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I hereby recommend that the thesis prepared under my supervision by Kenneth L. Mulstad,

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THE ACTION OF HYDROGEN PEROXIDE UPON GLYCOLIC AND LACTIC ACIDS

A dissertation submitted in partial fulfillment

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INTRODUCTION

The research discussed in this thesis is a continuation of the work started in this laboratory in 1927 on the action of hydrogen peroxide on simple carbon compounds. The first work (1), designed to determine the effect of variations in the acidity of the medium upon the oxidation of methyl alcohol with hydrogen peroxide, disclosed some perplexing anomalies. The yields of oxidation products which would naturally be expected were invariably greater than theoretical yield calculated from the reactions known to occur. Since the experimental procedure was so conducted that the amount of hydrogen peroxide added to the reaction mixture was definitely known, the only explanation possible was that simultaneously with the oxidation reaction, involving the active hydrogen peroxide, there were other reactions occurring which involved the formation of reducing substances, whose presence had escaped notice. This conclusion was verified in the discovery that hydrogen was produced as one of the reaction products and could be quantitatively determined.

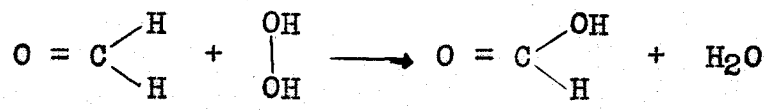
This unsuspected and unexplained liberation of hydrogen, which had not been previously recorded in the literature, led to a quantitative study of the reactions of methyl alcohol, formaldehyde, and formic acid with hydrogen peroxide, with a view of suggesting a possible reaction mechanism (2). The products of the reactions were quantitatively determined in each case, and the percentage quantities of hydrogen

peroxide equivalent to the yield of oxidation products calculated from the proposed reactions were found to agree, within the limits of experimental error, with the quantity of hydrogen peroxide used.

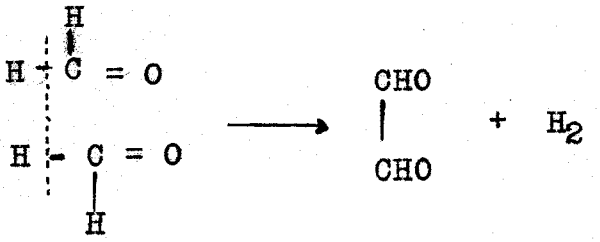
The quantitative study was then extended to members of the next higher corresponding series, namely ethyl alcohol, acetaldehyde, and acetic acid. The yields of reaction products were again found to agree with the theoretical yield calculated from the one or more postulated equations.

To account not only for the usual types of oxidation reactions effected by hydrogen peroxide, but also for the anomalous reactions which yield hydrogen from methyl alcohol and formaldehyde, and hydrogen and methane from acetaldehyde, a new type of reaction mechanism, termed "perhydrolysis", was proposed.

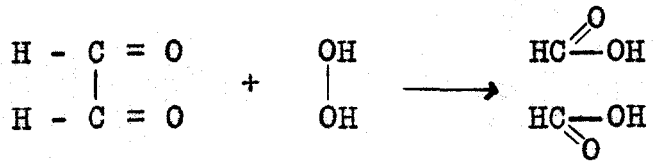
To illustrate this perhydrolysis mechanism of reaction, the action of hydrogen peroxide upon formaldehyde may be reviewed. It is here assumed that hydrogen peroxide displays a type of reaction which may be termed "perhydrolysis", that is, a double decomposition reaction involving hydrogen peroxide in precisely the same manner that hydrolysis is a double decomposition reaction involving water. Thus the normal oxidation of formaldehyde to formic acid is a perhydrolysis reaction.



To explain the apparently anomalous reaction, namely, the oxidation of formaldehyde to formic acid with the liberation of hydrogen, requires the assumption that hydrogen peroxide may act catalytically in the liberation of hydrogen from formaldehyde.



to be followed immediately by the perhydrolysis of the intermediately formed glyoxal, yielding two molecules of formic acid



These reactions may be summarized in one equation

$$2\ HCHO + H_2O_2 \longrightarrow H_2 + 2\ HCOOH.$$

The proposed mechanism has been confirmed by the quantitative data obtained in the case of formaldehyde, methyl alcohol, formic acid, acetaldehyde, and acetic acid. It is the object of this research to extend the quantitative study of the action of hydrogen peroxide on simple carbon compounds, to higher carbon compounds, with the view of establishing the further validity of the reaction mechanism termed "perhydrolysis".

The compounds studied in this investigation include glycolic and lactic acid, both of which yield compounds

containing the aldehyde radical on treatment with hydrogen peroxide, and consequently should yield hydrogen as a reaction product. The general reaction mechanism proposed for the oxidation of simple carbon compounds is in this research extended to glycolic and lactic acids, and is confirmed by the quantitative data obtained.

In the following dissertation, first there will be given a historical outline of previous work recorded in the literature, followed by the experimental procedure and interpretation in terms of the proposed reaction mechanism on the basis of the results obtained.

HISTORICAL

A. Glycolic Acid

The mechanism of the oxidation of glycolic acid has attracted the attention of may investigators, notably in the study of biological oxidation reactions. An explanation of the mechanism of the oxidation of acetic acid has been formulated through a study of its possible intermediate oxidation products which no doubt first involves the formation of glycolic acid. Many biochemical investigations have been concerned with the intermediate formation of glycolic acid in the oxidation of other organic compounds, but the following references undoubtedly cover the principal literature relating to the reaction of hydrogen peroxide upon glycolic acid. In no case has a thorough quantitative study of this reaction been attempted and in only one instance has the formation of hydrogen been recorded.

Fenton and Jones (3) studied the action of hydrogen peroxide on glycolic acid at low temperatures and found in the absence of ferrous salts that there was little action. In the presence of ferrous salts there is an instantaneous reaction, and a quantitative yield of glyoxylic acid was obtained which was separated and identified as the phenylhydrazone. No mention is made of the presence of other products. However, it is possible that under the conditions of the experiment, glyoxylic acid is the only product, even though glyoxylic acid is very susceptible to oxidation with hydrogen peroxide.

Heinrod and Levene (4) found that glycolic acid was oxidized in alkaline solution by thirty per cent peroxide solution to formic acid and carbonic acid. Glyoxylic acid was also found, as was determined. They state that no hydrogen escapes, nor was there any oxalic acid formed. They also suggest that glycolic acid may yield formaldehyde according to the reaction,

$$\begin{array}{l} CH_2OH \\ | \\ COOH \end{array} + \begin{array}{l} OH \\ | \\ OH \end{array} \longrightarrow \begin{array}{l} HCHO + H_2O \\ CO_2 + H_2O \end{array}$$

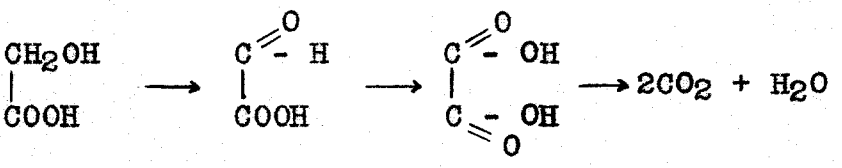
but they do not report its presence in the products of the reaction. It is probable that if any formaldehyde was formed, it would be immediately oxidized to formic acid.

In an attempt to explain the course of metabolism of amine acids in the body, Dakin (5) studied the action of hydrogen peroxide upon amino acids in the presence of traces of ferrous sulphate. He states that they are all readily converted at ordinary temperatures into carbon dioxide, ammonia, and an aldehyde. From glycocoll, he obtained ammonia, glyoxylic acid, carbon dioxide, formaldehyde, and formic acid. It is difficult to account for the production of formaldehyde without the simultaneous liberation of hydrogen, and from the analogy between glycolic acid and glycocoll it is doubtful, as will be shown later in this thesis, if any formaldehyde is formed.

From the results obtained with glycocoll, Dakin (6) applied the same interpretation to the reaction between

glycolic acid and hydrogen peroxide. He found that glycolic acid in the presence of ferrous sulphate gave a small amount of glyoxylic acid, but the main product was formaldehyde, which was almost entirely oxidized to formic acid. Here again no mention is made of the formation of hydrogen, and if formaldehyde is the main product of the reaction, hydrogen would certainly be one of the products of the reaction. As will be shown later, the production of hydrogen may be accounted for without the intermediate formation of formaldehyde, but conclusive evidence cannot be given at the present time.

Later Dakin (7), in pointing out the course of oxidation by chemical means, and through the agency of living organisms, states that the oxidation of saturated substances usually consists in replacement of hydrogen by hydroxyl with formation of products which may or may not be isolated. He then adds that glycolic acid in the body is oxidized through glyoxylic and oxalic acid stages according to the following scheme.



Dakin obtained the evidence of the intermediate formation of oxalic acid by finding it present in the urine during feeding experiments. The presence of oxalic acid has not been established in this research and although there is no doubt that there is an analogy between chemical oxidation and tissue oxidation, the mechanism in many cases is entirely

different.

Dakin (8) made a further study of the oxidation by hydroxy fatty acids by treating their ammonium salts with hydrogen peroxide. He found that glycolic acid yields primarily glyoxylic acid and formaldehyde, and these on further oxidation, yield formic acid which in turn is capable of further oxidation to carbon dioxide and water. Here as in previous investigations, no mention is made of the formation of hydrogen which should be formed if formaldehyde is an intermediate but which will be shown may be accounted for without the formation of formaldehyde.

Spoehr (9) in an attempt to offer further explanation of the action of hydrogen peroxide on sugars in alkaline solution, studied its action upon glycolic acid in alkaline solution. He found that at the end of four days, formic acid. carbon dioxide, and unchanged glycolic acid were the only products and each was quantitatively determined. He states that this proves that glyoxylic acid, which is first formed by oxidation, gives equal molecular proportions of formic and carbonic acids but no oxalic acid on further oxidation. Although he mentions no hydrogen as a product of the reaction, a partial quantitative study of the reaction was made which offers more conclusive evidence of the correctness of his interpretation. Furthermore it shows that biochemical and chemical oxidations are not alike if oxalic acid is a product of the oxidation of glycolic acid in the animal body.

Spoehr [10] made a further study of the oxidation of glycolic acid with hydrogen peroxide in the presence of ferrous sulphate. The products were the same as in the previous investigation, but the carbon dioxide and formic acid were not formed in equal molecular proportions. The carbon dioxide was present in much greater amounts, which shows the further oxidation of formic acid to carbon dioxide. No oxalic acid or hydrogen were formed.

Perhaps the most complete, as well as the most conclusive study of the action of hydrogen peroxide on glycolic acid has been made by Wieland [11]. He assumes that the main path of the oxidation is through glyoxylic acid, but at the same time a part may go through formaldehyde, even though formaldehyde could not be detected as one of the products. He assumes the formation of formaldehyde to account for the production of hydrogen which he obtained. He expresses the reaction in the following equations, representing hydrogen peroxide as a hydrogen acceptor.

I. $$O=C(H_2C-OH)-OH + OH|OH \longrightarrow O=C=O + H_2C=O + HOH + HOH$$

II. $$O=C(H)-CH(OH)-COOH + OH|OH \longrightarrow O=C(H)-COOH + H-C=O + HOH + HOH$$

The glyoxylic acid is further oxidized to carbon dioxide and formic acid, while the formaldehyde yields formic

formic acid and hydrogen. This dehydrogenation theory of Wieland seems logical but one is led to believe that the production of hydrogen always involves the intermediate formation of formaldehyde. In the interest of simplicity of explanation, it seems unnecessary to assume the formation of formaldehyde, as will be later pointed out in this thesis. Also acetaldehyde readily gives hydrogen as well as methane on treatment with hydrogen peroxide, and a simple logical explanation has been offered by Fry and Payne (2) which does not involve the formation of formaldehyde.

Hatcher and Holden (12) postulate the formation of glycolic acid as an intermediate in the oxidation of acetic acid but failed to obtain a test for it, which may be accounted for by its speed of oxidation. They maintain that the glycolic acid is changed into a peracid which in turn give glyoxylic acid and ultimately carbon dioxide and water, according to the following reactions,

$$CH_2(OH)COOH + H_2O_2 \longrightarrow CH_2(OH)CO_3H + H_2O$$

$$CH_2(OH)CO_3H \longrightarrow CHOCOOH + H_2O$$

$$CHOCOOH + H_2O_2 \longrightarrow CO_2 + HCOOH + H_2O$$

$$HCOOH + H_2O_2 \longrightarrow HCO_3H + H_2O$$

$$HCO_3H \longrightarrow H_2O + CO_2$$

No glyoxylic acid could be detected, which may also be accounted for by its extreme ease of oxidation. Fry and Payne (2) were also unable to detect glycolic and glyoxylic acids in the intermediate oxidation of acetic acid, although

the above explanation is probably correct since the formation of peracids is a common occurrence in the treatment of organic acids with hydrogen peroxide.

In an attempt to substantiate the proposed mechanism of the oxidation of acetic acid and to clear up the conflicting opinions of previous investigations, Hatcher and Holden (13) studied the action of hydrogen peroxide on glycolic acid. No formaldehyde or oxalic acid were formed, nor was there any hydrogen in the escaping gases. They state that the failure to find hydrogen in the gas is sufficient evidence for the absence of formaldehyde. It will be shown in the experimental part of this thesis that hydrogen is always one of the products of the reaction which thereby does not eliminate the formation of formaldehyde as one of the products. However, the exhausted qualitative tests applied to the reaction mixture by these investigators, establishes definitely that formaldehyde is not one of the products of the action of hydrogen peroxide on glycolic acid. In the review of the literature given by Hatcher and Holden no mention is made of the work of Wieland, which no doubt is the most conclusive up to the present time.

It is evident from the foregoing survey of the literature that no definite conclusions can be drawn as to the exact quantitative nature of the reaction between glycolic acid and hydrogen peroxide. The following reactions in summary have been postulated by previous investigators, but

the extent pf the occurrence of each has not yet been definitely determined.

$$CH_2OHCOOH + H_2O_2 \longrightarrow CHOCOOH + 2\,H_2O$$

$$CH_2OHCOOH + H_2O_2 \longrightarrow CH_2O + 2H_2O + CO_2$$

$$CH_2OHCOOH + 2H_2O_2 \longrightarrow HCOOH + CO_2 + 3H_2O$$

$$CH_2OHCOOH + 2H_2O_2 \longrightarrow (COOH)_2 + 3H_2O$$

$$CH_2OHCOOH + 3H_2O_2 \longrightarrow 2CO_2 + 5H_2O$$

$$2CH_2OHCOOH + 3H_2O_2 \longrightarrow 2HCOOH + H_2 + 2CO_2 + 4H_2O.$$

In order to determine the extent of the occurrence of these reactions, further quantitative investigations are necessary.

B. Lactic Acid

Although in the animal body the reaction,

$$CH_3CHOHCOOH + O \longrightarrow CH_3C^{O} - COOH$$

is assumed to occur, without direct evidence, no final conclusions have been drawn as to the mechanism of the oxidation in vitro with hydrogen peroxide. It is entirely possible for lactic acid to be oxidized in the body without assuming this intermediate formation of pyruvic acid, but rather with the formation of acetaldehyde. Evidence for this intermediate formation of acetaldehyde in biological processes is found in the work of Ringer and Frankel (14), who have shown that acetaldehyde when administered to phlorhizinized dogs, is converted to glucose. It is therefore not necessary to assume that pyruvic acid is an intermediate product in the oxidation conversion of lactic acid to glucose in biological processes, nor does this reaction appear in this research to occur to any degree in vitro when lactic acid and hydrogen peroxide interact.

It is reported by Hatcher and Toole (22) that Wurster (15) was the first to study the action of hydrogen peroxide on lactic acid. He found that lactic acid was fairly stable to oxidation by this compound.

With the aid of ferrous sulphate at a very low temperature, Fenton and Jones (3) obtained the phenylhydrazone of pyruvic acid. Since the same authors have shown that pyruvic acid in ice water is immediately attacked by hydrogen

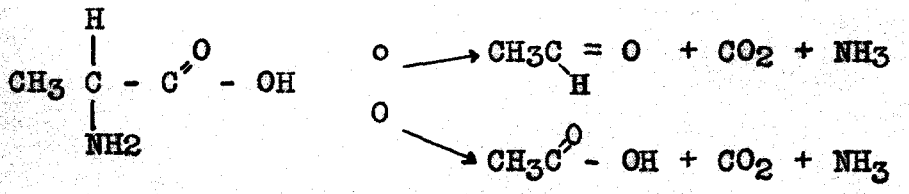
peroxide in the absence of ferrous salts, it is possible that pyruvic acid is not necessarily a normal oxidation product of lactic acid without ferrous salts as catalysts.

Holleman [16] in a study of the action of thirty per cent hydrogen peroxide on alpha ketonic acids, found that pyruvic acid is converted quantitatively into acetic acid and carbonic acids according to the equation

$$RCOCOOH + HOOH \longrightarrow RCOOH + CO_2 + H_2O$$

This observation further confirms the above suggestion that pyruvic acid is not necessarily a normal oxidation product of the action of hydrogen peroxide on lactic acid. It is accordingly preferable in this thesis to base the proposed mechanism of reaction upon the assumption that pyruvic acid is not necessarily an intermediate oxidation product rather than to assume that as fast as it is intermediately formed it is instantly converted to acetic and carbonic acids.

Dakin [5], in an attempt to follow the course of metabolism of alanine in biological oxidations, studied the action of hydrogen peroxide on this amino acid in the presence of ferrous sulphate. The products obtained were acetaldehyde, acetic acid, carbon dioxide, and ammonia, but no pyruvic acid was found. He represents the reaction as follows



This shows that hydrogen peroxide oxidizes alpha substituted acids without the intermediate formation of stable products.

It is possible that alanine is first converted to lactic acid with subsequent decomposition by means of hydrogen peroxide, for Ringer [17] has shown that alanine, on deaminization, yields lactic acid, which is the first reaction in the oxidation of alanine in the body.

From the results obtained with alanine and the similarity between this compound and lactic acid, Dakin [8] extended his studies to the oxidation of lactic acid with hydrogen peroxide. With the ammonium salt of lactic acid, acetaldehyde and carbonic acid are the first oxidation products. The acetaldehyde is partly oxidized to acetic acid, which may also yield formic acid, and carbonic acid on further oxidation. The formic acid may also be oxidized to carbonic acid. Dakin represents the course of the oxidation as follows:

$$CH_3CHOHCOOH + H_2O_2 \longrightarrow CH_3C(=O)H + CO_2 + 2H_2O$$

$$CH_3C(=O)H + H_2O_2 \longrightarrow CH_3C(=O)OH + H_2O$$

$$CH_3C(=O)OH + 2H_2O_2 \longrightarrow 2HCOOH + 2H_2O$$

$$HC(=O)OH + H_2O_2 \longrightarrow CO_2 + 2H_2O$$

The work of Dakin was only qualitative and no mention is made of any gaseous product except carbon dioxide. However, as will be shown in the Experimental Part IV of this thesis, the above equations probably represent the main course of the oxidation of lactic acid with hydrogen peroxide. The above equations receive further support from the fact that other oxidizing agents bring about the same results.

Kolbe [18] found that potassium lactate on electrolysis gives acetaldehyde and carbon dioxide while it is reported by Dakin [8] that Liebig found a similar change was brought about by manganese and lead peroxides and sulphuric acid.

Heinrod and Levene [4] obtained formic acid on treatment of lactic acid with hydrogen peroxide in alkaline solution. No mention is made of the formation of other products, but the formic acid probably results from the oxidation of acetic acid, as pointed out by Dakin, although it may be formed by the direct oxidation of lactic acid as will be noticed later.

The oxidation conversion of lactic acid to glucose in the animal body was originally believed to involve pyruvic acid as the first intermediate. The active compound in this conversion is now believed to be methyl glyoxal, for Dakin [19] has shown that methyl glyoxal is a product of the action of hydrogen peroxide on lactic acid but pyruvic acid is not a product. Therefore the formation of pyruvic acid is not necessary to account for the oxidation of lactic acid, either in the animal body or in vitro.

Effront [20] studied the oxidation of lactic acid at its boiling point by hydrogen peroxide and almost a quantitative yield of acetic acid was obtained according to the following equation:

$$CH_3CHOH\,COOH + 2H_2O_2 \longrightarrow CH_3COOH + CO_2 + 3H_2O$$

The acid obtained contained a small quantity of alcohol and aldehyde. He expresses the course of the reaction as first

involving a decomposition of lactic acid into ethyl alcohol and carbon dioxide, with the subsequent oxidation of the alcohol to acetic acid. In his experiments lactic acid is always present in large excess, which may account for the production of alcohol. An equally good explanation of the formation of ethyl alcohol is by the reduction of acetaldehyde, which may arise from the direct oxidation of lactic acid, with hydrogen peroxide reacting in its known capacity as a reducing agent, or from intermediately formed pyruvic acid, since pyruvic acid readily looses carbon dioxide with the formation of acetaldehyde. Conclusive evidence is still lacking for the correct explanation of the conversion of lactic acid to ethyl alcohol by means of hydrogen peroxide.

Ray (21) has verified the formation of acetaldehyde as the first product of the reaction of hydrogen peroxide with lactic acid. He attempted to formulate a mechanism of oxidation of lactic acid by a determination of the rate of evolution of carbon dioxide when sodium lactate is treated with hydrogen peroxide. He states that the hydrogen peroxide acts through the intermediate formation of organic peroxides, which will later be verified in the Experimental Part IV of this thesis. He further found that acetaldehyde is the end product of the oxidation of sodium lactate by means of hydrogen peroxide in an atmosphere of nitrogen. This differs from all other investigations recorded in the literature. In the experiments recorded by all other investigators, acetic acid is always one of the reaction products.

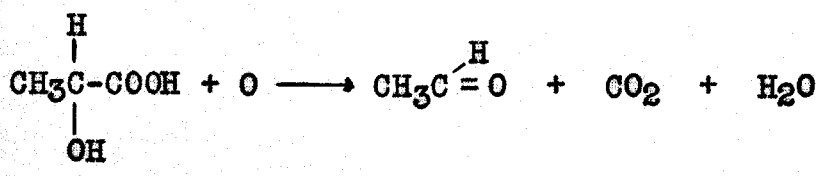
Hatcher and Toole [22] studied the oxidation of lactic acid with hydrogen peroxide at 100° Centigrade and found complete oxidation to three molecules of carbon dioxide. Acetaldehyde was present in very small amounts, and no formic or acetic acids remained at the conclusion of the oxidation. No mention is made of the liberation of hydrogen or methane. It is possible that in the presence of a large excess of hydrogen peroxide, carbon dioxide is the only product. If this is true, an accurate quantitative determination of lactic acid should be possible through a measure of the carbon dioxide. As far as is known to the author, no such quantitative determinations have been attempted.

Bernhauer and Nistler [23], working from the standpoint of the oxidation of sugars and their intermediate products, found that lactic acid gave acetic and formic acids when treated with hydrogen peroxide in the presence of calcium carbonate. No mention is made of the analysis of the gases, but the qualitative results reported are in accordance with those of previous investigators.

Battie, Smedley and Maclean [24] studied the effect of copper salts on the oxidation of lactic acid with hydrogen peroxide and found that reproducible results could be obtained. They found that in the presence of a large excess of hydrogen peroxide, in one hour's time, forty-three per cent of the lactic acid was converted to carbon dioxide. In the absence of copper salts only 0.14 per cent was converted. This

confirms the very early work of Wurster (15) that lactic acid is fairly stable to oxidation with hydrogen peroxide in absence of a catalyst.

In connection with the action of hydrogen peroxide on lactic acid, the results of the action of ultraviolet light on this compound are of interest. Numerous investigators, including Euler (25), Ganassini (26), and Burns (27) have investigated this problem. The main products of the action of ultraviolet light on lactic acid are acetaldehyde, acetic acid and carbon dioxide. In addition Burns reports a test for formaldehyde and Ganassini found pyruvic acid, which he states does not give acetaldehyde on further decomposition. The acetaldehyde, as in the case with hydrogen peroxide, arises from the following reaction:



The oxidation of lactic acid by means of ultraviolet light and by means of hydrogen peroxide, yield similar products, and since in both cases the active oxidizing agent is a peroxide of acetaldehyde, it is probable that the mechanism of the reaction in both cases is similar.

While the oxidation of lactic acid by means of hydrogen peroxide and other oxidizing agents is similar in that acetaldehyde is one of the first products which is converted on further oxidation into acetic acid, a striking exception is found in the work of Conant and Longberg (28)

in which they used ceric sulphate as an oxidizing agent. They report that lactic acid gives, first, acetaldehyde; and, if the aldehyde is not removed, the final products are glycolic aldehyde, glyoxylic acid, formic acid, and formaldehyde. Ceric sulphate possesses the property of effecting the alpha oxidation of acetaldehyde, which no other oxidizing agent effects to so marked a degree.

From the foregoing summary the following reactions are pertinent.

$$CH_3CHOHCOOH + H_2O_2 \longrightarrow CH_3COCOOH + 2H_2O$$

$$CH_3CHOHCOOH + H_2O_2 \longrightarrow CH_3CHO + CO_2 + 2H_2O$$

$$CH_3CHOHCOOH + 2H_2O_2 \longrightarrow CH_3COOH + CO_2 + 3H_2O$$

$$CH_3C(=O)-OH + 2H_2O_2 \longrightarrow 2HCOOH + 2H_2O$$

$$HC(=O)-OH + H_2O_2 \longrightarrow CO_2 + 2H_2O$$

$$CH_3CHOHCOOH + 6H_2O_2 \longrightarrow 3CO_2 + H_2O$$

On the occurrence of certain of these reactions, the proposed perhydrolysis reaction mechanism will be extended to the explanation of the action of hydrogen peroxide on lactic acid.

THEORY AND OBJECT OF THE INVESTIGATION

The primary objects in undertaking this investigation were:

(1) To determine both qualitatively and quantitatively the several products of oxidation resulting from the action of hydrogen peroxide upon: (A) glycolic acid; (B) lactic acid.

(2) To determine quantitatively the extent to which each of the postulated reactions occurs.

(3) From the results obtained in (1) and (2), to extend the perhydrolysis mechanism of reaction to an explanation of the action of hydrogen peroxide on glycolic and lactic acids.

Since it has been shown that aldehydes, or compounds yielding aldehydes as intermediate oxidation products, evolve hydrogen or methane on treatment with hydrogen peroxide, and since the simple aldehydes or compounds yielding these aldehydes have been investigated, it would seem desirable to extend the investigation and perhydrolysis reaction mechanism to higher carbon compounds.

Glycolic acid was chosen because it contains a primary alcohol group and should yield glyoxylic acid as the first oxidation product. The glyoxylic acid, containing an aldehyde group, would undergo the perhydrolysis mechanism of reaction to yield hydrogen as one of the reaction products. Glycolic acid presents further interest in that it is probably the first product resulting from the action of hydrogen peroxide on acetic acid. Therefore a complete mechanism of

reaction may be formulated for acetic acid through a study of the intermediate products.

Lactic acid was chosen because it presents particular interest in oxidation in the animal body. Furthermore, it is known that lactic acid is fairly unstable giving acetaldehyde and formic acid on decomposition. It was therefore decided to study lactic acid experimentally since it would be expected to undergo reactions similar to acetaldehyde, yielding hydrogen and methane.

It will be shown in the Experimental Part IV of this thesis that both glycolic and lactic acid give hydrogen on treatment with hydrogen peroxide, and in addition lactic acid yields methane.

A. Glycolic Acid

Since glycolic acid contains a primary alcohol group, the first reaction with hydrogen peroxide would yield glyoxylic acid according to the equation

$$CH_2OH\ COOH + H_2O_2 \longrightarrow CHO\ COOH + 2H_2O \qquad (a)$$

Fenton and Jones [3] obtained only glyoxylic acid and therefore assumed (a) as the only reaction taking place. However, the low concentration of hydrogen peroxide and the temperature under which their reactions were carried out, explain the results obtained. It does not seem possible, in view of the marked susceptibility to oxidation of the aldehyde radical, as has been shown, for glyoxylic acid to be a stable intermediate in this oxidation unless the hydrogen peroxide is present in extremely small concentrations.

Reaction (a) receives further support from the work of Dakin, Spoehr, and especially that of Hatcher and Holden [13].

In addition to reaction (a) another parallel reaction has been suggested by Dakin [6] and Wieland [11] which yields formaldehyde, and as the first product, which Wieland represents thus:

$$\begin{array}{l} O{=}C{-}OH \\ \quad | \\ H_2C{-}OH \end{array} + \begin{array}{l} OH \\ | \\ OH \end{array} \longrightarrow \begin{array}{l} O{=}C{=}O \\ \\ H_2C{=}O \end{array} + \begin{array}{l} HOH \\ \\ HOH \end{array} \qquad (b)$$

Evidence of reaction (b) given by Wieland is the production of hydrogen on treatment of glycolic acid with hydrogen peroxide. This is without doubt very good evidence, since formaldehyde readily yields hydrogen with hydrogen

peroxide. An objection to this assumption that formaldehyde is a product is found in the results of the quantitative study of the action of hydrogen peroxide on formaldehyde by Fry and Payne (2). These investigators have shown that the maximum amount of hydrogen is obtained when formaldehyde is present in large excess, and as the concentration of hydrogen peroxide is increased the yield of hydrogen is decreased. It is not probable that a sufficient concentration could accumulate to account for the production of hydrogen.

Furthermore, Hatcher and Holden (13) subjected their reaction mixtures to exhaustive qualitative tests for formaldehyde. They were unable to detect the presence of this compound but did obtain a test for glyoxylic acid. This work of Hatcher and Holden has been verified in the Experimental Part of this thesis, and a reaction mechanism proposed for the liberation of hydrogen. Reaction (b) is not necessary to correlate the quantitative yields of the oxidation products obtained.

The glyoxylic acid formed in (a) may undergo further oxidation to oxalic acid, as proposed by Dakin, since the course of the oxidation of glyoxylic acid in the body conforms to the following equations

$$CHO\ COOH + H_2O_2 \longrightarrow HOOCCOOH + H_2O \qquad (c)$$

No mention is made of the occurrence of reaction (c) in the literature, nor was oxalic acid detected in any of the reactions carried out in this work.

Since oxalic acid is not one of the products, the other possible reaction is one yielding formic and carbonic acid:

$$OHC\ COOH + H_2O_2 \longrightarrow HCOOH + CO_2 + H_2O \quad (d)$$

No doubt the main course of the action of hydrogen peroxide on glycolic acid is represented by equations (a) and (d), which combined, give the following equation (e)

$$CH_2OH\ COOH + 2H_2O_2 \longrightarrow 3H_2O + CO_2 + HCOOH \quad (e)$$

Concurrently with reaction (e) there occurs a reaction which results in the liberation of hydrogen. Since glyoxylic acid contains an aldehyde group, and previous investigations have shown that acetaldehyde as well as formaldehyde yield hydrogen on treatment with hydrogen peroxide, it is reasonable to suppose that glyoxylic acid undergoes the following reaction

$$2\ OHC\ COOH + H_2O_2 \longrightarrow H_2 + 2HCOOH + 2CO_2 \quad (f)$$

which is parallel to the reaction of hydrogen peroxide with formaldehyde.

$$2\ HCHO + H_2O_2 \longrightarrow H_2 + 2\ HCOOH.$$

There is only one reference in the literature (11) to the occurrence of reaction (f). This liberation of hydrogen has been verified in the Experimental Part IV, and the established fact that hydrogen is always evolved has an important bearing upon the subsequent extension of the theory of "perhydrolysis" to glycolic acid. Conclusive evidence of the occurrence of reaction (f) can only be obtained through a study of glyoxylic acid.

Since formic acid is quite readily oxidized by comparatively weak oxidizing agents, it would seem that the formic acid produced in reactions (e) and (f) would be wholly or in part oxidized to carbonic acid as follows:

$$HCOOH + H_2O_2 \longrightarrow 2H_2O + CO_2 \quad (g)$$

It has been conclusively proved by Fry and Payne (2) that this is the only reaction occurring when formic acid is treated with hydrogen peroxide in acid solution.

It will be shown in the Experimental Part IV that the consecutive reactions (a), (d), and (g) represent the main course of the reaction, with the occurrence of the simultaneous reaction (f) to a smaller extent. For convenience in reference to the experimental part these proposed reactions are here grouped.

$$CH_2OHCOOH + H_2O_2 \longrightarrow CHOCOOH + 2H_2O \quad (a)$$

$$OHCCOOH + H_2O_2 \longrightarrow HCOOH + CO_2 + H_2O \quad (d)$$

$$2OHCCOOH + H_2O_2 \longrightarrow H_2 + 2HCOOH + 2CO_2 \quad (f)$$

$$HCOOH + H_2O_2 \longrightarrow 2H_2O + CO_2 \quad (g)$$

B. Lactic Acid

The study of the oxidation of lactic acid is of interest since it contains a secondary alcohol group adjacent to a carbonyl group. It would seem natural to expect to find pyruvic acid as the first oxidation product, produced according to the equation:

$$CH_3CHOH\ COOH + H_2O_2 \longrightarrow CH_3CO\ COOH + 2H_2O \qquad (h)$$

Fenton and Jones [3] obtained pyruvic acid as the only product and assumed reaction (h) as the only one occurring, which Dakin [8] has attributed to the presence of the catalyst. From the results of other investigators, including those described in this thesis, reaction (h) probably does not occur except in the presence of a catalyst and at a very low temperature.

The other possible reaction which has been pointed out by Dakin [7], Effront [20], Ray [21], and Hatcher and Toole [22] is one yielding acetaldehyde and formic acid as follows:

$$CH_3CHOH\ COOH \longrightarrow CH_3CHO + HCOOH \qquad (i)$$

The acetaldehyde may undergo further oxidation to acetic acid

$$CH_3CHO + H_2O_2 \longrightarrow CH_3COOH + H_2O \qquad (j)$$

Reaction (j) is very fast and therefore only a very small amount of acetaldehyde is present in the solution at any time. A negligible amount remains at the completion of the reaction. Combining equations (i) and (j) gives the following equation (k).

$$CH_3CHOH\ COOH + H_2O_2 \longrightarrow CH_3COOH + HCOOH + H_2O \quad (k)$$

Reaction (k) receives support from the fact that in practically all cases recorded in the literature, acetic acid and formic acid are found to be reaction products.

Concurrently with reaction (k) there is a reaction resulting in the liberation of hydrogen which has not been recorded in the literature but which will be shown in the Experimental Part IV to occur. Since acetaldehyde readily yields hydrogen on treatment with hydrogen peroxide, a part of the acetaldehyde formed in (i) may react as follows:

$$2CH_3CHO + H_2O_2 \longrightarrow H_2 + 2CH_3COOH \quad (l)$$

In addition to the products formed according to the reactions (k) and (l), methane is also formed. Since Fry and Payne [2] have shown that acetaldehyde yields methane on treatment with hydrogen peroxide, the following reaction concurrent with (k) and (l) is also occurring:

$$2CH_3CHO + H_2O_2 \longrightarrow CH_4 + HCOOH + CH_3COOH \quad (m)$$

Reaction (m) has not been recorded in the literature as occurring when lactic acid is treated with hydrogen peroxide, but, as will be shown in the Experimental Part IV, it does take place.

The acetic acid produced in each of the above reactions may undergo further oxidation to formic acid as follows:

$$CH_3COOH + 2H_2O_2 \longrightarrow 2HCOOH + 2H_2O \quad (n)$$

and the formic acid is readily and finally oxidized to carbonic acid

$$HCOOH + H_2O_2 \longrightarrow 2H_2O + CO_2 \quad (g)$$

The following reactions are accordingly proposed for the action of hydrogen peroxide on lactic acid. The confirmation of their occurrence is described and explained in the Experimental Part IV of this thesis.

$$CH_3CHOH\ COOH + H_2O_2 \longrightarrow CH_3COOH + HCOOH + H_2O \qquad (k)$$

$$2CH_3CHOH\ COOH + H_2O_2 \longrightarrow H_2 + 2CH_3COOH + 2HCOOH \qquad (l)$$

$$2CH_3CHOH\ COOH + H_2O_2 \longrightarrow CH_4 + CH_3COOH + 3HCOOH \qquad (m)$$

$$CH_3COOH + 4\ H_2O_2 \longrightarrow 2CO_2 + 6H_2O \qquad (n)$$

$$HCOOH + H_2O_2 \longrightarrow CO_2 + 2H_2O \qquad (g)$$

EXPERIMENTAL

In practically all of the previous investigations recorded in the literature preceding the work started in this laboratory in 1929, involving the use of hydrogen peroxide as an oxidizing agent, no attempt has been made to correlate quantitatively the extent of oxidation with the amount of hydrogen peroxide consumed. Since the determination of the mechanism of a reaction can only be interpretated on the basis of a quantitative study of all the products formed and consumed, methods have been devised by which such determinations can be made.

Knowing the initial quantity of hydrogen peroxide present in a given reaction mixture, and determining the yields of the products of oxidation, it is possible to determine the total quantity of hydrogen peroxide entering into the reaction. This total quantity of hydrogen peroxide is the sum of several fractions which is equivalent to the products formed, in conformity with one or more equations. The initial quantity of peroxide taken does not enter totally into reaction with the carbon compound, some undergoing direct decomposition into water and molecular oxygen according to the well-known equation

$$2\ H_2O_2 \longrightarrow 2\ H_2O + O_2 \qquad (z)$$

The hydrogen peroxide equivalent to this oxygen, when subtracted from the initial quantity of hydrogen peroxide, gives the "active" hydrogen peroxide or that quantity which reacts with the carbon compounds present in the reaction

mixture. If a sufficient number of the products of the reaction can be quantitatively determined, then it is possible to calculate the quantity of these separate fractions of the hydrogen peroxide which effected the one or more postulated reactions, and the sum total of these fractions of hydrogen peroxide should check the "active" hydrogen peroxide as above determined.

The hydrogen peroxide used throughout this investigation was the so-called "one hundred volume" containing approximately thirty per cent by weight of hydrogen peroxide. This was obtained free from organic preservatives and the first lot contained a small amount of sulphuric acid as prepared by the Roessler-Hasslacher Chemical Company, Niagara Falls, New York, and kindly donated by their President, Dr. H. R. Carveth. The second lot of hydrogen peroxide used was free from sulphuric acid and was prepared especially for this research under the supervision of Dr. John Payne and donated by the above company. The total acidity of the peroxide was determined by titration, using methyl orange as indicator, after decomposing a given amount of the peroxide by boiling with silica.

The initial quantity of hydrogen peroxide used in each reaction mixture was determined by titration with potassium permanganate according to the standard method (1). The hydrogen peroxide is titrated immediately before use, due to the appreciable decomposition of the peroxide at room

temperature. When not in use the peroxide is maintained at a temperature below 40°F and is stable. As a matter of fact the peroxide increases in concentration between successive titrations. The amount of hydrogen peroxide decomposing according to equation (z) is measured, gasometrically from the volume of oxygen evolved. From the difference between the amount of hydrogen peroxide originally used and the amount equivalent to the oxygen evolved, the active hydrogen peroxide would be evident.

A. Glycolic Acid

1. Procedure

The apparatus consisted of a 250 cc. Pyrex reaction flask connected through a 30 cm. upright Liebig condenser to calcium chloride drying tubes and thence to a carbon dioxide absorption U-tube of 28 cm. length and filled with Ascarite. The carbon dioxide absorption tube was connected in turn to another calcium chloride safety tube and then to a gas collecting bottle, where the gases evolved in the reaction could be collected over saturated sodium chloride solution.

The use of Ascarite for determining the carbon dioxide has a decided advantage over soda lime. In earlier runs it was found that soda lime lost moisture on the continual passage of dry gases through it, and check determinations of carbon dioxide could not be made. The saturated sodium chloride solution has the advantage that hydrogen is much less soluble therein than in water.

Through the top of the condenser a tube with a stopcock led into the reaction flask so that a stream of pure nitrogen could be passed through the apparatus and displace all air before the reaction was started. All connections were glass sealed with the exception of those between the reaction flask and the condenser, and the two joining the carbon dioxide absorption tube to the apparatus. The connection between the reaction flask and condenser was sealed with extra hard De Khotinsky cement, which formed a perfectly gas tight joint

but still allowed the easy removal of these parts from the remainder of the apparatus. The carbon dioxide tubes were originally connected to the apparatus by De Khotinsky cement but it was discovered in the early part of this investigation that this method of connection did not allow for the easy removal of this part so that it could be weighed accurately. The carbon dioxide absorption tubes were then provided with mercury seals which allowed a more convenient removal and accurate weighing and more accurate determination of the carbon dioxide. No rubber tubing or rubber stoppers were permissible since both are known to be permeable to hydrogen.

Eastman's pure glycolic acid was dried in a desiccator over phosphorus pentoxide. Samples were then analyzed by titration with standard alkali, using phenolphthalein as an indicator. The glycolic acid was found to be practically one hundred per cent pure. The above described train of apparatus was set up in duplicate, care being taken in the selection of flasks of uniform structure and smooth internal surface, to prevent as far as possible decomposition of hydrogen peroxide, which is catalyzed by rough surface. The condensers were of the same size and a uniform stream of water was circulated through them. The neck of each reaction flask was provided with a copper coil through which water circulates. This prevented softening of the cement which forms the connection between the flask and condenser, and also prevents more effective cooling.

One fourth of a mole of glycolic acid was carefully weighed into each reaction flask. Enough sulphuric acid was then introduced to make the total amount, including that initially present in the hydrogen peroxide, equal 2.16 grams. Hydrogen peroxide was then added from a calibrated burette in sufficient quantity to give the desired concentration. Distilled water was then added to make the total volume of the reaction mixture 125 cc. The flasks were then connected to the upright condenser and sealed with De Khotinsky cement; both flasks were immersed in the same constant level water bath, all air was swept out of the apparatus by a stream of nitrogen and the water bath was heated to and maintained at boiling temperature until reactions were completed, as evidenced by cessation of evolution of gases.

The water bath was provided with a gas regulating device to supply a constant flow of gas to the burners. This was necessary in order that the bath may be heated to boiling at a constant rate and thereby obtain a uniform thermal decomposition of the hydrogen peroxide. Earlier runs showed that duplicate sets of runs could not be checked without this device.

After heating for five to nine hours, the reaction appeared to be complete as no more gas was evolved. Five grams of finely divided silica were then added through the nitrogen delivery tube and gently blown into the reaction flask by means of a stream of nitrogen. In this manner no gas was

allowed to escape from the apparatus. The silica brought about the decomposition of any remaining hydrogen peroxide in the reaction flask through the catalytic action of the large surface presented. After remaining in the presence of the silica for two hours (which time has been shown to completely decompose all peroxide (1)), the gas in the apparatus was slowly displaced into the gasometer by means of nitrogen. The passage of nitrogen was slow due to the large amount of carbon dioxide remaining in the flask and condenser. The passage of nitrogen was continued until the carbon dioxide absorption tubes were cooled and ample time had been allowed for sweeping all the gas into the gasometer.

The contents of the reaction flasks were transferred to volumetric flasks and then diluted to 250 cc. Ten cc. portions were titrated with standard alkali, using phenolphthalein as an indicator. Subtracting the acidity due to the sulphuric acid initially present in the peroxide from the total acidity thus found in each case, gave the amount of alkali equivalent to unchanged glycolic acid and other acids produced in the reaction.

The reaction flask contained in addition to unchanged glycolic acid, formic acid but no glyoxylic or oxalic acid could be detected. The solution gave no test for oxalic acid by reduction of dilute potassium permanganate. No glyoxylic acid was detected by the Hopkins-Cole reaction (29). The reaction mixture showed a slight trace of aldehydes with

fuchsin reagent, but no formaldehyde could be quantitatively determined.

The formic acid was determined gravimetrically by the mercuric chloride method (30). In this method, 10 cc. aliquot portions of the reaction mixture contained in 250 cc. Erlenmeyer flasks, were neutralized with dilute sodium hydroxide, diluted to 150 cc. and 10 cc. of fifty per cent sodium acetate solution added. Two cubic centimeters of 10 per cent hydrochloric acid were added together with 25 cc. of mercuric chloride reagent, prepared by dissolving 100 grams mercuric chloride and 150 grams sodium chloride in sufficient water to make one liter. The flasks were then heated on a boiling water bath for two hours, during which time the mercuric chloride was quantitatively reduced by the formic acid, to the insoluble mercurous chloride according to the equation,

$$HCOOH + 2HgCl_2 \longrightarrow 2HgCl + 2HCl + CO_2.$$

The mercurous chloride was filtered onto a Gooch crucible, washed with hot water, alcohol and ether and dried at 105° to constant weight. Subtracting the acidity due to the formic acid and the sulphuric acid from the total acidity in each case gave the amount of glycolic acid remaining at the completion of the reaction.

The increase in weight of the Ascarite bulbs gave the amount of carbonic acid produced.

Having determined the glycolic acid remaining unattacked, the amount of formic acid produced and remaining unattacked, and the yield of carbonic acid, the gas collected

during the reaction was analyzed for possible gaseous products of the reaction.

The yield of gaseous products consisted only of oxygen and hydrogen. The presence of hydrogen indicates the occurrence of reaction (f) noted in Part III. The amount of hydrogen peroxide decomposing into molecular oxygen and water during the reaction was then determined from a measure of the oxygen collected in each case. The percentage of oxygen in the gas was ascertained by use of the standard Hempel gas analysis apparatus, the oxygen being absorbed over phosphorus. From the contraction in volume, the percentage of oxygen and hence the total volume collected was readily obtained. This volume was reduced to standard conditions of temperature and pressure, and its hydrogen peroxide equivalent calculated. Deducting this calculated amount of hydrogen peroxide from the initial quantity of hydrogen peroxide used, gave the amount of active hydrogen peroxide. The hydrogen was determined by the pipette explosion method, and the number of moles corresponding to the total hydrogen liberated was calculated.

2. Data

The results obtained in duplicate runs involving 1/8, 1/4, 1/2, 3/4, and one mole of hydrogen peroxide with 1/4 mole of glycolic acid are shown in Table I, which records as moles the quantities of reacting substances and the products formed.

Table II gives the percentage of hydrogen peroxide equivalent to the products of the reaction as calculated from the several equations for the proposed reactions. The amounts of peroxide reacting according to the various equations are separately recorded and also totaled in order to account for the total quantity of hydrogen peroxide used in each of the duplicate sets of runs.

Table III records separately the percentage amounts of glycolic acid reacting according to the various equations as well as the total percentage amount of glycolic acid accounted for.

Table IV records the percentage yields of carbon dioxide equivalent to reactions (g), (d), and (f).

Table V records the qualitative tests performed on the reaction mixture and the conclusions drawn.

Table I

MOLAR QUANTITIES OF HYDROGEN PEROXIDE AND GLYCOLIC ACID USED AND OF PRODUCTS FORMED

Run	Initial H_2O_2 moles	Initial $CH_2OHCOOH$ moles	HCOOH found moles	H_2CO_3 found moles	H_2 found moles	O_2 found moles	$CH_2OHCOOH$ found moles
Ia	0.1250	0.2500	0.0142	0.0267	0.0024	0.0410	0.229
Ib	.1250	.2500	.0141	.0268	.0023	.0408	.225
IIa	.2500	.2500	.0271	.0566	.0055	.0801	.2044
IIb	.2500	.2500	.0246	.0563	.0047	.0799	.2106
IIIa	.5000	.2500	.0353	.1000	.0083	.1786	.1787
IIIb	.5000	.2500	.0339	.0979	.0087	.1726	.1781
IVa	.7500	.2500	.0374	.153	.0134	.2605	.158
IVb	.7500	.2500	.0363	.154	.0132	.2622	.156
Va	1.0000	.2500	.0399	.194	.0138	.3489	.1270
Vb	1.0000	.2500	.0416	.208	.0161	.3447	.1219

Table II

PERCENTAGE QUANTITIES OF HYDROGEN PEROXIDE USED EQUIVALENT TO YIELDS OF PRODUCTS FORMED

Run	1 % H_2O_2 used H_2 Eq.(f)	2 % H_2O_2 used HCOOH Eq.(e)	3 % H_2O_2 used H_2CO_3 Eq.(g)	4 % H_2O_2 used O_2 Eq.(z)	5 Total % H_2O_2 used Eqns. (f) (e)(g)(z)
Ia	5.759	25.44	5.200	65.60	102.00
Ib	5.520	25.28	5.040	65.28	101.12
IIa	6.601	24.72	5.921	64.07	101.31
IIb	5.640	24.80	6.322	63.93	100.69
IIIa	4.979	20.40	6.460	71.44	103.28
IIIb	5.223	19.398	6.398	69.04	100.06
IVa	5.359	18.13	7.68	69.45	100.62
IVb	5.279	18.40	7.40	69.90	100.98
Va	4.14	17.88	7.71	69.78	99.51
Vb	4.83	18.56	8.34	68.94	100.67

Table III

PERCENTAGE QUANTITIES OF GLYCOLIC ACID USED
EQUIVALENT TO YIELDS OF PRODUCTS FORMED

Run	1 % $CH_2OHCOOH$ used H_2 Eqn. (f)	2 % $CH_2OHCOOH$ used HCOOH Eqn. (e)	3 % $CH_2OHCOOH$ used Unchanged $CH_2OHCOOH$	4 Total % $CH_2OHCOOH$ used Eqns. (f) and (e).
Ia	1.920	6.360	91.60	99.88
Ib	1.841	6.322	90.01	98.17
IIa	4.400	12.36	81.75	98.51
IIb	3.760	12.40	84.24	100.40
IIIa	6.640	20.40	71.48	98.52
IIIb	6.960	19.40	71.24	97.60
IVa	10.72	27.20	63.22	101.14
IVb	10.56	27.60	62.44	100.60
Va	11.04	35.76	50.81	97.61
Vb	12.88	37.12	48.75	98.75

Table IV

PERCENTAGE YIELDS OF CARBON DIOXIDE
EQUIVALENT TO REACTIONS (g) (d) and (f)

Run	1 % CO_2 reaction (g)	2 % CO_2 reaction (d)	3 % CO_2 reaction (f)
Ia	23.22	58.80	17.98
Ib	23.89	58.96	17.17
IIa	25.97	54.61	19.44
IIb	28.24	55.07	16.69
IIIa	32.40	51.00	16.60
IIIb	32.68	49.53	17.77
IVa	37.90	44.58	17.52
IVb	38.32	44.54	17.14
Va	39.69	46.08	14.22
Vb	39.90	44.61	15.48

Table V

Test	Results of Test on Reaction Mixture	Conclusions
Hopkins-Cole	Negative	No glyoxylic acid
Schiffs reagent	Slight test	Trace of aldehyde
Resorcinol test	Negative	No formaldehyde
Phenol test	Negative	No formaldehyde
Permanganate	No decolorization	No glyoxylic acid or only in traces.

3. Summary

The results given in Table I verify the prediction made in Part III (A) that the reaction between glycolic acid and hydrogen peroxide should result in the liberation of hydrogen from the intermediately produced glyoxylic acid, according to reaction (f). The results of the tests on the reaction mixture do not definitely eliminate the occurrence of reaction (b). Both glyoxylic acid and formaldehyde are extremely susceptible to oxidation and it is therefore doubtful if either could be detected as an intermediate. However, in view of the fact that acetaldehyde has been shown to yield hydrogen without the production of formaldehyde, and that glyoxylic acid is a more logical oxidation product of glycolic than is formaldehyde, reaction (b) is eliminated from the reaction mechanism. The results further show that no oxalic is produced and therefore reaction (c) does not take place.

In view of the results obtained, the following equations, noted separately in Part III, represent the possible reactions occurring along with the decomposition of hydrogen peroxide into molecular oxygen and water.

$$CH_2OH\ COOH + 2\ H_2O_2 \longrightarrow 3\ H_2O + CO_2 + H\ COOH \qquad (e)$$

$$2\ CH_2OH\ COOH + 3\ H_2O_2 \longrightarrow 4\ H_2O + 2CO_2 + 2H\ COOH + H_2 \qquad (f)$$

$$H\ COOH + H_2O_2 \longrightarrow 2\ H_2O + CO_2 \qquad (g)$$

$$2\ H_2O_2 \longrightarrow H_2O + O_2 \qquad (z)$$

Formic acid may be produced by either reaction (e) or (f), or both. If reaction (f) is the only one occurring, there should be formed one mole of hydrogen for every two moles of formic acid. Furthermore, three moles of hydrogen peroxide should be required for the completed reaction. From Table I it is evident, however, that in no case was this ratio found to exist. In all instances the ratio of formic acid to hydrogen is seen to be greater than two to one, indicating that reaction (e) is also taking place. Assuming that reactions (e) and (f) proceed simultaneously, the extent of the occurrence of reaction (f) may be directly calculated from the yield of hydrogen, and hence the quantity of formic equivalent to this yield of hydrogen, when subtracted from the total yield of formic acid, gives the quantity of formic acid produced according to equation (e) and thus indicates the extent of the occurrence of reaction (e).

The formic acid formed according to equation (e) equals the formic acid experimentally determined minus that produced by reaction (f) plus that oxidized according to reaction (g). Therefore to establish the extent of the occurrence of reaction (e), the extent of the occurrence of reaction (g) must be determined. From the yield of formic acid, hydrogen, and carbon dioxide, this may be accomplished as follows:

Let $x = CO_2$ (e) and $a = HCOOH$ (e)

$y = CO_2$ (g) $b = HCOOH$ (g)

$z = CO_2$ (f) $c = HCOOH$ (f)

$z = 2 \times H_2$ $c = 2 \times H_2$

$\frac{a}{x} = \frac{1}{1}$ $\frac{b}{y} = \frac{1}{1}$

From these equations (x) (y), (a) and (b) may be calculated, and hence the extent of the occurrence of reactions (e) and (g).

Table II records the extent of the occurrence of reactions (e) (f) and (g) in terms of percentage of hydrogen peroxide. The last column, 5, in each case indicates the total percentage of the initial amount of hydrogen peroxide used in the reaction mixture, (a part of which effects oxidation according to (e) (f) and (g), while the remainder suffers simple decomposition into oxygen and water), accounted for on the basis of these reactions from the yield of oxidation products obtained.

Column 5, Table II, indicates the validity of the reactions postulated as well as a check on the accuracy of the experimental methods. It will be noted that within the limits of experimental error, these values check very closely with the initial quantities of hydrogen peroxide used. This is direct evidence of the fact that the reactions postulated are occurring to the extent calculated from the yields of the oxidation products.

Table III records the extent of the occurrence of reactions (e) (f) and (g) in terms of percentage of glycolic acid used. This is made possible through a determination of the amount of glycolic acid which remained at the end of the reaction. Column (5) of Table III indicates the total percentage of the initial amount of glycolic acid used in the reaction mixture as accounted for on the basis of reactions

(e) (f) and (g) from the respective yields of oxidation products obtained. The data given in Column (5), Table III, is very significant in that they give a further check on the type of reactions occurring, and also on the accuracy of the experimental methods. It will also be noted, as in the case with the calculations based on hydrogen peroxide, that the sum of the quantities of reacting glycolic acid agree well with the initial quantity of glycolic acid used.

Regardless of the concentration of hydrogen peroxide used in the several runs, the results shown in Tables I, II, and III show conclusively that reactions (e) and (f) are always concurrent. The data in Table III moreover show that reaction (f) is favored by low concentration of hydrogen peroxide.

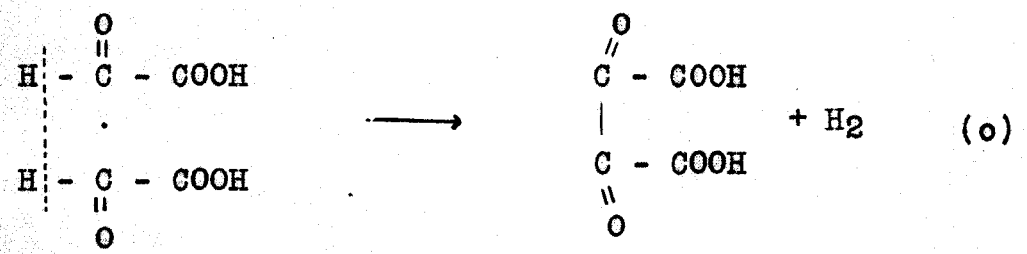
The secondary reaction (g) proceeds only to a small per cent at low concentration of hydrogen peroxide, but increases with increasing concentration of hydrogen peroxide as would be expected.

Table IV, Column 3, indicates that reaction (f) remains practically constant in extent of occurrence. The slight decrease in occurrence at higher concentration of hydrogen peroxide shows the increased tendency of hydrogen peroxide to act as an oxidizing agent in concentrated solutions. With increasing concentrations of hydrogen peroxide reaction (g) increases in extent, which would be expected from the susceptibility of formic acid to oxidation.

The liberation of hydrogen on treatment of glycolic acid with hydrogen peroxide involves the preliminary formation of glyoxylic acid according to equation (a),

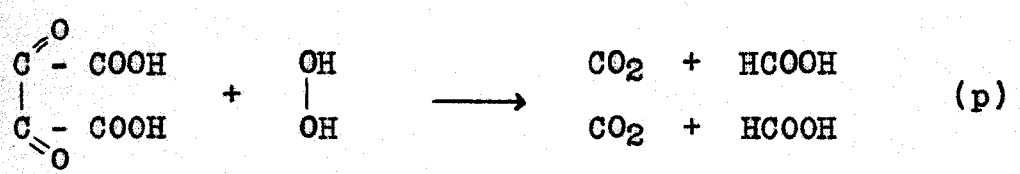
$$CH_2OHCOOH + H_2O_2 \longrightarrow CHOCOOH + 2H_2O.$$

Through the catalytic action of hydrogen peroxide, one molecule of hydrogen is liberated from two molecules of glyoxylic acid with the intermediate formation of dihydroxy tartaric acid. The liberation of hydrogen may be pictured as follows:



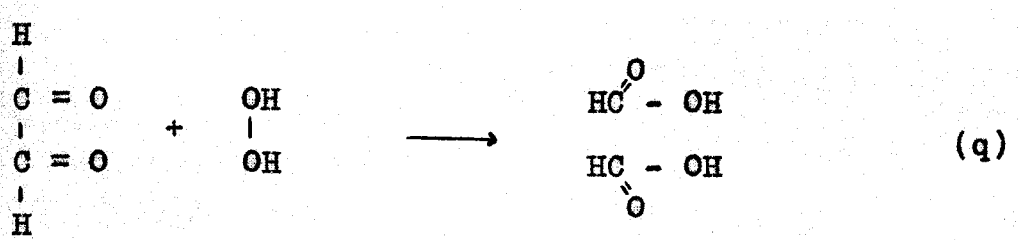
(o)

The dihydroxy tartaric acid thus formed undergoing perhydrolysis with the liberation of carbon dioxide and formation of formic acid.



(p)

Or perhaps the dihydroxy tartaric acid first decomposes into carbon dioxide and glyoxal, the latter undergoing perhydrolysis to formic acid, as follows, would effect the same end.



(q)

The summation of equations (o) and (p) gives equation (f).

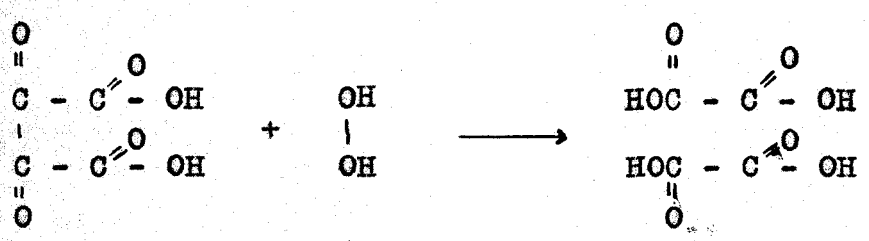
$$2\ CHOCOOH + H_2O_2 \longrightarrow H_2 + 2CO_2 + 2HCOOH \qquad (f)$$

This reaction is perfectly analagous to the liberation of hydrogen from formaldehyde and verifies the conclusions of Fry and Payne [2] that the liberation of hydrogen involves two molecules of the aldehyde and not one, which has been expressed in the following summation equation:

$$2HCHO + H_2O_2 \longrightarrow 2HCOOH + H_2$$

That no dihydroxy tartaric acid or glyoxal was isolated may be due to the fact that the speeds of the perhydrolysis reaction (p) and (q) are greater than the reaction (o) and the decomposition of dihydroxy tartaric into carbon dioxide and glyoxal.

No oxalic acid was found, as anticipated through the perhydrolysis of dihydroxy tartaric acid without the elimination of carbon dioxide as follows:



That no oxalic acid was found supports the previous statement that the dihydroxy tartaric acid is converted very rapidly through the loss of carbon dioxide into glyoxal which then undergoes perhydrolysis.

B. Lactic Acid

1. Procedure

The reaction between lactic acid and hydrogen peroxide occurs at such a rate that it can be studied in the same manner as the previously described reactions with glycolid acid. It was found on conducting preliminary runs that the quantity of sulphuric acid previously used (2.16 grams) increased the decomposition of lactic acid into acetaldehyde and formic acid by hydrogen peroxide, to such an extent as to be practically the only reaction occurring. However, in the basence of sulphuric acid the decomposition is very slight and the oxidation proceeds smoothly. It was necessary to modify the apparatus slightly, due to the fact that some acetaldehyde is produced, which is not further oxidized. Being very volatile, it passed through the condenser and collected in the ascarite tubes, thereby interfering with the carbonic acid determinations.

Since the acetaldehyde escaping oxidation is very small, no attempt was made to estimate it but only to prevent it from entering the soda lime tubes. This difficulty was overcome by introducing into the apparatus, between the condenser and the first calcium chloride tube, a Millikan wash-bottle containing eighty-five per cent phosphoric acid. (Preliminary experiments had shown that phosphoric acid is able to absorb completely very large quantities of acetaldehyde).

Since the large sized mercury sealed Ascarite tubes used in the experiments with glycolic acid were difficult both

to handle and to weigh, two smaller Ascarite bulbs were substituted. These were connected to the calcium chloride tubes on both sides with extra thick walled tubing. The tubing was wired with heavy copper wire and coated with Duco cement which prevents the escape of any hydrogen through the tubing.

The lactic acid used in the investigation, Merck's C.P. grade, contained a trace of hydrochloric acid, sulphuric acid, and also a trace of iron. The lactic acid was analyzed by titration with standard alkali, using phenolphthalein as an indicator, and also by the oxidation method (31). Concordant results were obtained.

The volume necessary to yield .25 moles of lactic acid calculated from the titration value was introduced into the reaction flask along with sufficient water to make the total volume equal to 125 cc.

A series of five duplicate runs were conducted using one fourth mole of lactic acid in every case, and varying the amounts of hydrogen peroxide from 1/8, 1/4, 1/2, 3/4 to one mole, respectively. The speed of the decomposition of the hydrogen peroxide is very slow, indicating the formation of stable compounds of a peroxide nature. The reactions were allowed to proceed for twelve hours, at the end of which time the oxidations were practically complete. This was shown by the fact that no more gas was passing through the Millikan wash bottle. On the completion of the reaction, five grams of finely divided silica was introduced into the reaction flasks. There was no evolution of oxygen, which shows that no free

hydrogen peroxide remained. However, on removing the contents of the reaction flask and testing it for peroxide, a pronounced positive reaction was noted. This fact further supports the previous statement that some of the hydrogen peroxide is present in the reaction mixture as a fairly stable peroxide of the organic compounds present.

An attempt was made to locate a substance which would catalytically decompose the peroxides remaining at the end of the reaction into oxygen and the organic compound in order that a determination of the active hydrogen peroxide could be made. Manganese dioxide was the only compound found that would successfully accomplish this reaction, but it could not be used since it would oxidize the formic acid remaining in the reaction flask at the end of the reaction.

It therefore became necessary to estimate the hydrogen peroxide present in the form of organic peroxides at the end of the reaction by some other means. This was successfully accomplished by adding an aliquot of the reaction mixture to a concentrated (about twenty per cent solution) potassium iodide solution in thirty per cent acetic acid and titrating the liberated iodine according to the method described by Clover and Houghton (32). The quantity of hydrogen peroxide held as organic peroxides as thus determined was added to the quantity of hydrogen peroxide equivalent to the oxygen liberated. This total quantity of hydrogen peroxide could then be subtracted from the initially used quantity of hydrogen peroxide, thereby revealing the "active" quantity of hydrogen

peroxide used up in the several reactions.

At the end of the reaction, the reaction-mixture was found to contain formic, acetic, and unchanged lactic acids. No pyruvic acid could be detected by the Hopkins Cole reaction (29) nor did the reaction mixture yield a positive phenylhydrazone test for pyruvic acid.

The reaction mixture was diluted to 250 cc. and ten cc. portions titrated with standard alkali. The formic acid was determined by the mercuric chloride method (30) in another ten cc. aliquot of the reaction mixture. The unchanged lactic acid was determined in a third aliquot by the oxidation method (31). Subtracting the acidity due to the formic and lactic acids from the total acidity gives the acidity due to the acetic acid formed in the reaction. It should be pointed out that the determination of formic and lactic acid and hence acetic acid involve considerable error. Preliminary determination of formic acid in the presence of lactic acid indicated no error, but when peroxides of organic acids are also present the decomposition of lactic acid into acetaldehyde and formic acid is increased. Therefore the formic acid determined by the mercuric chloride method represents not only that produced in the reaction but also a small amount formed by the decomposition of lactic acid during the determination. Furthermore, the methods described in the literature (33), (34), (35), & (31) for the determination of lactic acid have been tested on solutions containing known

weights of lactic acid, and found to be unreliable. The method of oxidation with potassium permanganate (31) was chosen because it involves the least error, about three to five per cent.

The gaseous products were analyzed for hydrogen, oxygen, methane, and carbon monoxide. The latter was absent. Oxygen was quantitatively determined in the usual manner, while the hydrogen and methane was obtained together by explosion and subsequent removal of the carbon dioxide thus produced, by absorption in sodium hydroxide. Since no carbon monoxide was present, the decrease in volume on absorption in sodium hydroxide gave the volume of methane in the sample. Two thirds of the value obtained by subtracting twice the volume of methane from the decrease in volume on explosion gave the volume of hydrogen in the sample. The total volume of hydrogen and methane obtained was calculated and after reduction to standard conditions of temperature and pressure, was converted to molar quantities.

That compound producing carbon dioxide was only methane was proven by determining the amount of oxygen consumed in the explosion. This amount agreed with that calculated for a mixture of hydrogen and methane.

2. Data

The results of five duplicate runs are given in Table VI. Columns (1) and (2) record the initial quantities of hydrogen peroxide and lactic acid used. Columns (3)-(10) record the molar yields of the various reaction products.

Table VII records the percentage of the total hydrogen peroxide calculated as necessary on the basis of the proposed reactions, to account for the yield of the various products. Thus Column (1) indicates the percentage of the initial amount of hydrogen peroxide necessary to account for the extent of reaction (l), as measured by the yield of hydrogen. Column (6) gives likewise the percentage of the initial quantity of hydrogen peroxide necessary to account for the extent of reaction (m), as measured by the yield of methane. Column (2) shows the percentage of the initial quantity of hydrogen peroxide necessary to bring about the oxidation of the "active" lactic acid, that is, the amount which actually entered into reaction with the hydrogen peroxide. This calculation is made possible by a determination of the lactic acid remaining at the completion of the reaction. Subtracting from the "active" lactic acid that quantity equivalent to reactions (l) and (m) give the amount reacting according to equation (k) and hence the calculation of the percentage of the initial amount of hydrogen peroxide effecting reaction (k). In Column (3) is given the percent of the hydrogen peroxide initially used which would be necessary to oxidize a part of the acetic acid by equation (n). This quantity of acetic acid oxidized

equals that produced by reactions (k), (l), and (m) minus the amount remaining at the completion of the reaction. Column (4) records the percentage of the initial hydrogen peroxide necessary to bring about the oxidation of a part of the formic acid according to reaction (g). This quantity of formic acid, as in the case of acetic acid, is that amount produced by reactions (k), (l), and (m) minus that amount remaining at the completion of the reaction. In Column (5) is recorded the percent of the initial amount of hydrogen peroxide which is equivalent to the organic peroxides remaining at the completion of the reaction, while Column (7) gives the percent of the initial quantity of peroxide decomposing into molecular oxygen and water.

The last column, (8), is a summation of the percentages given in Columns 1-7 inclusive, and should approximate 100% if all reaction products have been correctly accounted for in conformity with the equations for the proposed reactions.

Table VI

MOLAR QUANTITIES OF HYDROGEN PEROXIDE AND LACTIC ACID USED AND OF PRODUCTS FORMED

	1.	2.	3.	4.	5.	6.	7.	8.	9.	10.
Run	Initial H_2O_2 mole	Initial $CH_3CHOHCOOH$ mole	HCOOH found mole	H_2CO_3 found mole	CH_3COOH found mole	$CH_3CHOHCOOH$ found mole	H_2 found mole	O_2 found mole	CH_4 found mole	Peroxides found mole
Ia	0.1250	0.2500	0.0112	0.0544	0.0538	0.2018	0.0000	0.0020	0.0019	0.0281
Ib	.1250	.2500	.0113	.0560	.0537	.2017	0.0000	.0020	.0019	.0243
IIa	.2500	.2500	.0164	.1139	.0984	.1498	.0007	.0030	.0033	.0570
IIb	.2500	.2500	.0194	.1145	.1018	.1537	.0007	.0038	.0041	.0579
IIIa	.5000	.2500	.0254	.1983	.1505	.0794	.0024	.0212	.0000	.0616
IIIb	.5000	.2500	.0216	.1875	.1516	.0859	.0029	.0243	.0000	.0840
IVa	.7500	.2500	.0261	.2716	.1769	.0426	.0027	.0655	.0000	.0901
IVb	.7500	.2500	.0272	.2718	.1755	.0416	.0026	.0624	.0000	.0906
Va	1.0000	.2500	.0309	.3090	.1909	.0184	.0024	.1035	.0000	.1233
Vb	1.0000	.2500	.0270	.3086	.1954	.0197	.0023	.1068	.0000	.1244

Table VII

PERCENTAGE QUANTITIES OF HYDROGEN PEROXIDE USED EQUIVALENT TO YIELD OF PRODUCTS FORMED

Run	1. % H_2O_2 used H_2 Eq.(l)	2. % H_2O_2 used $CH_3CHOHCOOH$ Eq.(k)	3. % H_2O_2 used CH_3COOH Eq.(n)	4. % H_2O_2 used HCOOH Eq.(g)	5. % H_2O_2 used peroxides	6. % H_2O_2 used CH_4 Eq.(m)	7. % H_2O_2 used O_2 Eq.(z)	8. Total % H_2O_2 used
Ia	0.00	35.52	0.00	31.12	22.51	1.52	3.20	93.87
Ib	.00	35.60	.00	31.12	19.86	1.52	3.20	91.20
IIa	.28	36.88	.00	34.84	22.81	1.32	2.43	98.56
IIb	.28	34.68	.00	32.41	23.14	1.64	3.10	95.25
IIIa	.47	33.18	16.08	29.04	12.32	.00	8.48	99.57
IIIb	.57	31.68	10.00	28.50	16.79	.00	9.73	96.27
IVa	.36	26.92	16.27	24.17	12.01	.00	17.47	97.20
IVb	.35	27.09	17.54	24.16	12.07	.00	16.63	97.84
Va	.24	22.67	16.28	26.07	12.33	.00	20.70	92.29
Vb	.23	22.58	13.96	26.34	12.44	.00	21.36	90.91

Conclusions

The production of both hydrogen and methane as products of the action of hydrogen peroxide upon lactic acid was predicted through the extension of the perhydrolysis mechanism of reaction, as in the case of formaldehyde and acetaldehyde to lactic acid. It is assumed that lactic acid is just decomposed into formic acid and acetaldehyde, and the latter undergoes reactions with hydrogen peroxide with the liberation of hydrogen and methane. This assumption is supported by the fact that acetaldehyde may be detected in the reaction flasks at any time and a small quantity escapes oxidation which passes out of the reaction flask and is collected in eighty five per cent phosphoric acid.

From the results given in Table V and the fact that neither formic nor acetic acids yield hydrogen or methane on oxidation to carbonic acid (2), the following equations noted in Part III represent the possible reactions occurring when lactic acid is oxidized by means of hydrogen peroxide.

$$CH_3CHOHCOOH + H_2O_2 \longrightarrow CH_3C(=O)OH + HCOOH + H_2O \quad (k)$$

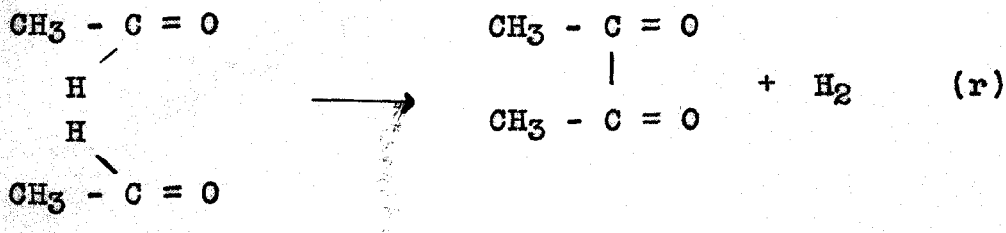
$$CH_3COOH + 4H_2O_2 \longrightarrow 2CO_2 + 6H_2O \quad (n)$$

$$HCOOH + H_2O_2 \longrightarrow CO_2 + 2H_2O \quad (g)$$

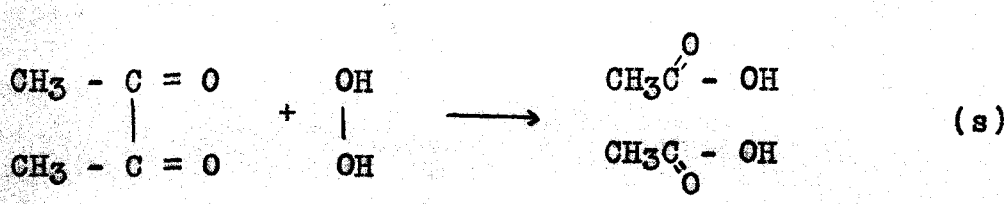
$$2CH_3CHOHCOOH + H_2O_2 \longrightarrow H_2 + 2CH_3COOH + 2HCOOH \quad (l)$$

$$2CH_3CHOHCOOH + H_2O_2 \longrightarrow CH_4 + 3HCOOH + CH_3COOH \quad (m)$$

The liberation of hydrogen results from the intermediate formed acetaldehyde and may be pictured as follows:



(r)



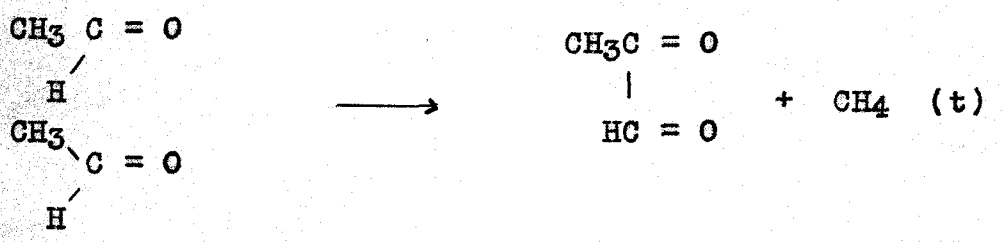
(s)

The summation of equations (r) and (s) gives equation (1)

$$2CH_3CHO + H_2O_2 \longrightarrow H_2 + 2CH_3COOH \qquad (1)$$

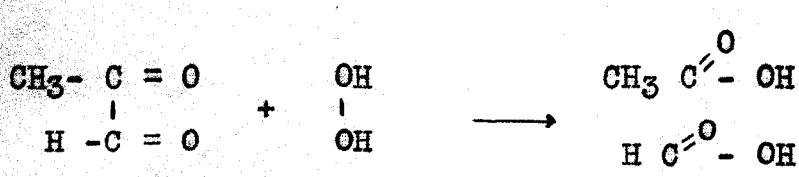
This reaction is perfectly analogous to the liberation of hydrogen from formaldehyde and has been established by Fry and Payne (3). The catalytic action of the hydrogen peroxide brings about a joining of two molecules of acetaldehyde with a liberation of one molecule of hydrogen. The diacetyl thus formed undergoes perhydrolysis as shown in equation (p), giving two molecules of acetic acid.

The liberation of methane is pictured similarly, with methane splitting our instead of hydrogen.



(t)

The methyl glyoxal thus formed undergoing perhydrolysis into one molecule of acetic acid and one molecule of formic acid as follows:



(u)

The summation of equations (t) and (u) give equation (m)

$$2CH_3CHO + H_2O_2 \longrightarrow CH_4 + HCOOH + CH_3COOH \quad (m)$$

Concurrent with the above reactions is the direct oxidation of acetaldehyde to acetic acid according to equation (k)

(k)

Reaction (k) is the predominating reaction at all concentrations of hydrogen peroxide as shown in Table VII. The extent of its occurrence decreases with increasing concentrations of hydrogen peroxide which is in conformity with the results of Fry and Payne (2).

Increase in concentration of hydrogen peroxide produces a decrease in extent of reaction (g) which is again in conformity with the results obtained by Fry and Payne (2) on the action of hydrogen peroxide on acetaldehyde.

A further proof that the proposed mechanism of the oxidation of lactic acid is correct, is found in a consideration of reactions (l) and (m). Reaction (l) increases to a maximum and then decreases, while reaction (m) decreases markedly with increasing peroxide concentration. (There is probably a very small amount of methane formed at concentration of hydrogen peroxide greater than .25 moles but not sufficient quantity to be determined). This again agrees with the results obtained,

from the action of hydrogen peroxide on acetaldehyde, by Fry and Payne (2).

It will be noted from Column (8) in Table VII that the total hydrogen peroxide accounted for from the yield of oxidation products only approximates 100% in three of the runs. This deviation may be accounted for by the fact that there is no accurate method for the determination of lactic acid. Results of preliminary blank runs on samples containing known amounts of lactic acid by the oxidation methods showed the determination to be uniformly 3-5% low. The acetic acid is determined by subtracting the acidity due to the unchanged lactic acid, and the formic acid produced in the reaction from the total acidity, that remaining being due to acetic acid. Since the determination of lactic acid is low, the corresponding yield of acetic acid will be high, and the extent of the occurrence of reaction (n) calculated from the yield of acetic acid will be less than the reaction which actually takes place. Therefore the per cent of the initial quantity of hydrogen peroxide equivalent to reaction (n) is always low and consequently the total per cent of hydrogen peroxide accounted for is correspondingly low.

This fact, however, has no effect upon the interpretation of the mechanism of the reaction for the liberation of hydrogen and methane.

GENERAL SUMMARY AND CONCLUSIONS

The extension of the quantitative study of the action of hydrogen peroxide on simple carbon compounds to glycolic and lactic acids, with the object of further verifying the perhydrolysis mechanism of reaction, has led to the postulation of a variety of reactions. The equations have been verified with respect to the products obtained, and the quantities of hydrogen peroxide participating in the reactions. The general summary, extension of the perhydrolysis mechanism of reaction, and conclusions are as follows.

A. The action of hydrogen peroxide on glycolic acid in slightly acid media at 100°C has been studied, and the following reactions have been proposed and verified.

$$CH_2OHCOOH + 2H_2O_2 \longrightarrow 3H_2O + CO_2 + HCOOH \qquad (e)$$

$$2CH_2OHCOOH + 3H_2O_2 \longrightarrow 4H_2O + 2CO_2 + 2HCOOH + H_2 \qquad (f)$$

$$HCOOH + H_2O_2 \longrightarrow 2H_2O + CO_2 \qquad (g)$$

Reactions (e) and (f) are concurrent, each being followed to a greater or lesser extent, depending upon the hydrogen peroxide concentration, by reaction (g). Reaction (f) is favored by low concentration of hydrogen peroxide, while reaction (g) increases in extent with increasing concentrations of hydrogen peroxide.

The perhydrolysis mechanism of reaction has been extended to glycolic acid to explain the liberation of hydrogen from intermediately formed glyoxylic acid.

B. In the reaction between lactic acid and hydrogen peroxide the following reactions have been shown to be concurrent.

$$CH_3CHOHCOOH + H_2O_2 \longrightarrow CH_3C(=O) - OH + HCOOH + H_2O \qquad (k)$$

$$CH_3COOH + 4H_2O_2 \longrightarrow 2CO_2 + 6H_2O \qquad (n)$$

$$HCOOH + H_2O_2 \longrightarrow CO_2 + 2H_2O \qquad (g)$$

$$2CH_3CHOHCOOH + H_2O_2 \longrightarrow H_2 + 2CH_3COOH + 2HCOOH \qquad (l)$$

$$2CH_3CHOHCOOH + H_2O_2 \longrightarrow CH_4 + 3HCOOH + CH_3COOH \qquad (m)$$

The scheme of the reaction has been explained on the basis of the decomposition of lactic acid into acetaldehyde and formic acid with the subsequent reactions of hydrogen peroxide on these compounds. Reaction (k) is the predominate reaction at all concentrations while reactions (l) and (m) are favored by low concentrations of hydrogen peroxide.

The perhydrolysis mechanism of reaction has been extended to the action of hydrogen peroxide on lactic acid, to not only explain the normal reactions which would be expected but also the reactions resulting in the liberation of hydrogen and methane.

C. The new type of reaction mechanism, termed perhydrolysis, proposed by Fry and Payne (2) to explain some of the normal and also apparently anomalous reactions of hydrogen peroxide, has been shown to be capable of extension to higher carbon compounds. It is hoped that other compounds will be studied from this point of view in the future.

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