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THE OXIDATION OF ALPHA- AND BETA-HYDROXYBUTYRIC ACIDS WITH HYDROGEN PEROXIDE

BY EDGAR J. WITZEMANN

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In this paper the results of the oxidation of α - and β -hydroxybutyric acids are described, with special reference to the results of the oxidation of butyric acid, described in the two preceding papers.

Introduction

The results in the two preceding papers indicate that butyric acid probably undergoes two distinct types of oxidation in the phosphate-peroxide systems. One of these types has long been known to occur in the body and was reproduced *in vitro* by Dakin¹ and Neubauer,² that is, the so-called β -oxidation by which the "acetone bodies" are formed. The other type appears to differ in some important respect. Dakin has shown long ago that when hydrogen peroxide acts upon ammonium butyrate products attributed to an α -oxidation are also formed, and the results in this case were such that they conformed with the commonly accepted idea that when a fatty acid is oxidized on the alpha carbon atom it is shortened one carbon atom at a time. Such a behavior was of little or no value in aiding in the interpretation of biological oxidation of fatty acids and naturally led to emphasis of the β -oxidation that also occurs under these conditions, which does permit an explanation of the known facts. The current views on the subject can be briefly summarized by stating that unless it can be shown that products of α -oxidation of fatty acids can lose two carbon atoms at a time their importance as intermediate stages in fatty acid oxidation cannot be great. The data reported in this paper on the behavior of α -hydroxybutyric acid, as well as butyric acid in the phosphate-peroxide system, show that a process of α -oxidation with the loss of two carbon atoms does exist. Hence, the common arguments³ against considering α -oxidation as a possible bodily process seem to be invalidated, if it can eventually be shown that the phenomena here reported have a general significance.

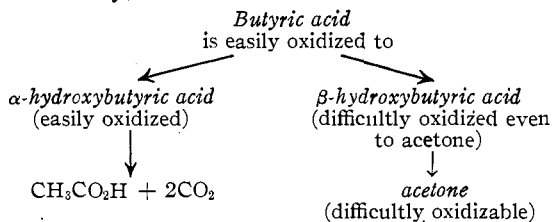
α -Hydroxybutyric acid is easily oxidized in the phosphate-peroxide systems used and gives rise to the formation of acetic acid and two molecules of carbon dioxide, instead of propionic acid and one molecule of carbon dioxide. On the other hand β -hydroxybutyric acid is practically not attacked, except to be oxidized to some extent to the corresponding β -

¹ Dakin, *J. Biol. Chem.*, **4**, 77 (1908).

² Neubauer, *Deutsch. Arch. klin. Med.*, **95**, 211 (1908-1909).

³ Dakin, "Oxidations and Reductions in the Animal Body," Longmans, Green and Co., London, 2nd. ed., 1922, p. 45.

keto acid, which is recovered as acetone. In other words, α -hydroxybutyric acid is an easily oxidized compound while β -hydroxybutyric acid is very resistant to oxidation in these phosphate-peroxide systems. All things considered, the oxidation of butyric acid in these systems resembles that of α -hydroxybutyric acid more, from the standpoint of facility, than it does that of β -hydroxybutyric acid. Moreover, the main portion of the oxygen consumed in butyric acid oxidation is used in producing the same products that are obtained in the oxidation of α -hydroxybutyric acid. The results also show that in the case of α -hydroxybutyric acid, and in the portion of the butyric acid that undergoes extensive oxidation, the fatty acid chain is shortened by two carbon atoms, before we obtain a fragment that is more resistant to oxidation than the parent molecule. We have not proved directly that the intermediate mechanism of the oxidation of α -hydroxybutyric acid and of butyric acid is the same in this case, nor have we proved that α -hydroxybutyric acid is an intermediate product in the oxidation of this portion of the butyric acid, but the results suggest that such an assumption is not far from the truth. If such an assumption is permissible we could represent the oxidation of butyric acid in these systems schematically, thus:



This diagram represents the essential facts as established. The only assumption contained in it is that α - and β -hydroxybutyric acids are the first intermediate products; as a matter of fact, the corresponding keto acids may be formed at once from butyric acid.

From the biological viewpoint the results in these papers are of importance for several reasons.

1. The results so far obtained suggest that products of α -oxidation of fatty acids are of importance in the chemical manipulation of fatty acids in the organism, and that this may occur in a way that has not heretofore received much experimental support. This suggestion is also supported by the well-known importance of the alpha carbon atom in lactic acid and in the amino acids.

2. There is nothing known to the writer that would diminish the known importance of β -oxidation in the organism. The results here given, however, suggest that α -oxidation of the type here described may exist side by side with β -oxidation in the organism and that its existence will be more difficult to prove owing to the greater reactivity of the products of α -oxidation.

3. Owing to the well-known fundamental importance of various phosphoric acid compounds in organisms, these results are also of significance in suggesting ways in which these groups may influence the process of oxidation in the organism by virtue of catalytic effects of the phosphoric acid radical.

1. Test of the Oxidation of *l*, β -Hydroxybutyric Acid in the Phosphate Mixture

The *l*, β -hydroxybutyric acid used in these experiments was isolated from diabetic urine and purified as the calcium-zinc double salt. A solution of 2 g. of calcium-zinc *l*, β -hydroxybutyrate in hot water was treated with a slight excess of sodium carbonate. The mixed carbonates precipitated were filtered off. The solution was treated with hydrochloric acid a drop at a time until neutral to litmus and was then made up to 100 cc. Four experiments were set up.

(a) 25.6 cc. of 0.33 *M* dipotassium phosphate soln., 6.4 cc. of 0.33 *M* monopotassium phosphate soln., 3 cc. of water, 20 cc. of the sodium *l*, β -hydroxybutyrate soln., 20 cc. of 2.3% hydrogen peroxide.

(b) 25.6 cc. of 0.33 *M* disodium phosphate soln., 6.4 cc. of 0.33 *M* monosodium phosphate soln., also the last 3 items as in (a).

(c) 35 cc. of water with the last two items as in (a).

(d) 55 cc. of water, 20 cc. of sodium *l*, β -hydroxybutyrate soln.

After these mixtures had stood in the incubator for five days at 37°, about 0.5 of powdered manganese dioxide was added to Expts. a, b and c which contained much unchanged peroxide. After standing overnight the mixtures were analyzed by the method previously used in the glucose experiments¹ and the results calculated for comparison as though oxidation by permanganate to carbon dioxide had been complete. The results for unchanged *l*, β -hydroxybutyric acid obtained in this way in two trials are as follows: first trial, (a) 0.154, (b) 0.162, (c) 0.177, (d) 0.190 g.; second trial, (a) 0.1334, (b) 0.1279, (c) 0.1011, (d) 0.1131. In the second trial only 1.25 g. of the calcium-zinc salt was used and the final solution was again made up to 100 cc.

Considering that Expt. d was a blank in each case it may be concluded that not much oxidation with peroxide occurred. This conclusion is justified even though the entire mechanism of the permanganate oxidation is unknown.

It should be noted that in the analysis of the *l*, β -hydroxybutyric acid solutions described above this acid is oxidized very slowly by permanganate in strongly alkaline solutions even when heated. In this respect it was less reactive than α -hydroxybutyric acid as observed in the experiments described below. Those solutions which had been subjected to treatment with peroxide reduced a little permanganate rapidly.

⁴ Witzemann, *J. Biol. Chem.*, **45**, 1 (1920).

2. Test of the Oxidation of *dl*, α -Hydroxybutyric Acid in the Phosphate Mixture

The *dl*, α -hydroxybutyric acid used was synthesized from butyric acid through α -bromobutyric acid and purified as the zinc salt, $(\text{CH}_3\text{CH}_2\text{CHOHCO}_2)_2\text{Zn} \cdot 2\text{H}_2\text{O}$. Two g. of this salt in 75 cc. of water was freed from zinc with sodium carbonate and made up to 100 cc..

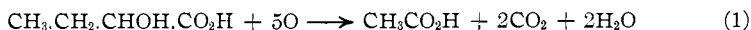
Expts. e, f, g and h were set up like the correspondingly lettered experiments with the β -hydroxy acid except that 20 cc. of the sodium α -hydroxybutyrate solution was used.

After the mixtures had stood for five days in the incubator, peroxide was absent from the mixtures used in Expts. e and f, while Expt. g contained much peroxide; 0.5 g. of manganese dioxide was then added. The next day the solutions were analyzed and the results calculated by the method used before, when (e) 0.027, (f) 0.037, (g) 0.079 and (h) 0.157 g. of α -hydroxybutyric acid were recovered.

From these results it is obvious that much oxidation occurred in the experiments containing the phosphates and considerable oxidation occurred even in Expt. g where no phosphates were present.

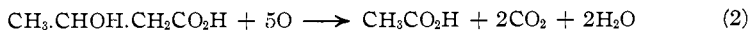
These preliminary tests show that α -hydroxybutyric acid is easily oxidized by peroxide in the presence of the phosphate mixtures while β -hydroxybutyric acid is difficultly oxidized.

The permanganate consumed in both cases was calculated as though complete oxidation to carbon dioxide was produced with permanganate. The results in Expts. d and h indicate that this did not occur. If it is assumed however that α -hydroxybutyric acid is oxidized thus,



by permanganate in alkaline solution then the permanganate consumed (0.866 g.) in Expt. h corresponds to 0.413 g. of zinc α -hydroxybutyrate, while sodium α -hydroxybutyrate corresponding to 0.400 g. was used.

When a similar calculation is made for the two results in Expt. d the permanganate consumed (1.041 and 0.619 g., respectively) is found to correspond to 0.42 and 0.27 g. of the calcium-zinc *l*, β -hydroxybutyrate, while sodium *l*, β -hydroxybutyrate corresponding to 0.40 and 0.25 g. of the calcium-zinc salt, respectively, was used. The reaction in this case is



These results show that the data conform, within the limits to be expected, with a loss of two carbon atoms at a time, in both of these acids, when oxidized by alkaline potassium permanganate. This result was to be expected with β -hydroxybutyric acid. On the contrary, propionic acid and carbon dioxide are commonly supposed to be formed with α -hydroxybutyric acid thus,



Since this reaction requires only two-fifths as much oxygen as Reaction 1 the permanganate consumed could oxidize 2.5 times as much α -hydroxybutyric acid as was used, according to Reaction 3. This is obviously impossible. This fact is, therefore, really the best evidence, although indirect, for the truth of Reaction 2 that was obtained in any of the experiments.

3. Oxidations with Permanganate

It is a well-known fact that when it is desired to trace the course of an oxidation quantitatively it is frequently important to know just how much oxygen is utilized. This is particularly true when it is difficult to determine the products quantitatively. Owing to the great ease of spontaneous decomposition of hydrogen peroxide this substance does not lend itself well to such experiments. Potassium permanganate may, however, be used for this purpose. Since the object of these experiments was to study the mechanism of the oxidation of the α - and β -hydroxybutyric acids, and particularly to determine whether the former may lose two carbon atoms at a time, this reagent recommended itself; it was in permanganate oxidations of acetone⁵ that the shift from the loss of one to the loss of two carbon atoms in pyruvic acid on adding alkali was first observed.

Oxidation of *l*, β -Hydroxybutyric Acid with Potassium Permanganate.—A solution of 1.2 g. of calcium-zinc *l*, β -hydroxybutyrate in water was treated with an excess of sodium carbonate.

The calcium and zinc precipitates were filtered off and washed. The solution was barely acidified with sulfuric acid, boiled to remove carbon dioxide and then made barely alkaline to litmus with sodium hydroxide. The solution was next diluted to 500 cc. and treated at room temperature with a few centigrams of potassium permanganate. The permanganate color remained unchanged for several weeks, which proved that no oxidation occurred. No further attempt was made to force the oxidation.

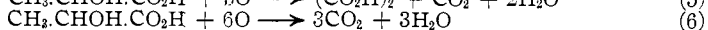
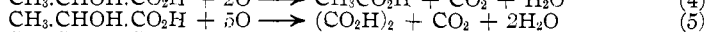
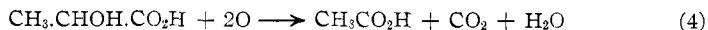
On the basis of this experiment and the results described above it was concluded: (1) that sodium *l*, β -hydroxybutyrate is not oxidized at room temperature by permanganate; in this respect it behaves exactly like acetone toward permanganate;⁵ (2) that even in the presence of alkali it is oxidized relatively slowly by permanganate; (3) that under the latter conditions it is oxidized mostly to acetic acid and carbon dioxide.

Oxidation of *dl*, α -Hydroxybutyric Acid with Potassium Permanganate.—No data upon the oxidation of α -hydroxybutyric acid with permanganate were available. For the sake of comparison the oxidation of lactic acid with permanganate was first studied.

Lactic Acid. A.—The oxidation of lactic acid in alkaline solution with permanganate has been fully studied by Evans and Witzemann.⁶ It was conclusively shown that lactic acid under the conditions used is oxidized in accordance with three reactions.

⁵ Witzemann, *THIS JOURNAL*, **39**, 2657 (1917).

⁶ (a) Denis, *Am. Chem. J.*, **38**, 577 (1907). (b) Evans and Witzemann, *THIS JOURNAL*, **34**, 1086 (1912).



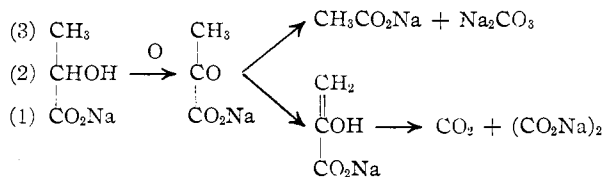
In these experiments 87% of the lactic acid was oxidized according to Reaction 5, 3% according to Reaction 4 and 10% according to Reaction 6. These experiments were satisfactory and were not repeated.

B.—Equally definite results on the oxidation of lactic acid in neutral solution were not available. The following experiment was therefore carried out.

A solution of 2 g. of zinc lactate, $(\text{CH}_3\text{.CHOH.CO}_2)_2\text{Zn.2H}_2\text{O}$, in hot water was treated with a slight excess of sodium carbonate. The zinc precipitate was filtered off and the solution made up to 500 cc.

Potassium permanganate crystals from a weighed portion were added at room temperature until the color was discharged only after standing for some days. The colorless supernatant liquid was then analyzed for acetic, oxalic and carbonic acids by methods previously described. The oxalic acid formed was not less than 0.0292 g. (2.2% yield), or more than 0.0549 g. (4.2% yield), depending on the method used for determining it; 0.6948 g. (80.9%) and 0.7464 g. (86.9%) of acetic acid were found by distillation with phosphoric acid. The volatility curve on fractionation was typical for acetic acid;⁶ 1.0475 g. of carbon dioxide was found. One carbon atom of the zinc lactate used could have furnished 0.636 g. of carbon dioxide. The extra carbon dioxide found is more than enough to account for the carbon of the two remaining atoms that was not recovered as acetic and oxalic acids.

These results clearly prove that although the mechanism of oxidation again involves the three reactions given under (A) there is a distinct shift in their quantitative importance. The importance of Reaction 4 is in this case much greater than that of Reaction 5. Reactions 4 and 5 may be represented by a scheme analogous to that previously used for acetone.



The results in (A) and (B) show that increasing alkalinity causes a shift in the main point of rupture of the carbon chain from the bond between atoms 1 and 2 to that between 2 and 3. Just how gradually this shift may occur is brought out clearly in the earlier experiments on acetone.⁵ There are therefore at least two organic compounds known (acetone and lactic acid and probably propylene glycol as well)⁶ that are oxidized by way of pyruvic acid,⁷ in which this shift in the major point of cleavage of the carbon chain has been shown to occur.

⁶ (c) Witzemann, *THIS JOURNAL*, **41**, 1946 (1919).

⁷ The experimental justification for considering pyruvic acid an intermediate in the oxidation of lactic acid with permanganate rests on the experiments of Beilstein and Wiegand [*Ber.*, **17**, 840 (1884)].

α -Hydroxybutyric Acid.—In the preceding experiments with lactic acid the problem of determining the point of rupture of the carbon chain was simplified by the fact that there are but two hydrocarbon groups in lactic acid, one of which is partly oxidized (CHOH) and the other of which (CH₃) is relatively inert. When the CHOH group is oxidized to the CO group the hydrogen of the methyl group becomes somewhat more labile. Alkalinity favors the mobility of this methyl hydrogen. In the case of α - or β -hydroxybutyric acid there are two carbon atoms with potentially labile hydrogen in addition to the CHOH group. Moreover it is well known that the hydrogen on the CH₂ is much more labile in both of these acids than the hydrogen on the CH₃ group at the end of the molecule. This factor may reasonably be expected to complicate the results from the oxidation of α -hydroxybutyric acid.

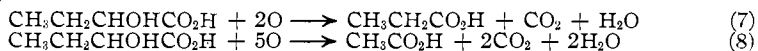
C. In the Absence of Caustic Alkali.—Two g. of zinc α -hydroxybutyrate was treated in aqueous solution with 0.70 g. of sodium carbonate and then enough to produce sufficient excess.

The zinc precipitate was filtered off and the solution diluted to 550 cc. and treated at once with 3 g. of potassium permanganate at room temperature. After reduction was complete another gram was added. Since this remained incompletely reduced 0.93 g. of sodium hydroxide was added and then gradually 0.4 g. of potassium permanganate until reduction was complete only after standing for some days. In this way a total of 4.4 g. of potassium permanganate was added.

Two hundred cc. of the supernatant liquid was distilled in the presence of an excess of phosphoric acid. Fifty cc. of water was added to the residue and the remaining volatile acids were distilled. The combined distillate on titration with alkali in the presence of phenolphthalein required alkali corresponding to 0.521 g. of sodium hydroxide for the entire distillate. If one molecule of a volatile acid is formed by the oxidation of each molecule of α -hydroxybutyric acid used, 0.540 g. of sodium hydroxide would have been required.

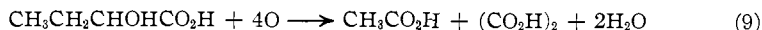
On fractionation, 225 cc. of the 255 cc. of the combined distillates required 0.1 *N* sodium hydroxide solution for the four 50cc. fractions as follows: (1) 6.92, (2) 6.96, (3) 6.90, (4) 7.15, (residue) 4.45 cc. This is not the volatility curve of pure acetic acid. It was suspected that this was a mixture of about two parts of propionic acid with one part of acetic acid. Such a mixture containing 0.186 g. of propionic acid and 0.075 g. of acetic acid (that is, 2:1) in 225 cc. on fractionating similarly required 0.1 *N* sodium hydroxide solution as follows: (1) 7.99, (2) 7.90, (3) 7.70, (4) 7.70, (residue) 4.55 cc. The volatility curve for a molecularly equivalent amount of propionic acid (that is, 0.278 g.) in the same volume follows: (1) 9.90, (2) 9.00, (3) 8.34, (4) 7.30, (residue) 3.30 cc. The similar curve for 0.225 g. of acetic acid is: (1) 5.75, (2) 6.55, (3) 7.33, (4) 9.35, (residue) 9.22 cc.

These data show that one molecule of a volatile acid is formed from each molecule of α -hydroxybutyric acid in accordance with two reactions as follows,



and that the reaction goes about 66% according to (7) and 33% according to Reactions 8 and 9. The amount of oxalic acid formed was 0.0810 g.

or 6.9% of the calculated yield (1.17 g.) and it is probably formed according to the reaction,



0.707 g. of carbon dioxide was formed. The calculated amount of carbon dioxide according to Reaction 8 is 1.146 g.

A simple calculation shows that the results given above account for all but a few per cent. of the α -hydroxybutyric acid used.

The potassium permanganate required to oxidize the α -hydroxybutyric acid according to Reaction 7 is 2.74 g., according to Reaction 8, 6.86 g., and according to Reaction 9, 5.48 g. On calculating the permanganate required for the products formed the quantity obtained in this way is not more than 10% more than the amount used, which is a good check considering the uncertainties of the analytical data and the fact that atmospheric oxygen tends to cooperate in such oxidations.^{5,8}

D. In the Presence of Caustic Alkali.—In order to have one more straw to examine in determining the direction of the wind, α -hydroxybutyric acid was oxidized in the presence of an excess of alkali.

The sodium α -hydroxybutyrate was prepared from 2 g. of the zinc salt as in Paragraph C. Two g. of sodium hydroxide (4 + molecular equivalents) was added and the solution diluted to 500 cc.; 4.6 g. of potassium permanganate was added a little at a time at room temperature during two weeks until perceptible reduction was extremely slow.

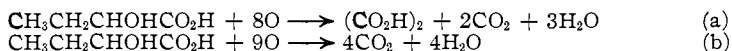
One hundred cc. of the supernatant solution with 50 cc. of water and an excess of phosphoric acid gave a distillate which on dilution to 225 cc. and fractionation in 50cc. fractions required amounts of 0.1 *N* sodium hydroxide solution as follows: (1) 1.47, (2) 1.65, (3) 2.15, (4) 2.40, (residue) 4.30 cc. The total alkali, 11.82 cc., corresponds to 0.354 g. of acetic acid or a 45.5% yield, assuming that each molecule of the α -hydroxy acid yields one molecule of acetic acid. A second determination gave 0.400 g. or a 51.2% yield. Two oxalic acid determinations gave 0.8757 g. and 0.8725 g. This is a 74.7 and 74.4% yield, respectively, on the assumption that each molecule of α -hydroxy acid gives one molecule of oxalic acid. Two determinations of carbon dioxide gave 0.725 g. and 0.830 g. which corresponds to a 63.2% and 72.4% yield, respectively, assuming that each molecule of α -hydroxy acid gives two molecules of carbon dioxide.

The solution was still capable of reducing some permanganate when hot, but the amount of reduction was not determined.

If the lower values are taken, 91.6% of the α -hydroxy acid used is accounted for. The higher values account for 99.1% of the acid used. Either of these groups of results is considered satisfactory. It is futile to attempt to determine, in those cases in which alkali was used, whether the permanganate consumed is sufficient to account for the oxidation observed, as was shown in work on the oxidation of glucose and acetone.^{5,8}

These results although only approximately quantitative show that in the presence of alkali propionic acid is no longer formed. Further than this the oxidation cannot be definitely interpreted. For instance, it is quite probable that Reactions 8 and 9 are surely involved but Reactions a and b may also take place.

⁸ Witzemann, *THIS JOURNAL*, **38**, 150 (1916).



It is obvious from previous experience on the oxidation of glucose and acetone,^{5,8} that the formation of oxalic acid is mainly determined by the presence of sufficient alkali to give normal alkali oxalates. The acid oxalates are oxidizable to carbon dioxide by permanganate.

4. Peroxide Oxidation of α - and β -Hydroxybutyric Acids

It was desired to confirm and extend the experiments in Sections 1 and 2 on the peroxide oxidation of these acids by identifying and recovering the products of oxidation.

β -Hydroxybutyric Acid.—A solution of 1.2 g. of the calcium-zinc salt was precipitated with sodium carbonate as usual.

The slight excess of carbonate was removed with phosphoric acid and the solution was boiled to remove carbon dioxide. Then 128 cc. of 0.33 *M* disodium phosphate solution, 32 cc. of 0.33 *M* monosodium phosphate solution and 125 cc. of 2.3% peroxide were added and the whole was made up to 425 cc. After 20 days at room temperature (in order to prevent the loss of acetone) the peroxide concentration was 0.50% (0.67% at the beginning). One hundred cc. of the solution with 50 cc. of water and 2 g. of manganese dioxide distilled for acetone as usual gave 0.1376 g. or 25.5% of the theoretically possible acetone. A very small amount of volatile acids was obtained in which acetic acid could not be definitely identified. Fifty cc. of the solution was treated overnight with manganese dioxide to decompose the peroxide and then oxidized with permanganate first in the presence of alkali and then in the presence of acid, as in Sections 1 and 2, in order to determine the unoxidized material. The portion taken reduced 0.398 g. of potassium permanganate, which for the entire solution corresponds to 1.34 g. of the calcium-zinc salt (1.2 g. used). On repeating this experiment, a 13.2% and 13.8% conversion into acetone was observed after 12 and 14 days, respectively, at room temperature.

These results confirm the results in Section 1. They show that under these conditions β -hydroxybutyric acid is not perceptibly oxidized except to some extent to acetone and carbon dioxide.

α -Hydroxybutyric Acid.—Two g. of zinc α -hydroxybutyrate was precipitated with sodium carbonate and prepared for use as before.

The solution with 128 cc. of 0.33 *M* disodium phosphate solution, 32 cc. of 0.33 *M* monosodium phosphate solution and 125 cc. of 2.3% peroxide was made up to 425 cc. and kept in the incubator for five days. At this time the solution, which at the beginning contained 0.67% of hydrogen peroxide, contained only 0.260%.

A mixture of 105 cc. of the solution, 50 cc. of water and 2 g. of manganese dioxide was distilled as usual and gave a distillate that showed the typical volatility curve of acetic acid, which is quite unlike that of propionic acid. The results corresponded to 0.4309 g. of acetic acid (yield, 55.2%) for the entire solution. The solution was used up in confirming this result.

The same experiment was repeated. When the mixture was removed from the incubator it showed 0.04% of hydrogen peroxide (originally 0.782%). The distillate from 100 cc. was made up to 225 cc. and fractionated in 50cc. portions as usual: (1) 2.82, (2) 3.02, (3) 3.17, (4) 3.63, (residue) 4.76 cc. of 0.1 *N* sodium hydroxide were required, respectively. These results yield a typical volatility curve for this oxidation

mixture and correspond to a 56.8% conversion of the α -hydroxybutyric acid into acetic acid. The weight of carbon dioxide found was 0.643 g., while 0.650 g. of carbon dioxide corresponds to the amount of acetic acid formed if the oxidation proceeds according to Reaction 8. Formic and oxalic acids were not found in appreciable amounts. Unchanged α -hydroxybutyric acid was determined with permanganate in the usual way and the oxidizable material present was found to correspond to 61.8% of the α -hydroxybutyric acid used, assuming as usual that it reacts entirely according to the reaction cited above. The total amount of α -hydroxybutyric acid recovered corresponds to 118.6%, which is a good result when the large analytical errors are considered. Not so much oxidation occurred as in the smaller phosphate experiments described in Section 2, for some unknown reason. A similar effect of volume was observed in the glucose experiments with phosphates.⁴

The results show that the small tendency of α -hydroxybutyric acid to lose but one carbon atom to give propionic acid in neutral permanganate oxidation is even less in the neutral phosphate mixture. In this case no indications of the formation of propionic acid were obtained. The α -oxidized fatty acid was oxidized, with the loss of two carbon atoms at a time.

5. The Oxidation of the α - and β -Ketobutyric Acids

No data upon the oxidation of α -ketobutyric acid were obtained. Only a few results were obtained with the β -keto acid (aceto-acetic acid).

Oxidation of Acetone and Aceto-acetic Acid.—The oxidation of acetone in neutral and alkaline permanganate solution has been worked out in detail.⁵ It was shown that in neutral solution at room temperature acetone is quite stable toward permanganate, but is progressively more and more oxidized as the available alkali is increased. Acetone is not so stable toward peroxide in the presence of disodium phosphate but it is by no means easily oxidized as is shown by the following experiments.

A.—Twenty-five cc. of an acetone solution (± 0.25 g. of acetone) with 32 cc. of 0.33 *M* disodium phosphate solution and 50 cc. of 3% hydrogen peroxide at room temperature for five days gave 0.1866 g. of unchanged acetone when worked up in the usual way.

B.—A blank experiment with 25 cc. of the same acetone solution, 32 cc. of 0.33 *M* disodium phosphate solution and 50 cc. of water analyzed at the same time gave 0.2098 g. of unchanged acetone.

The acetone lost in Expt. A was 11.0% of that used. A repetition of the experiment gave a loss of 11.7% of the acetone used.

Engfeldt⁹ has recently investigated the oxidation of aceto-acetic acid with permanganate in the presence and absence of acid and alkali. Consequently, only a few experiments in the presence of phosphates were made with sodium aceto-acetate and these gave results similar to those obtained with acetone above.

A solution of 10.0 g. of ethyl aceto-acetate with 4.6 g. of potassium hydroxide (a slight excess) was made up to 112 cc. and placed in a refrigerator for a week. Five cc. portions of this solution were treated as in Expts. A and B above and allowed to stand at room temperature for ten days. The solution to which peroxide was added contained

⁹ Engfeldt, *Z. physiol. Chem.*, **112**, 176 (1921).

only a trace of peroxide at the end of this time. The distillates from the two solutions, when titrated with iodine-potassium iodide solution for acetone (the alcohol also reacts in the same way), showed even less oxidation than in the experiments with acetone described above.

The results were satisfactory in indicating that potassium aceto-acetate (as well as ethyl alcohol) is difficult to burn under these conditions.

Since it was not possible to experiment with the α -ketobutyric acid, these experiments were not extended. The results here mentioned for acetone and aceto-acetic acid together with the data given above for β -hydroxybutyric acid, when compared with the behavior of α -hydroxybutyric acid as well as lactic and pyruvic acids, demonstrate that the β -hydroxy and β -keto fatty acids (that is, the "acetone bodies") are much less easily oxidized than the corresponding α -hydroxybutyric acid and the α -keto acid of lactic acid (pyruvic acid). In fact, it is no doubt the relatively high stability of the β -keto-aliphatic acids in comparison with the α -keto-aliphatic acids that makes the former substances well known, in great variety, while the latter, except pyruvic acid, are almost unknown. Moreover, pyruvic acid would presumably be the most stable of the series, on account of the relatively limited mobility of hydrogen in the methyl group in comparison with hydrogen in the CH_2 group (compare above). Consequently, although there may still be some question as to whether β -hydroxybutyric or β -ketobutyric acid (aceto-acetic acid) is formed first¹⁰ in butyric acid oxidation, there can be no question that both would be much more difficult to oxidize under known conditions than the corresponding alpha compounds.

Summary

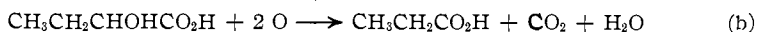
1. The oxidation of α - and β -hydroxybutyric acids with hydrogen peroxide in the presence of phosphates, and with potassium permanganate in the presence and absence of alkali, was studied in order to interpret results on the oxidation of butyric acid previously described.

2. It was found that α -hydroxybutyric acid is more easily oxidized under all conditions tested than β -hydroxybutyric acid, as well as the chemically related aceto-acetic acid and acetone.

3. Unlike lactic acid which reacts almost entirely with permanganate in the absence of alkali, thus



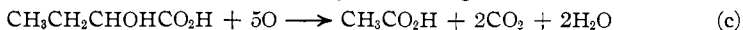
giving acetic acid and carbon dioxide, α -hydroxybutyric acid in only one experiment underwent oxidation, thus



giving propionic acid and carbon dioxide in sufficient amounts so that

¹⁰ Blum, *Münch. med. Wochenschr.*, **57**, 683 (1910). Friedmann and Maase, *Biochem. Z.*, **27**, 474 (1910). Dakin, *J. Biol. Chem.*, **8**, 98 (1910). Wakeman, Dakin, *ibid.*, **8**, 105 (1910). Marriott, *ibid.*, **18**, 241 (1914). Wilder, *ibid.*, **31**, 59 (1917).

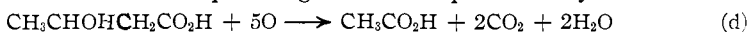
its presence among the other products could be proved. Generally, so much acetic acid was formed, mostly according to the reaction



that no evidence of the formation of propionic acid was obtained.

4. With hydrogen peroxide in the presence of phosphates no evidence of propionic acid formation from α -hydroxybutyric acid could be obtained. Oxidation to acetic acid and carbon dioxide took place according to Reaction c, at room temperature, in the absence of alkali.

5. β -Hydroxybutyric acid was oxidized with permanganate with a measurable velocity only in the presence of an excess of alkali. It was not appreciably oxidized with peroxide in the presence of phosphates. The results with alkaline permanganate correspond closely to the reaction



6. Former experiments on the oxidation of acetone with potassium permanganate showed that, in the oxidative breakdown of pyruvic acid which is an intermediate in the oxidation of acetone, lactic acid and α -propylene glycol, there is a shift from a loss of one carbon atom as carbon dioxide, by the carbon chain, to a loss of two carbon atoms as oxalic acid, on increasing the concentration of alkali in the solution, and a corresponding diminution of the production of acetic acid. This change is definitely associated with the formation of isopyruvic acid by the shifting of a hydrogen atom in the methyl group of pyruvic acid.

7. A corresponding enolization is thought to be involved in the oxidation of α - and β -hydroxybutyric acids when the chain is shortened by two carbon atoms. The greater lability of the hydrogen on the CH_2 group in α -ketobutyric acid accounts for the greater tendency of this intermediate to enolize and consequently lose two carbon atoms rather than one, as it is commonly thought to do.

8. The results detailed in this paper give definite chemical grounds based on experiments, as closely analogous to biological conditions as is so far possible, for considering α -hydroxy- and α -keto fatty acids as normal intermediates in oxidative metabolism. Such a suggestion harmonizes general chemical experience with biological observation and sets the "acetone bodies" in a separate compartment both chemically and biologically. The "acetone bodies" are known to be burned in the organism with relatively great difficulty and have now been shown to be difficultly oxidized chemically in comparison with other possible intermediates, that have now been shown to explain the known facts of fat oxidation equally well. These experiments do not reveal how glucose burning in the organism can prevent the formation or complete the oxidation of the "acetone bodies."

MAYO CLINIC
ROCHESTER, MINNESOTA