_2\text{H}^\cdot$ radicals.

The oxygen produced in the second chain propagation reaction explains its evolution in the oxidation of Fe^{++} to Fe^{+++} which could not be accounted for by the simple compensating reactions outlined above. In the gasometric experiments described in this paper the corresponding ratio $\Delta\text{O}_2/E_0$, the amount of oxygen evolved in reaching the equilibrium state, to the molar concentration of catalyst initially present can be evaluated. This is important because if the ratio is less than one then the principle of compensating reactions provides an adequate kinetic mechanism: if it is greater than one then either a chain mechanism or another more complex reaction mechanism must be adopted.

In the appendix to this paper the mathematical expression for the ratio $\Delta\text{O}_2/E_0$ is derived from the general system of two compensating reactions. If A and B are the two oxidation states of the catalyst, it takes part in two opposing monomolecular reactions which can be represented:

The parameters k_1 and k_2 contain peroxide and possibly H^+ ion concentrations together with the reaction velocity constants. When oxygen is evolved in the first reaction and not in the second it is shown that the ratio

$$\frac{\Delta\text{O}_2}{E_0} = \frac{k_1^2}{(k_1 + k_2)^2}, \quad \text{i.e. } \frac{\Delta\text{O}_2}{E_0} < 1.$$

If the compensating reactions are more complicated and oxygen is evolved in both steps then the ratio is still less than one. This ratio can be calculated for the experiments in which ferrous iron and catalase were used.

Ferrous iron (1.0 and 0.05M- H_2O_2),

$$\Delta\text{O}_2 = 50 \mu\text{l.} = 2.2 \times 10^{-6} \text{ mol.}$$

$$\text{Fe}_0^{2+} = 6.1 \times 10^{-5} \text{ mol.}$$

$$\text{Fe}_0^{2+}/\Delta\text{O}_2 = 0.037.$$

Thus, with the experimental conditions employed, there is no evidence for a chain reaction and a set of compensating reactions is sufficient.

Catalase

$$E_0 = 4.1 \times 10^{-13} \text{ mol.}$$

$$\Delta\text{O}_2 (1.0\text{M-}\text{H}_2\text{O}_2) = 100 \mu\text{l.}, \quad \frac{\Delta\text{O}_2}{E_0} = 1.1 \times 10^7.$$

$$\Delta\text{O}_2 (0.2\text{M-}\text{H}_2\text{O}_2) = 200 \mu\text{l.}, \quad \frac{\Delta\text{O}_2}{E_0} = 2.2 \times 10^7.$$

This is a most striking result, for the ratio is of the order ten million, rather than less than one. It is apparent that the system of two compensating reactions is not an adequate kinetic mechanism. The applicability of the chain mechanism or another more complex mechanism must be examined.

It can be argued from the experiments of Haber & Weiss (1934), on the decomposition by ferrous ions, that this result means that with catalase a chain reaction occurs with a chain length of ten million. However, a comparison of the results with catalase and with ferrous iron presents a serious difficulty. It follows from the mathematical treatment of the compensating reactions given in the appendix that the time for half the initial rapid evolution to occur, $t_{\frac{1}{2}}$, is given by

$$t_{\frac{1}{2}} = \frac{\ln 2}{(k_1 + k_2)}.$$

So, from the experimental results, the sum of $k_1 + k_2$ can be evaluated. With 1.0M- H_2O_2 the values are:

$$\text{Catalase: } t_{\frac{1}{2}} = 10 \text{ sec.}, (k_1 + k_2) = 0.069 \text{ sec.}^{-1}.$$

$$\text{Ferrous iron: } t_{\frac{1}{2}} = 5 \text{ sec.}, (k_1 + k_2) = 0.139 \text{ sec.}^{-1}.$$

The sum $k_1 + k_2$ is of the same order for both ferrous iron and catalase. Now according to the Haber & Weiss (1934) mechanism, where the catalyst both starts and stops reaction chains k_1 and k_2 , the parameters involving velocity constants, H_2O_2 and possibly H^+ ion concentrations, refer to the chain-starting and chain-stopping reactions. The measurements of $\Delta\text{O}_2/E_0$ suggested that, whilst the ferrous iron reaction does not involve long chains (a sequence of reactions provides an adequate mechanism), the catalase reaction has a chain length of 1.1×10^7 with 1.0M- H_2O_2 . The corresponding chain length for the Fe^{++} ion reaction, 0.037, can be interpreted on the Haber & Weiss mechanism as a more rapid removal of chain centres by reaction with the Fe^{++} ion in the termination process compared with the alternative reaction of the chain centres with H_2O_2 in the propagation reaction. On this basis the ratio of the velocity constants $(k_1^2)_{\text{Fe}}$ and $(k_2^2)_{\text{E}}$ for the termination reactions in the Fe^{++} ion and enzyme systems can be estimated. If k_p is the velocity constant for the propagation reaction, then

$$\frac{k_p [\text{H}_2\text{O}_2]}{(k_2^2)_{\text{Fe}} [\text{Fe}^{++}]} = 0.037 \quad \text{and} \quad \frac{k_p [\text{H}_2\text{O}_2]}{(k_2^2)_{\text{E}} [E]} = 1.1 \times 10^7.$$

Substituting the experimental values for H_2O_2 , Fe^{++} and catalase concentrations, the ratio $(k_2^2)_{\text{Fe}}/(k_2^2)_{\text{E}}$ is found to be 2.0. This implies that the velocity constants of the chain initiation and termination reactions for catalase and ferrous iron are all of the same order. Whilst the H^+ ion concentration is considerably different in the two cases it can hardly account for this odd conclusion. In all comparable

reactions of the iron protoporphyrin protein complexes their velocity constants greatly exceed those with free iron. Thus the simple chain theory does not offer a satisfactory explanation for the action of catalase and the possibility of other explanations should be explored. One alternative mechanism was suggested by Manchot during the course of his work on induced reactions.

Manchot & Lehmann (1928) described experiments with ferrous iron and H_2O_2 , the results of which are very similar to those of Haber & Weiss (1934). In the oxidation of Fe^{++} to Fe^{+++} in dilute solution he found that, for each equivalent of Fe^{++} oxidized, three equivalents of peroxide were decomposed, two equivalents of which could react with acceptors. In more concentrated peroxide solutions as many as twentyfour equivalents of peroxide were decomposed for the oxidation of each equivalent of Fe^{++} . He explained the result in dilute solution by the intermediary formation of Fe_2O_6 , and in the more concentrated peroxide solutions the increased peroxide decomposition is satisfactorily explained by a reaction between the intermediate and H_2O_2 , in which the latter reduces the former back to the ferrous state.

In the absence of more kinetic data it is impossible to reach any definite conclusion regarding the mechanism of the decomposition. Detailed kinetic studies of the reaction with catalase and ferrous iron will be described in future papers.

SUMMARY

1. The evolution of O_2 from H_2O_2 by haemin, ferrous phthalocyanine, ferrous and ferric iron and catalase has been measured manometrically and by

the 'boat technique' of Meldrum & Roughton (1934), using a pressure gauge for following rapid rates.

2. There is an initial rapid evolution of O_2 with ferrous iron and catalase but not with the other catalysts. In general, this transition from α - to β -activity might be due to the destructive fission of the catalyst, or to a special kinetic feature of the decomposition with a particular catalyst. Since ferrous iron shows this activity it is not necessarily due to catalyst destruction, for none can occur here.

3. By comparing the catalytic effect of catalase with haemin and iron phthalocyanine it has been shown that complete destruction of the enzyme cannot account for the initial activity, and it is unlikely that partial destruction is responsible.

4. The principle of compensating reactions, which attributes the $\alpha\beta$ -activity to the setting up of an equilibrium between two oxidation states of the catalyst, can explain the ferrous iron catalysis but not the reaction with catalase. This follows from the values of $\Delta\text{O}_2/E_0$, the ratio of the equivalents of oxygen evolved in the initial rapid reaction to the equivalents of catalyst present, which are 0.037 and 1.1×10^7 respectively for the given experimental conditions.

5. For the catalase reaction the familiar chain mechanism leads to the improbable conclusion that the reaction-velocity constants for chain initiation and termination are each of the same order of magnitude with catalase and ferrous iron.

I am very grateful to Prof. D. Keilin, F.R.S., for his stimulating interest and kind encouragement in this work: and I wish to thank Dr E. F. Hartree for much advice on manometric methods and the preparation of catalase.

Appendix. Compensating Reactions and the Initial Evolution of Oxygen

The relation between the number of equivalents of oxygen evolved in the initial rapid reaction and the equivalents of catalyst present can be obtained in the following way:

A and B represent the two oxidation states of the catalyst whose initial concentrations and concentrations at time t are given by a and b , and $(a-x)$ and $(b+x)$ respectively. At time t the rates of conversion of A into B and B into A are given by $k_1(a-x)$ and $k_2(b+x)$, the parameters k_1 and k_2 containing peroxide and possibly hydrogen-ion concentration terms. This is the simplest representation of two compensating reactions; if either involves a sequence of reactions then k_1 and k_2 refer to the rate determining steps in such a sequence.

Moelwyn-Hughes (1940) has shown that in such a system of two compensating monomolecular reactions (monomolecular with respect to the catalyst in the peroxide decomposition)

$$x = \frac{k_1 a - k_2 b}{k_1 + k_2} (1 - e^{-(k_1 + k_2)t}).$$

Now if $b = 0$, that is the catalyst is present initially in the ' A ' state, then the amount of A left at any time is $(a-x)$ where

$$(a-x) = a \frac{k_2 + k_1 e^{-k_1 + k_2 t}}{k_1 + k_2}.$$

Changing to a more familiar nomenclature, if E_0 is the initial concentration of the catalyst (in state A) and E is its concentration at time t ,

$$E = E_0 \frac{k_2 + k_1 e^{-k_1 + k_2 t}}{k_1 + k_2}.$$

Now if the k_1 reaction is the one in which oxygen is evolved, the rate at any time t , is given by

$$R_t = k_1 E = \frac{E_0 k_1 [k_2 + k_1 e^{-\overline{k_1+k_2}t}]}{k_1 + k_2}, \quad (1)$$

and R_0 and R_e , the initial and equilibrium rates when $t=0$ and $t=\infty$ respectively, are

$$R_0 = k_1 E_0, \quad (2)$$

$$R_e = E_0 \frac{k_1 k_2}{k_1 + k_2}. \quad (3)$$

The relation between the amount of oxygen evolved in reaching the equilibrium and the mol. of catalyst present is obtained by integrating equation (1):

$$\left. \begin{aligned} R_t &= \frac{dO_2}{dt} = \frac{E_0 k_1 k_2}{k_1 + k_2} + \frac{E_0 k_1^2}{k_1 + k_2} e^{-\overline{k_1+k_2}t}, \\ dO_2 &= \frac{E_0 k_1 k_2 dt}{k_1 + k_2} + \frac{E_0 k_1^2}{k_1 + k_2} e^{-\overline{k_1+k_2}t} dt. \end{aligned} \right\} \quad (4)$$

Integrating this expression

$$O_2 = \frac{E_0 k_1 k_2 t}{k_1 + k_2} - \frac{E_0 k_1^2}{(k_1 + k_2)^2} e^{-\overline{k_1+k_2}t} + I, \quad (5)$$

where I is the integration constant. This can be evaluated, for when $t=0$, $O_2=0$, therefore

$$I = \frac{E_0 k_1^2}{(k_1 + k_2)^2}.$$

Substituting this value in equation (5):

$$O_2 = \frac{E_0 k_1 k_2 t}{k_1 + k_2} + \frac{E_0 k_1^2}{(k_1 + k_2)^2} (1 - e^{-\overline{k_1+k_2}t}). \quad (6)$$

This is the mathematical expression, according to these simple compensating reactions, for the amount of oxygen evolved at any time. It shows that the experimental curve can be considered in two parts, a straight line through the origin (first term equation (6)) and a curve which rises rapidly to a constant value for O_2 (second term, exponential, equation (6)). This is illustrated in Fig. 11, which is based on Exp. c in Fig. 2. Curve A is the experimental curve, B the linear part and C the exponential part: $A = B + C$.

The amount of O_2 evolved in reaching the equilibrium ΔO_2 is given mathematically by the exponential term in equation (6) when $t=\infty$, and

Fig. 11. Analysis of experimental curve (Fig. 2 c). Experimental curve, A . Linear portion, C . Exponential portion, B . $A = B + C$.

experimentally by the extrapolation of curve A or C back to the ordinate, i.e.

$$\Delta O_2 = \frac{E_0 k_1^2}{(k_1 + k_2)^2}.$$

Hence the relation between the oxygen evolved and the number of mol. of catalyst present is

$$\frac{\Delta O_2}{E_0} = \left(\frac{k_1}{k_1 + k_2} \right)^2. \quad (7)$$

If the compensating reactions are slightly more complicated, and oxygen is evolved in both steps, it can be shown that:

$$R_t = \frac{E_0}{k_1 + k_2} [2k_1 k_2 + (k_1^2 - k_1 k_2) e^{-\overline{k_1+k_2}t}],$$

$$R_0 = k_1 E_0,$$

$$R_e = \frac{2E_0 k_1 k_2}{k_1 + k_2},$$

$$\frac{\Delta O_2}{E_0} = \frac{k_1 (k_1 + k_2)}{(k_1 + k_2)^2}.$$

Thus it is an essential feature of the mechanism based on compensating reactions that the ratio $\Delta O_2/E_0$ should be less than one.

REFERENCES

- Abel, E. (1920). *Z. phys. Chem.* **96**, 1.
 Baxendale, J. H., Evans, M. G. & Park, G. S. (1946). *Trans. Faraday Soc.* **42**, 155.
 Bertalan, J. V. (1920). *Z. phys. Chem.* **95**, 328.
 Bray, W. C. (1932). *Chem. Rev.* **10**, 106.
 Bray, W. C. & Livingston, R. S. (1923). *J. Amer. chem. Soc.* **45**, 1251.
 Bredig, G. & Berneck, M. V. (1899). *Z. phys. Chem.* **31**, 258.
 Duclaux, J. (1922). *Bull. Soc. chim. Fr.* **31**, 961.
 Haber, F. & Weiss, J. (1934). *Proc. Roy. Soc. A*, **147**, 332.
 Haber, F. & Willstätter, R. (1931). *Ber. deutsch. chem. Ges.* **64** B, 2844.
 Hale, D. R. (1929). *J. phys. Chem.* **33** (2), 1633.
 Keilin, D. & Hartree, E. F. (1945). *Biochem. J.* **39**, 148.
 Manchot, W. (1901). *Z. anorg. Chem.* **27**, 420.
 Manchot, W. & Lehmann, G. (1928). *Liebigs Ann.* **460**, 179.
 Meldrum, N. U. & Roughton, F. J. W. (1934). *J. Physiol.* **80**, 113.
 Moelwyn-Hughes, E. A. (1940). *Physical Chemistry*, 1st ed. Cambridge: University Press.
 Morgulis, S., Beber, M. & Rabkin, I. (1926). *J. biol. Chem.* **68**, 521.
 Münzer, E. & Neumann, W. (1917). *Biochem. Z.* **81**, 319.
 Stern, K. (1942). *A Symposium on Respiratory Enzymes* 1st ed. Madison, U.S.A.: University of Wisconsin Press.