THE RÔLE OF CERTAIN METALLIC IONS AS OXIDATION CATALYSTS.

By S. F. COOK.

(From the Laboratory of General Physiology, Harvard University, Cambridge.)

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It has long been known that metals in some way are very closely connected with the processes of respiration and oxidation in both plants and animals, and the mode of their action has been the subject of a great deal of research and discussion. The most important of these metals is undoubtedly iron, and the recent work of Warburg (1925) and Meyerhof (1924) has gone far toward elucidating its mode of action.\(^1\) Iron as an oxygen carrier is present in the hemoglobin of the blood of mammals and has been the subject of much investigation. It is not within the province of this paper, however, to discuss this aspect of the matter, treatment of which may be found in text-books of general physiology.

Next to iron, in occurrence and in importance, is copper, although the latter has not received the careful and attentive research which has been bestowed on the former.\(^2\)

\(^1\) The book by Meyerhof (Chemical dynamics of life phenomena, 1924) contains an excellent summary of investigations up to a very recent date. Reference may also be made to the individual papers of these authors, and particularly, in English, of Warburg (1925). Crozier (1924-25) has analyzed the data of various investigators who have worked with oxidation systems, from the point of view of the effect of temperature. He finds that many oxidation reactions, including several which are undoubtedly catalyzed by iron, have a critical thermal increment close to 16,000. Hecht (1925-26) explains on this basis the latent period of the photic response in Ciona, the critical thermal increment of which is nearly 16,000, as a reaction catalyzed by iron and probably an oxidation. The fact that many oxidation systems and many reactions catalyzed by iron have the same increment may be taken as further evidence that iron is intimately connected with oxidation catalysis.

\(^2\) The toxic effects of copper have been widely observed and discussed (see papers by Cook, 1925-26). In fact, the striking and obvious toxic action of copper has more or less obscured the possibility that this element may be of fundamental importance in the catalysis of normal oxidation reactions.
That copper occurs normally in a large number of organisms has been shown by Maquenne and Demoussy (1920) for plants and Muttkowski (1920-21) and Rose and Bodansky (1920) for animals. In animals it is usually found in the hemocyanin, or oxygen-carrying fluid, of arthropods and crustaceans. Hemocyanin is considered analogous to hemoglobin, with copper taking the place of the iron. Henze (1904-05), working on octopus blood, decided that oxidations were catalyzed by the copper. Alsberg and Clark (1914), using Limulus, concluded that “With the aid of copper, oxygen may, perhaps, be transferred catalytically within the organism.” Glaser (1923) found considerable copper in Arbacia eggs; he thinks its function is partially to inactivate certain enzymes.

Of the other metallic elements manganese (Bertrand, 1897, and later papers) is the only one which has been mentioned as a substitute for iron and copper. McHargue (1926) states that manganese is found very frequently in chlorophyl-bearing tissues and assigns to this metal an important rôle in photosynthesis. In molluscan bloods Mn may appear to take the place of Cu. However, its function, if not its occurrence, is rather problematical, and it does not take rank in prominence with the other two. Some reasons will be advanced, based on the present work, for believing that iron and copper, if not the only two elements possible, are nevertheless the two elements which are, chemically, especially well adapted to the rôle of oxidation catalysts.

One method of approach to the problem of biological oxidations is the measurement of respiration in organisms. Another is the attempt to duplicate, as far as possible, the conditions existing in the cell by means of inorganic chemical systems where the conditions may be controlled. The first method has led to the conclusion that iron, and to a lesser extent copper, is the catalytically active substance. The second method is more apt to furnish data regarding the mechanism of the reactions involved and is the one used in the present investigation.

II.

In order to duplicate the essential conditions in living systems it is necessary to have an oxygen-rich substance (a peroxide), a catalyst, and an easily oxidizable substance. Ray (1923-24) has proceeded according to this principle and has investigated the system iron-hydrogen peroxide-unsaturated fatty acids from the point of view of
the effect of anesthetics. It was thought best here to use a combination which reacted quite rapidly, and therefore pyrogallol was used as the oxidizable substance. One of the end-products of the reaction is carbon dioxide, the rate of production and amount of which may be accurately measured.

The experimental measurements were made with an Osterhout respiration machine the principles of which have been described in an earlier paper (Cook, 1925–26). Briefly, there is a closed system of tubes through which air is forced by a pump, from a reaction chamber to a tube containing an indicator (phenolsulfonphthalein) and thence by another route to the reaction chamber again. The indicator is decolorized by the carbon dioxide and the color is restored by switching the current of air through a U-tube containing sodium hydroxide.

In carrying out the present series of experiments a definite amount of pyrogallol was dissolved in water in a large test-tube (the reaction chamber); the tube was placed in its proper position in the circuit of the machine, and the carbon dioxide present in the solution was cleared out. Then through a separatory funnel a mixture of the metal salt and hydrogen peroxide was run into the reaction chamber. These two constituents were mixed immediately before being run in, so that there might be as little reaction between them as possible before striking the pyrogallol solution; the error here involved is entirely negligible because the reaction between the metal and the peroxide is relatively slow. Then the machine was started and the carbon dioxide produced was measured practically from the start of the reaction.

In order that there might be no possibility of the walls of the container exercising a catalytic effect, or otherwise disturbing the reaction, the inside of the reaction chamber was coated with paraffin and the coating renewed frequently. Furthermore, the indicator solution was replaced after every experiment in order to guard against any contamination by volatile organic acids which might be produced during the oxidation of the pyrogallol. Finally the reaction chamber was placed in a water bath and the temperature was kept uniformly at 25°C. in all the experiments here reported.

Unless the concentration of the reactants was purposely varied the mixtures were made up by dissolving 0.1 gm. of pyrogallol in 44 cc. of water (distilled) to which was added 1 cc. of hydrogen peroxide and 10 cc. of the metal salt in the desired concentration. The hydrogen peroxide was all taken from the same bottle and retained its strength at approximate constancy throughout the entire series of experiments.

Since the hydrogen ion concentration of the medium affects the rate of oxidation of pyrogallol it was ascertained colorimetrically that a mixture of 0.1 gm. of pyrogallol and 1 cc. of the peroxide in 44 cc. of water has a pH of approximately 5. Since most of the metal salts here used have an acid reaction there is no doubt that all the present experiments were performed in an acid medium. The pyrogallol
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will not absorb oxygen as rapidly in an acid as in an alkaline medium, but since the hydrogen ion concentration did not vary to any great extent, and always remained on the acid side of neutrality, the relative values obtained are not invalidated. It would of course be impossible to conduct experiments by the indicator method with an alkaline medium in the reaction chamber.

It is customary when using the indicator method with an organism to express the results as a rate curve based on the normal rate of respiration as 100 per cent. Such a procedure is impossible here since there is no "normal rate." In fact there is no production of carbon dioxide previous to the start of the reaction. It is necessary, therefore, to find some other method of expressing the results. This may be done by using the absolute amounts of carbon dioxide produced. Ray (1923-24) has developed this method with the assistance of E. J. Cohn and has calculated the actual quantity of the gas. Here it is not essential to know the actual quantities in mg. Arbitrary units are satisfactory since all the results are relative and may be compared with each other even if we do not know the exact amounts. If the same amount of sodium bicarbonate is always present in the indicator solution and the buffer standards always remain constant, then it will always take the same amount of carbon dioxide to decolorize the indicator. We may then take this amount as a unit and calculate the number of units produced in a given time, or the length of time to produce 1 unit. But we must remember that the reaction is also proceeding during the time that the color of the indicator is being restored and include this time. For example, if it takes 30 seconds to decolorize and 30 seconds to restore the color then 2 units of carbon dioxide are formed per minute. Or if in 10 minutes there are four periods of decolorization 2 minutes long and four periods of 30 seconds to restore the color, then 5 units will have been formed during the 10 minutes.

Using this method the total amount of carbon dioxide produced can be plotted against the time and an integral curve obtained. If the equation of this curve is known then the rate curve can be derived therefrom.

III.

Since this investigation has been primarily on the effect of copper, this element will be treated in a separate section. Fig. 1 shows typical curves obtained with 1 cc. of hydrogen peroxide, 0.1 gm. of pyrogallol, and various concentrations of copper chloride, in 44 cc. of water. The curves represent the total amounts of carbon dioxide produced, plotted against time. The slope of such a curve at any

3 Pyrogallol is oxidized by hydrogen peroxide and metal salts separately at such a slow rate (if at all) that it cannot be detected by this method. Hence we may say for practical purposes that there is no production of carbon dioxide unless all three constituents are present.
point indicates the rate of production of carbon dioxide. It will be
seen that with very dilute copper the rate is at first relatively rapid,
but soon falls off until it approaches zero. With concentrated solu-
tions the rate decreases continually, but since the earlier portion of
the curves is most important it was not considered necessary to follow
the course of the reaction entirely to completion. In fact to do so
with concentrated copper solutions would require many hours if not
days of continuous observation. The upper curves, therefore, are
incomplete.

In order to compare and analyze curves of this sort the equations should be
known. The attempt was made to fit the curves with the equations of a mono-
molecular and bimolecular chemical reaction. Although in some instances an
approximate constant can be obtained, the correspondence is not very close, and
these equations have very little significance in the present connection. However,
the curves can be fairly closely fitted by the general equation for the hyperbola \( y = x/(a + bx) \). In all the experimental cases, when \( x/y \) is plotted against \( x \) the resulting figure is a straight line. There are individual deviations of slight extent but these are irregular in their occurrence and indicate that the experimental curves are fitted only approximately by the general equation \( y = x/(a + bx) \). Even though the correspondence is only approximate, however, it is sufficiently close to permit the use of the equation in a purely empirical way for the comparison of the curves. In considering the present data the special equation may be used

\[
a = t/(p + bt),
\]

where \( a \) is the amount of carbon dioxide produced after time, \( t \), and \( p \) and \( b \) are constants.

The constants \( p \) and \( b \) must be determined for each different curve and when determined they can be an index to the characteristics of the curve, within the same limits as were suggested with respect to the accuracy of the general equation as applied to these cases. The constants, with these reservations, may be used to compare the action of different concentrations of the reagents. To evaluate the constants the method of least squares was used. In the experimental curves we are particularly interested in the total amount of carbon dioxide which will be formed if the reaction is allowed to run to completion, and the rate, specially the initial rate, of the production. These two quantities may be calculated from the formula

\[
a = t/(p + bt)
\]

when the constants are known.

From an inspection of the curves it can be seen that as time proceeds the amount of carbon dioxide, called \( a \), will approach a limiting value. Then, in the formula,

\[
a = \frac{\sum (xy)}{\sum (y^2)}
\]

and

\[
b = \frac{\sum (x^2y) - \sum (x^2) \sum (y)}{(\sum (x^2))^2 - \sum (x^2y) \sum (y^2)}
\]
allow \( t \) to become very large. Since \( p \) is constant, \( t \), and also \( bt \), will become so large that \( p \) may be neglected. Then \( a(t_{\text{lim}}) = t/bt \). Cancelling \( t \), \( a(t_{\text{lim}}) = 1/b \). The limiting value of \( a \) and the total amount of carbon dioxide (expressed in arbitrary units of course) will therefore equal \( 1/b \). This furnishes a convenient method for comparing the total amount of the action under different conditions.

In considering the rate of the activity we may deal with either a derivative or a tangent depending on whether we are considering an equation or a curve. With the experimental curves the tangents may be ascertained by means of instruments, and the rate curves then plotted if desired. The initial rate may be observed directly.

With the equation the procedure is different. If \( a = t/(p + bt) \) then \( da/dt = p/(p + bt)^2 \). By substituting values for \( t \) the value of \( da/dt \), or the rate, may be plotted. Now let \( t \) become exceedingly small compared with \( p \), and the differential equation approaches the value \( da/dt(t_{\text{lim}}) = p/p^2 \) or \( 1/p \). Therefore \( 1/p \) represents the initial rate of the production of carbon dioxide.

Applying these methods it is possible to get information concerning the concentration effects of the various reagents. Fig. 2 shows three curves obtained by plotting the values of \( 1/b \) against the concentrations of the reagents. Fig. 3 shows similar curves for the initial rates, as obtained from the equation and also from the tangents to the experimental curves.⁶

When the concentration of the pyrogallol or of the copper is varied we find that the limiting value of \( a \) varies as a constant fractional power of the concentration, or \( a(t_{\text{lim}}) = C^k \). This relation has been found very frequently in biological work, and owing to its similarity to the adsorption isotherm it has caused many phenomena to be ascribed to adsorptive processes. The present case makes it plain that adsorption cannot always be called upon to explain every process where the effect is proportional to some fractional power of the concentration. For here we have nothing but a chemical system. Pyrogallol and hydrogen peroxide do not show colloidal properties, and it is very unlikely that copper chloride exists in anything but an ionic or molecular form. Hence if adsorption is responsible for the fractional

⁶ The figures obtained by these two methods are almost identical in their general character, the differences being due to the fact that different units are employed in the calculations. This close correspondence between the two sets of curves is evidence of the soundness of the method of calculation used in determining \( 1/p \). The values calculated for \( 1/b \) may also be accepted with confidence, since the manner of derivation is the same.
power relation then it must be in a sense very different from that commonly employed.

With hydrogen peroxide a curious relation comes to light. When its concentration is varied and the logarithm of the concentration is

![Diagram](image)

**FIG. 2.** Effect of concentration on the total amount of CO₂ produced. In all cases the ordinate is the logarithm of the amount of CO₂ as expressed by means of the logarithm of \(1/b(nm)\) (see text). The abscissa is:

- in Curve A, the logarithm of the concentration of CuCl₂;
- in Curve B, the logarithm of the concentration of H₂O₂ + the logarithm of the amount of CO₂;
- in Curve C, the logarithm of the concentration of the pyrogallol.

plotted against the ratio of the logarithm of the concentration to the logarithm of the effect a straight line is obtained. Expressed as an equation \(\log a = \log C/(m + n \log C)\). That this relation has been
previously observed may be shown by four instances where data from Snapper (1912), Dreyer and Walker (1914), Nothmann-Zuckerkandl (1912), and Plavec (1900), when plotted, give a straight line under similar conditions. However, the relation is unusual and no interpretation is available at present.

Fig. 2, Curve A, shows the effect of varying the concentration of copper. It will be noticed that while in general the law $a_{\text{lim}} = C^k$ is obeyed, still, at 0.0001 m, there is a distinct break and the exponent $k$ is different on each side. This is a situation which exists quite frequently in biological data. It might be maintained that at this critical concentration some change of phase relations occurs, such as the appearance of a new hydrate, dissociation product, or the like,
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which would suddenly alter the effective concentration of the copper. While it is impossible to offer an adequate and detailed explanation of this phenomenon, nevertheless certain interesting possibilities are suggested, particularly with regard to phase relations in biological experiments.

Fig. 3, Curve A, shows the effect of changing the concentration of copper on the initial rate of oxidation. Here the relation is $\frac{da}{dt}$ (initial) = $k \log C$ or $\tan$ (initial) = $k \log C$. The lines are not perfectly straight, indicating some deviation from the simple law, but the fit is close enough to show that there is a continuous and definite variation of initial rate with concentration. On the other hand, there is no such clear-cut variation with the pyrogallol or peroxide. The variation, if any, is slight and seems to pass through a maximum. If three points can be said to determine a straight line in such a case then the relation is the same as that with copper, i.e., $\frac{da}{dt}$ (initial) = $k \log C$. But here there is a break where $k$ changes from plus to minus. If the data cannot be said to warrant such a conclusion then the initial rate must be considered independent of the concentration and the apparent linear arrangement of the points purely fortuitous.

Whatever the interpretation of the data, the difference between copper and the other two reagents is striking. It suggests that the mode of action of copper is unique, and that it undergoes changes, or takes part in reactions, in which the other two constituents are not involved.

IV.

After copper the first metal investigated was iron. With hydrogen peroxide and pyrogallol it causes a rapid evolution of carbon dioxide. Contrary to what might have been expected, iron was but slightly more effective than copper. This doubtless is due, however, to the conditions existing in this particular system. Under other circumstances iron is a much more effective oxidation catalyst than copper. At the same time it should be borne in mind that as a toxic agent copper is far more powerful than iron.

The form of the time curve with iron was substantially the same as that with copper. The concentration effect was not investigated. One phenomenon worth mentioning appeared in connection with the
experiments on iron. If several experiments were performed, using the same concentrations of all reagents, each experiment showed a larger production of carbon dioxide than the one preceding. This rendered it almost impossible to duplicate an experimental result. When the inside of the reaction chamber was coated with paraffin and the paraffin renewed occasionally, this difficulty vanished. The results became reproducible. Such a situation indicates that part of the iron was adsorbed or deposited on the glass wall of the tube and influenced subsequent reactions. No deposition of iron took place on the paraffin. Although no perceptible influence was ever exercised by the walls of the tube in the experiments on copper, paraffin was used as a safeguard.7

Several experiments were performed with silver nitrate and gold chloride. The action of these two elements being very similar only one, gold, will be discussed in detail. When the reaction with gold chloride starts, the production of carbon dioxide is relatively rapid (although not quite so rapid, for equivalent concentration, as with

7 There are numerous instances recorded concerning the effect of the glass wall of the containing vessel on chemical reactions.
iron or copper). This period of great activity is followed by a much longer period of slight activity (see Fig. 4). The curve rises quite steeply for about 10 minutes, at the end of which time the rise becomes much more gradual. It appears as if there were two distinct curves, the first ending after about 10 minutes and merging into the second which continues for at least 2 hours. Examining the reagents it is found that the gold, which is introduced as a clear, yellow, solution of gold chloride, has precipitated as purple colloidal gold. If the reaction is followed in a test-tube it is observed that the precipitation begins after about 5 minutes and is apparently complete at 10 minutes. This precipitation corresponds in time to the sudden falling off in the rate of oxidation. Therefore the conclusion seems evident that the gold in solution catalyzes the oxidation of the pyrogallol to a marked extent, whereas the precipitated gold acts as a far less efficient catalyst if indeed it catalyzes the reaction at all. This matter will be discussed further in the next section.

Silver precipitates in a manner almost identical with that of gold, and the same considerations apply as with the latter metal.

Of several other metals none had a very marked effect. Cobalt and manganese caused a very slight production of carbon dioxide. They were each about as effective at a concentration of 0.01 M as copper at 0.00001 M; in other words, copper is about one thousand times as powerful a catalyst. Magnesium, mercury, cadmium, zinc, tin, and nickel had no detectable effect whatever. Hydrogen, in hydrochloric acid, was likewise without effect. The metals investigated may therefore be arranged in the following manner:

Group I: Catalysts (in order of effectiveness), Fe, Cu, Au, Ag, Co, Mn.

Group II: Non-Catalysts, Mg, Hg, Cd, Zn, Sn, Ni, H.

The theoretical significance of this grouping will be discussed later.

When we come to consider the probable mechanism of this oxidation system two facts stand out as of primary importance:

1. All the experiments were performed in an acid medium. Hopkins (1925) has found in studying the mechanism of oxidation by means of glutathione that there is a marked difference in the course of the
reaction depending on whether the medium is acid or alkaline, and Meyerhof (1923) maintains that oxygen transfer by sulfhydryl groups occurs only in acid and best at pH 3 to 5. (On catalysis by Fe in this connection, cf. Harrison, 1924.) These and other instances make it apparent that a change from an acid to an alkaline medium may profoundly alter the mechanism of an oxidation. Therefore it should be borne in mind in the discussion of the present data that all conclusions apply to experiments performed in an acid solution and that very different results might be obtained with experiments performed in an alkaline solution.

2. The experimental curves do not follow the course of a monomolecular or of a bimolecular reaction. Since the possibility of a reaction of a higher order is remote, it is apparent that we have here a complex of reactions of different types. To construct a hypothetical arrangement which would duplicate all the experimental data would be very difficult, but a simple system can be set up which will give a general representation of what is found experimentally.

Let us consider the action of copper. There are two distinct phases to the oxidation of pyrogallol by this metal. First, the hydrogen peroxide must be decomposed in order to liberate the oxygen; and second, the oxygen must combine with the pyrogallol to produce carbon dioxide and other substances. Considerable research has been performed on the problem of the decomposition of hydrogen peroxide by metals. Bredig and Ikeda (1901) studied the decomposition of peroxide by platinum and considered that the action was due to the formation and reduction of an oxide of platinum. Oliveri-Mandalà (1920) found that a similar catalytic effect is produced by iridium, as had Bredig and Fortner (1904) with palladium. Berthelot (1901) found that silver forms a peroxide and afterward a superoxide with hydrogen peroxide, both of which decompose and liberate oxygen. Baeyer and Villiger (1901) started with the oxide AgO and produced free oxygen with hydrogen peroxide. Colloidal silver was precipitated during the reaction. Hedges and Myers (1924) studied a periodic decomposition of hydrogen peroxide by silver, platinum, gold, and the enzyme catalase. These authors do not believe in the formation of an intermediate oxide.

In connection with the problem of “promoter action” the effect of
copper and iron, as well as other substances, has been extensively investigated. Bray and Livingston (1923) worked with bromine. Their opinion is that the hydrogen peroxide is decomposed by the bromine molecule which is thereby ionized and which is later restored to the molecular state. The ion is therefore the intermediate product. Von Bertalan (1920)\(^8\) says: "Die Zersetzung des Wasserstoffperoxyds durch Eisenionen verläuft als eine typische monomolare katalytische Reaktion." He considers the ion as the effective catalyst. Mummery (1913)\(^9\) says: "The catalytic decomposition of hydrogen dioxide by iron salts may be ascribed to the formation of higher perhydrols which are to be regarded as derivatives of hydrogen trioxide." Bohnson (1921) says that with iron the intermediate product is a hydrated ferric peroxide or "ferric acid." It is of interest that his data relating concentration to velocity constant (he finds the reaction to be of the first order) show breaks in continuity of very much the same sort as the break in Fig. 2, Curve A, where copper is the catalyst. Both ferric chloride and ferric sulfate exhibit this phenomenon. Bohnson also found that the specific reaction velocity decreases with time owing to the hydrolysis of the catalyst, and that free acid retards the action. Bohnson and Robertson (1923) conclude that the reaction, with iron, is an oxidation of the ferric ion, Fe, to a peroxide, FeO\(_4\), and subsequent reduction to the ionic form. They base their opinion on thermodynamic reasoning. Goard and Rideal (1924) state that the intermediate compound varies according to the acidity of the solution; that in neutral solution it is Fe\(_2\)O\(_6\) and in acid solution it is H\(_2\)FeO\(_4\). Similarly, Spitalsky and Petin (1924) ascribe the activity of iron to intermediate products which vary in composition with the hydrogen ion concentration. Robertson (1925) has summed up the discussion and has given an explanation of the so called promoter action of metals. For the simple combination of copper and hydrogen peroxide he suggests the following equations:

\[
\begin{align*}
\text{Cu} + \text{H}_2\text{O}_2 & \rightarrow \text{CuO}_2 + 2 \text{H} \\
\text{CuO}_2 + \text{H}_2\text{O}_2 & \rightarrow \text{Cu} + 2 \text{H}_2\text{O} + \text{O}_2
\end{align*}
\]

\(^8\) von Bertalan (1920), p. 328.
\(^9\) Mummery (1913), p. 889.
The CuO₂ is copper peroxide and when in acid solution may exist as H₂CuO₃.

In view of the above accumulation of opinion and evidence it seems permissible to accept Robertson's equations as the basis for the first step in the oxidation of pyrogallol, and to emphasize the following three points:

1. The metal functions in the ionic form.
2. There is always an intermediate product formed.
3. This intermediate product is a peroxide.

This system applies when there is simply a metal and hydrogen peroxide present. When an oxidizable substance is added it is necessary to alter the supposed course of the reaction. The fact that hydrogen peroxide will color guaiac in the presence of a metal salt has long been known. A recent study is that of Aloy and Valdigué (1923) on copper. Karczag (1921) has investigated the oxidation of dyestuffs by metal salts and hydrogen peroxide. Another investigation bearing on the present problem is that of Bredig and Ikeda (1901), who found that many organic substances, including pyrogallol, "poisoned" the reaction between the metal and the peroxide.

When an organic substance such as pyrogallol is oxidized by means of this system one of two things must happen. Either the oxygen which is liberated from the hydrogen peroxide unites with the pyrogallol, or the oxygen is transferred directly from the metallic peroxide without any separate existence. The latter supposition is more probable and is entirely in line with current theories of oxidation. If we accept it then we must notice two facts:

1. The second stage of Robertson's reactions is altered because the oxygen in the CuO₂ does not escape as molecular oxygen but is transferred directly to the pyrogallol.
2. If this transfer released the ionic copper in its original state then the copper could go through the same cycle again and the reaction would continue until either the pyrogallol or the hydrogen peroxide was exhausted. But we know from experiments with low concentrations of copper that this is not the case. For instance, if we use 0.00001 M copper the production of carbon dioxide soon stops. If we then add more peroxide or pyrogallol there is no effect; but if we add more copper the oxidation begins again. This fact demonstrates
conclusively that the copper is steadily removed from the active state. The removal may take place in various ways but the simplest assumption is that the metal is bound up irreversibly with the pyrogallol. That some such process occurs is suggested by the results of Bredig and Ikeda (1901) on the "poisoning" effect of pyrogallol.

Let us call pyrogallol PC. Then

\[
\text{CuO}_2 + \text{PC} \rightarrow \text{CuP} + \text{CO}_2
\]

The carbon dioxide is measured and the inactive, or bound, copper remains in the solution. But we must complete the original equation. This we may do and write the entire scheme:

\[
\begin{align*}
(1) & \quad \text{Cu} + \text{H}_2\text{O}_2 \rightarrow \text{CuO}_2 + 2 \text{H} \\
(2) & \quad 2 \text{H} + \text{H}_2\text{O}_2 \rightarrow 2 \text{H}_2\text{O} \\
(3) & \quad \text{CuO}_2 + \text{PC} \rightarrow \text{CuP} + \text{CO}_2.
\end{align*}
\]

Of course this scheme may differ in detail from what actually takes place. The real reactions are doubtless more complicated than is indicated here. But it is possible to go a certain distance toward duplicating the experimental results by means of the theoretical equations. Considering reactions (1) and (2) it is evident that there is a bimolecular reaction between the copper and the hydrogen peroxide. But there are two molecules of hydrogen peroxide decomposed for every molecule of copper peroxide formed, owing to the reduction of the second molecule of hydrogen peroxide by the free hydrogen. Using the ordinary notation, if \(a\) is the initial concentration of copper, \(b\) the initial concentration of the hydrogen peroxide, and \(y\) the amount of copper peroxide formed after time \(t\), then the rate of the reaction is proportional to the copper and hydrogen peroxide present. Of the latter, part is used in step (1) to form \(\text{CuO}_2\) and \(\text{H}\), and part in step (2) to form \(\text{H}_2\text{O}\). These two parts are equal. Then we may write \(dy/dt = K_1 (a - y) (b - 2y)\). This equation may be integrated and expressed in the following form:

\[
y = \frac{a b (1 - e^{K_1 (a - b)t})}{b - 2 a e^{K_1 (a - b)t}}.
\]
If $x$ is the amount of carbon dioxide formed after time $t$, and $c$ is the concentration of the pyrogallol, then $x$ is proportional to the amount of pyrogallol and the amount of CuO₂ (or $y$) present after $t$ units of time. This amount of CuO₂ is equal to the difference between the quantity produced from $a$ and $b$ and the quantity which has been transformed into $x$. Then $\frac{dx}{dt} = K_2 (y - x) (c - x)$, or

$$x = \frac{y \epsilon (1 - \epsilon K_2 (y - c) t)}{\epsilon - \frac{y \epsilon K_2 (y - c) t}{c - x}}.$$ 

Since $y$ is a variable quantity its value must be determined for every value of $t$ and substituted in the above equation.

![Graph](image)

Fig. 5. The solid line represents a typical experimental curve (CuCl₂ 0.001 M H₂O₂ 1 cc., pyrogallol 0.001 gm.). The points are obtained by means of the calculation outlined in the text whereby two bimolecular reactions are assumed. In this case $a = 0.5$, $b = 2$, $c = 0.08$, $K_1 = 0.2$, and $K_2 = 0.2$.

By the use of these equations the experimental curves may be approximately duplicated. Fig. 5 shows an example of an experimental curve selected at random and fairly closely fitted. The fit could be made much closer by more exact selection of the constants. This method of curve duplication does not afford absolute proof that the equations underlying the calculations are precisely those which underlie the experimental results. However, they must be fairly close to the truth. That the actual state of affairs is more complicated than has been suggested here is shown by one fact. In the calculated system the effect varies directly with the concentration, not as a fractional power thereof. In order to bring the calculated results into accordance with the experimental results it would be necessary
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to assume further complications such as side reactions, opposing reactions, etc. But the experimental data do not clearly indicate the precise nature of these additional reactions.

The definite conclusions which may be drawn are as follows: The oxidation of pyrogallol is due to the decomposition of the hydrogen peroxide by the copper ion, with the subsequent peroxidation of the copper. The copper peroxide then transfers its oxygen to the pyrogallol which is broken down into carbon dioxide and other products, including some form of combined copper.19

This system coincides with the Engler theory of respiration, in which there is an autoxidizable substance, a peroxide, and a catalyst. Atmospheric oxygen is absorbed and transferred by means of the peroxidase (catalyst) through the peroxide from the autoxidizable substance to the substance which is eventually to be oxidized. Here, of course, we start with a peroxide but the principle is the same. Wieland (1921) takes the view that hydrogen, not oxygen, is transferred, but he states that with metals and hydrogen peroxide metallic peroxides

1 The question of the atmospheric oxidation of pyrogallol and the nature of the products when the reaction is catalyzed by a metal has received attention from chemists. The two best known oxidation products are carbon dioxide and purpurogallin. The latter is specially likely to appear when iron is present. Salts of iron as well as of other metals may combine directly with pyrogallol, the reaction usually showing a characteristic color. Thus Jacquemin (1873) found that ferrous sulfate gives a blue color and ferric sulfate a red one.

de Clermont and Chautard (1882) got purpurogallin from pyrogallol in acid solution with AgNO₃ and KMnO₄. Wolff (1908) used ferrocyanide with hydrogen peroxide and obtained purpurogallin. Smirnow (1925) determined the effect of peroxidases by measuring the formation of purpurogallin, and found that manganese and iron catalyze the reaction although he does not consider that these two metals are necessary to a peroxidase. He quotes André (André, G., 1924, Chimie végétale, i, 210) to the effect that pyrogallol oxidizes according to the equation:

\[ 4 \text{C}_6\text{H}_8\text{O}_3 + 9 \text{O} \rightarrow 2\text{C}_6\text{H}_4\text{O}_6 + 4 \text{CO}_2 + 4 \text{H}_2\text{O} \]

where \(\text{C}_6\text{H}_4\text{O}_6\) represents purpurogallin. He also quotes Willstätter and Stoll (Willstätter, R., and Stoll, A., 1918, Ann. Chem., cxxxvi, 62), who state that 2 \(\text{C}_6\text{H}_4\text{O}_3\) + \(\text{O}_3\) \(\rightarrow\) \(\text{C}_6\text{H}_6\text{O}_6\) + \(\text{CO}_2\) + \(\text{H}_2\text{O}\). The latter formula (\(\text{C}_6\text{H}_6\text{O}_6\)) is given as the correct formula for purpurogallin in Beilstein, F., 1923, Handbuch der organischen Chemie, 4th edition, vi, 1072. In the experiments here recorded the concentrations of the reagents were too low for noticeable quantities of purpurogallin to be produced.
are formed. He therefore agrees in essentials with the intermediate peroxide theory.

VI.

Throughout the preceding discussion the assumption has been made that the metals act in the ionic form. This assumption is based on the results of Bohson, Robertson, etc., according to whom the metals (in decomposing hydrogen peroxide) take part in ordinary chemical reactions. There is no reason to suppose that there is any change in the state of iron or copper when pyrogallol is present. Nevertheless there is evidence to show that certain metals in the colloidal state can also decompose hydrogen peroxide. Certain metals, furthermore, are precipitated in the colloidal form by pyrogallol. Bredig and Reinders (1901) decomposed hydrogen peroxide with colloidal gold, as did Galecki (1925). Bredig and Ikeda (1901) used colloidal platinum, Duclaux (1923) used colloidal iron, and Zenghelis and Papaconstantinos (1920) used colloidal rhodium. As to the effect of pyrogallol, in addition to calling attention to the reduction of silver bromide by pyrogallol in photography, reference may be made to Garbowski (1903), who precipitated gold, platinum, and silver, and to Henrich (1903), who precipitated gold, platinum, silver, and mercury with pyrogallol.

We have a very clear example of the different effect of ionic and colloidal metal in the experiments performed on gold which were outlined in a previous section. In these experiments we can watch the rate of oxidation change while the colloidal gold is being precipitated. The first few readings are obtained with ionic gold and show a rapid action. After the gold has been precipitated the action still continues but at a greatly reduced rate. There exist at this point two possibilities: either the gold is all precipitated and the slow action is due to the colloidal metal, or not all the gold is precipitated, the colloidal metal has no effect, and the slow rate is due to a very much reduced quantity of the ionic metal. To throw light on this matter a standard quantity of gold chloride was added in a test-tube to the usual amount of hydrogen peroxide and pyrogallol and after the gold had been precipitated the entire mixture was dialyzed for a week.
Then the colloidal gold was recovered, added to more hydrogen peroxide, and pyrogallol and the resulting action tested in the respiration machine. There was no detectable production of carbon dioxide. The conclusion follows that colloidal gold does not catalyze the oxidation of pyrogallol in measurable quantities, despite the fact that it decomposes hydrogen peroxide.

The question arises why colloidal gold does not oxidize pyrogallol. It will be remembered that to account for the action of copper it was assumed that the copper forms a peroxide which carries the oxygen from the hydrogen peroxide to the pyrogallol. It does not liberate molecular oxygen which then, of itself, combines with the pyrogallol. The formation of the intermediate peroxide is essential. Now it is clear that while ionic gold can react to produce a gold peroxide, the metal in the colloidal form cannot do so. Therefore as the gold in solution becomes aggregated in solid particles the concentration of the effective gold is reduced. That the ions are not completely eliminated and that all the gold is not changed into the colloidal form (as is demonstrated by the fact that some oxidation persists) is probably due to the fact that the solution is acid. That the degree of dispersion, size of particles, and general properties of colloidal metals are dependent to a large degree on the acidity of the medium has been shown by numerous investigators. The presence of the pyrogallol doubtless has considerable influence and may tend to prevent the complete precipitation of the gold in solution.

Obviously the mode of action of the metal in the colloidal state is very different from the mode of action in the ionic form. As the present case shows, ionic gold will catalyze the oxidation of pyrogallol while colloidal gold will not. But the latter will decompose hydrogen peroxide as well as the former. The decomposition, however, must be due to an entirely different mechanism in the two cases. The intermediate peroxide theory, which is very satisfactory with the metal in solution, offers no explanation whatever for the action of the colloidal metal since the colloid cannot be expected to enter into simple stoichiometric relations with the other constituents, including the formation of metallic peroxides. If, as has been assumed, the oxidation of pyrogallol depends on the presence of such peroxides, it is quite evident
why colloidal gold can decompose hydrogen peroxide but fails to oxidize pyrogallol.

VII.

We are now in a position to make further comparison between the several metals which have been tested. A division has already been made between the effective catalysts, copper, iron, gold, silver, cobalt, and manganese, and the non-effective metals which include all the others tried. Among the first four there are marked differences depending on the ease with which the colloidal metal is precipitated. As a check on this point test-tube experiments were made which showed that gold and silver precipitated very easily, copper very slowly (a matter of days), and iron not at all. Iron is therefore the most powerful catalyst because it readily forms a peroxide and has the least tendency to be precipitated in the colloidal form by hydrogen peroxide and pyrogallol. Gold and silver are effective catalysts (though not so effective as iron and copper), but are prevented from exercising this function because they are so easily precipitated. Mercury, zinc, and the others, are ineffective because they do not form intermediate peroxides.

Herein may lie the reason why iron, and secondarily copper, are almost universally found in living cells as the metals which catalyze respiration. The assumption is legitimate that the cell contains organic peroxides similar to hydrogen peroxide and reducing (easily oxidizable) substances like pyrogallol. If this is true, and there is no strong evidence against it, then iron and copper are probably the only two metals which could effectively catalyze the oxidations in the cell. For metals like cobalt and manganese would be too weak in their action, mercury, zinc, etc., would not carry oxygen at all, and gold or silver would pass immediately into the ineffective colloidal state. Therefore iron and copper best fulfil the requirements for a metallic catalyst.

One final theoretical aspect of this question deserves brief consideration. If we examine the periodic table of the elements as revised to conform to the most recent ideas in physical chemistry, we find that all the active elements here investigated, i.e. iron, copper,
gold, silver, cobalt, and manganese will be found close together in the table. Below is a reproduction of a portion of the table showing the arrangement of electrons in the atoms of the elements (according to Langmuir), found in Taylor's Treatise on physical chemistry (1924):[1]

<table>
<thead>
<tr>
<th>Group</th>
<th>IIIa</th>
<th>IIIb</th>
<th>IVa</th>
</tr>
</thead>
<tbody>
<tr>
<td>VII</td>
<td>Mn</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Fe</td>
<td>Ru</td>
<td>Os</td>
</tr>
<tr>
<td>VIII</td>
<td>Co</td>
<td>Rh</td>
<td>Ir</td>
</tr>
<tr>
<td></td>
<td>Ni</td>
<td>Pd</td>
<td>Pt</td>
</tr>
<tr>
<td>I</td>
<td>Cu</td>
<td>Ag</td>
<td>Au</td>
</tr>
<tr>
<td>II</td>
<td>Zn</td>
<td>Cd</td>
<td>Hg</td>
</tr>
</tbody>
</table>

The groups refer to the standard groups in the periodic table. (The numerals at the head of the columns refer to the number of shells of electrons.) Thus we see that Group I, which includes copper, silver, and gold, has an outer shell of one electron, and Group VIII has an outer shell which lacks completeness by one electron. It is in these two groups that the effective elements Fe, Cu, Ag, Au, and Ru, Rh, Pd, Os, Ir, and Pt, which are probably also effective, occur. The only exceptions are manganese which is in Group VII, and nickel which is in Group VIII but is not effective. In general the situation is striking. Those elements which are effective oxidation catalysts have their outer shell either composed of just one electron or lacking just one electron. Furthermore, as the number of shells increases the greater becomes the tendency for the element to pass into the colloidal state with hydrogen peroxide and pyrogallol. It is impossible to draw any conclusions at present from these facts, but they are very suggestive and indicate the possibility of future theoretical developments.

**SUMMARY.**

1. When iron and copper are allowed to act on hydrogen peroxide and pyrogallol, enough carbon dioxide is produced to be readily measured.

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2. The curve of the production of carbon dioxide may be fitted by an empirical equation, by the use of which the initial rate and the total amount of the oxidation may be determined.

3. The effect of the concentration of the reagents is different in each case, the effect varying as a fractional power of the copper and pyrogallol concentrations and as a logarithmic function of the hydrogen peroxide concentration.

4. When gold or silver is used the rate changes suddenly during the course of the reaction due to the precipitation of colloidal metal.

5. Mercury, cadmium, zinc, tin, and some other metals have no effect.

6. A theoretical set of equations is assumed to account for the action of the metals.

7. The metals are assumed to act by means of the formation of intermediate peroxides.

8. Experiments on the action of gold indicate that the metals are active in the ionic and not in the colloidal state.

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