THE CATALYTIC EFFECT OF AMMONIA ON THE
OXIDATION OF BUTYRIC ACID WITH
HYDROGEN PEROXIDE.

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The results described in this paper bring to light what appears
to be a specific effect of ammonium hydroxide on the oxidation
of butyric acid by hydrogen peroxide. The results are of such
a nature that it appears that they aid in interpreting fatty acid
oxidation in acidosis of the diabetic type.

The experiments here described grew out of a discordance
between the author's own previous results on the influence of
varying amounts of potassium hydroxide on the oxidation of
butyric acid with hydrogen peroxide and those of Dakin on the
oxidation of ammonium butyrate with hydrogen peroxide. In
my own experiments it was found that the acetone yield increased
as the amount of potassium hydroxide present diminished, but
was never large. Dakin did not vary the amount of ammonium
hydroxide used, but obtained many times as much acetone as
was obtained under similar conditions with potassium hydroxide
in my own experiments. Under these conditions, it seemed
advisable to run simultaneous comparative experiments on the
influence of ammonium and potassium or sodium hydroxides,
particularly since specific effects of the salts of these bases on
biological processes have become better known since the time of
Dakin’s publications. Both Dakin's and my own previous
experiments were fully confirmed. The new experiments devel-

3 Cf. Amberg, S., and Helmholz, H. F., J. Pharm. and Exp. Therap.,
1918, xii, 19 for data and many references.
oped give additional interest to the careful experimental studies of Dakin on the oxidation of butyric acid. On the basis of the results described here, it seems not impossible that he was studying the actual mechanism of fatty acid oxidation in the diabetic type of acidosis, or at least a mechanism generically related to it.

EXPERIMENTAL.

Methods of Analysis.—When the large number of possible products of oxidation of butyric acid is considered, it is obvious that a complete quantitative analysis by the crude methods available, even for such of these compounds as are chemically known, is still out of the question. The problem, therefore, was to make such analytical determinations as would give an unmistakable indication of the state and trend of the oxidation. Suitable simple ways of determining unchanged butyric acid, acetone, and carbon dioxide were adopted and were found to give the needed information.

A portion of the oxidation solution (generally 100 or 110 cc.), with 50 cc. of water and 2 gm. of powdered manganese dioxide, was placed in a long necked distilling flask attached to a long condenser. It is necessary to use some catalyst that decomposes peroxide rapidly so that the results may be comparable, but especially to prevent the volatilization of the peroxide, which interferes with the acetone titration. A Wolff bottle connected by means of an adapter constituted the receiver. The other tubulature of the Wolff bottle was connected to two low wide mouthed wash bottles. Two wash bottles, containing sodium hydroxide and barium hydroxide, respectively, were attached to the distilling flask. During distillation a suction pump attached at the lower end circulated CO₂-free air into the apparatus and transported the CO₂ evolved on warming the mixture in the flask into the barium hydroxide solution. When only about 50 cc. remained in the distilling flask distillation was interrupted. The flask contents were filtered to remove the manganese dioxide, the filter was washed, about 50 cc. of water were added, the solution was strongly acidified with phosphoric acid, and distillation was resumed. In this way small additional amounts of CO₂ were obtained and also unchanged butyric acid as well as other volatile acids were driven over.
The CO₂ formed was determined by filtering off and weighing the BaCO₃ precipitated. The acetone was determined by using an aliquot portion of the distillate for the Lieben iodoform titration and the iodine consumed was calculated as acetone. This does not give the correct amount of acetone, since other iodoform-producing compounds are also present. The results, however, are satisfactory for comparative purposes.

The rapid determination of unchanged butyric acid in the distillate, even approximately, gave more difficulty until the rough method given below was adopted. The other possible volatile acids that would most likely interfere are propionic, acetic, and formic acids. Fortunately, all these acids in dilute aqueous solutions, such as were used here, have a typical form of volatility curve as was shown in another connection in a previous paper. On the basis of these curves and in the absence of a better method, the attempt was made to utilize this characteristic behavior on distillation to identify the main volatile acid products and to determine them approximately at the same time.

In order to determine whether this volatility curve can be used for the determination of unchanged butyric acid three distillations with known amounts of butyric acid were carried out. The amount of butyric acid given in the table was made up to 225 cc. with distilled water. Four fractions of 50 cc. were distilled off and titrated with 0.1 N NaOH.

<table>
<thead>
<tr>
<th>Butyric acid used, gm</th>
<th>A</th>
<th>B</th>
<th>C</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.25</td>
<td>0.25</td>
<td>0.125</td>
</tr>
<tr>
<td>Fraction 1, cc</td>
<td>10.50</td>
<td>10.92</td>
<td>5.38</td>
</tr>
<tr>
<td>&quot; 2, cc</td>
<td>8.04</td>
<td>8.06</td>
<td>4.08</td>
</tr>
<tr>
<td>&quot; 3, cc</td>
<td>5.60</td>
<td>5.52</td>
<td>2.75</td>
</tr>
<tr>
<td>&quot; 4, cc</td>
<td>3.30</td>
<td>3.20</td>
<td>1.64</td>
</tr>
</tbody>
</table>

More concordant results than these were repeatedly obtained without any special care. It will be noted that if the amount of butyric acid had been unknown in any case it could have been

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Fig. 1. Volatility curves.
determined approximately from the titration values in the other columns.

The results are given graphically in Curves I, II, and III in Fig. 1 for comparison with the curves of unknowns.

The method proved satisfactory for the purposes of this paper and was valued because the determination could be made in 15 or 20 minutes.

The data on the butyric acid recovered in such experiments are of especial importance for two reasons. (1) They enable one to determine approximately how much oxidation of all kinds has taken place. (2) Acetone is subject to oxidation in alkaline solutions with hydrogen peroxide and the acetone found would not necessarily be a satisfactory index of the amount of oxidation that occurred. Owing, moreover, to the fact that enolization precedes oxidation in the case of acetone the velocity of this oxidation of acetone would probably be greater when potassium is used than when ammonium hydroxide is used and so lower yields of acetone would be expected with the former. The data as determined prevent any such misunderstanding of the results.

In the preliminary qualitative experiments given below only the first distillation was carried out and no effort was made to determine the unchanged butyric acid and the carbon dioxide formed. Later the methods described above were developed and used.

It is well known that ammonium reacts in various ways with hypochlorous acid and alkali hypochlorites, hypobromites, and hypiodites in such a way that oxidation of ammonia results. This process is apparently perceptible on adding 0.1 N iodine solution to a solution strongly alkaline with sodium hydroxide containing small amounts of ammonia. Test experiments showed that although small, the error due to such reactions in the titration of acetone was increasingly significant as the amount of acetone present diminished. This error can be avoided by adding excess phosphoric acid to the distillate containing ammonia and distilling again. Moreover, if the excess is sufficient the butyric acid is quantitatively recovered. In all experiments reported in this

\[ Witzemann, E. J., J. Biol. Chem., 1918, xxxv, 89. \]
\[ Witzemann, E. J., J. Am. Chem. Soc., 1917, xxxix, 2657. \]
Oxidation of Butyric Acid

paper 10 cc. of syrupy phosphoric acid were used in this extra distillation for the removal of ammonia.

For the sake of convenience and accuracy the butyric acid used in all experiments was converted into a 5 per cent aqueous solution so that 5 cc. corresponded to 0.25 gm. of the acid. Similar solutions of ammonium, potassium, and sodium hydroxides were prepared of which 5 cc. corresponded to 0.25 gm. of butyric acid.

Influence of Ammonium Hydroxide.—In the following experiments with mixtures of butyric acid and potassium butyrate no marked difference in the yield of acetone was observed. Aqueous solutions, 100 cc. in volume, containing 50 cc. of 3 per cent hydrogen peroxide and butyric acid as given below were allowed to stand 42 to 45 hours at room temperature and analyzed. (a) Contained 0.25 gm. of butyric acid as the K salt. (b) Contained 0.20 gm. of butyric acid as the K salt and 0.05 gm. of free butyric acid. (c) Contained 0.15 gm. of butyric acid as the K salt and 0.10 gm. of free butyric acid. (d) Contained 0.25 gm. of free butyric acid.

In all cases the iodine consumed by the distillate corresponds to about 0.001 to 0.002 gm. of acetone. In other words, the acetone formed is practically imperceptible in all cases.

A similar series of experiments was done with ammonium butyrate. The ammonium butyrate was prepared by neutralizing a butyric acid solution of known strength with ammonium hydroxide using some excess of ammonia. 100 cc. of solution contained 50 cc. of 3 per cent hydrogen peroxide and

\[
\begin{align*}
(1) & \quad \text{With 0.25 gm. of butyric acid as NH}_4 \text{ salt} & \text{yields 0.0133 gm. of acetone.} \\
(2) & \quad \text{With 0.20 gm. of butyric acid as NH}_4 \text{ salt and 0.05 gm. of free butyric acid} & 0.0123 \quad \text{“ “ “} \\
(3) & \quad \text{With 0.15 gm. of butyric acid as NH}_4 \text{ salt and 0.10 gm. of free butyric acid} & 0.0053 \quad \text{“ “ “} \\
(4) & \quad \text{With 0.25 gm. of free butyric acid} & 0.0041 \quad \text{“ “ “}
\end{align*}
\]

These results show that the presence of the ammonium radical causes a marked increase in the yield of acetone. This result is in sharp contrast to what was regularly observed with potassium
butyrate as compared with butyric acid, as indicated by results such as those given above.

Another series with 0.25 gm. of butyric acid and 50 cc. of 3 per cent hydrogen peroxide in 100 cc. of solution and containing ammonium hydroxide as follows:

(5) With 50 cc. of NH₄OH solution yielded 0.0096 gm. of acetone.
(5 cc. = 0.25 gm. of butyric acid.)

(6) With 25 cc. of NH₄OH solution yielded 0.0076 " " "
(7) " 10 " " NH₄OH " " 0.0069 " " "
" " 0.0075 " " "
" " 0.0093 " " "

(8) " 7.5 " " NH₄OH " " 0.0075 " " "
(9) " 6.25 " " NH₄OH " " 0.0030 " " "
(10) " 5.00 " " NH₄OH " " 0.0021 " " "
" " 0.0017 " " "

(11) " 2.5 " " NH₄OH " " 0.00138 " " "
(12) " 0.0 " " NH₄OH " " 0.0016 " " "
" " 0.00153 " " "

These experiments constitute parts of three different series in which all of the reagents used and the solutions for titration were different. Although there is apparently considerable variation, the results conform with the preceding series. The results with (5), (6), and (7) suggest that apparently the maximum effect of the ammonium hydroxide is being exerted since the increase in the acetone is nowhere nearly proportional to the increase in ammonium hydroxide.

Repetition of (5), (6), and (10), brought out the fact that after 67 hours at room temperature, all peroxide had disappeared from (5); all but a trace from (6), while (10) still contained much peroxide. On distilling the mixtures in the presence of 2 gm. of manganese dioxide, the following results were obtained:

(5) 0.01887 gm. of acetone (11.5 per cent yield).
(6) 0.0238 " " " (15.7 " " " )
(10) 0.00523 " " " (3.2 " " " )

By means of other experiments which will not be described here, it was found that ammonium hydroxide catalyzes the rapid decomposition of hydrogen peroxide while potassium hydroxide does not do so. The smaller amount of oxidation in (5) as compared with (6) is due to the fact that more oxygen was lost into
the air in the latter case. This simply means that the oxidation reaction is not increased at the same rate by additional ammonium hydroxide as the decomposition reaction when the concentration of the other components remains the same.

The evolution of oxygen is visible in both (5) and (6); but not in (10).

The difference in the effect of potassium hydroxide as compared with ammonium hydroxide is strikingly brought out by the following experiments in which 0.25 gm. of butyric acid and 50 cc. of 3 per cent hydrogen peroxide in 100 cc. of solution,

(13) With 5 cc. of KOH solution yielded 0.00109 gm. of acetone. 
(14) With 5 cc. of NH₄OH solution “ 0.00126 “ “ “ 

(15) With 20 cc. of KOH solution “ 0.00092 “ “ “ 
(16) “ 20 “ “ NH₄OH “ “ 0.00707 “ “ “ 

0.00672 “ “ “ 
0.0338 “ “ “ *

* This result with the others in (16) show the extreme variations observed.

The results with (14) and (16) vary considerably with conditions, but can be duplicated for any set of conditions. Those for (13) and (15) are not subject to marked variations. Moreover, on standing longer than 2 days the results for (13) and (14) deviate farther and farther as the slow oxidation in (14) progresses. The final result in (14) after standing several weeks tends to approach the highest results obtained in (16). From this, one may conclude that the effect of ammonium hydroxide for a given time interval varies in the same way as the concentration and is in general influenced in the normal way by those factors that regulate the velocity of chemical reactions.

Interpretative Experiments.—In order to determine more accurately what happened in the above Experiments 15 and 16 with potassium and ammonium hydroxide, respectively, were repeated. After standing at room temperature nearly 3 days (16) was free from peroxide while (15) still contained unchanged hydrogen peroxide. The evolution of oxygen from both was clearly perceptible during the first day or more.
When (16) was analyzed by the method described above 0.0338 gm. of acetone or a 20.6 per cent yield was obtained. With 225 cc. or nine-elevenths of the distillate the Volatility Curve IV in Fig. 1 was obtained, which corresponds to a recovery of 0.1331 gm. or 52.4 per cent of the butyric acid used. The form of the curve shows that some lower fatty acid, probably acetic acid, was present. 0.219 gm. of barium carbonate, equivalent to 0.047 gm. of carbon dioxide or 4.7 per cent complete oxidation to carbon dioxide, was obtained. If it is assumed that all the carbon dioxide arises by oxidation of butyric acid to carbon dioxide and acetone thus: \( \text{CH}_3\text{CH}_2\text{CH}_2\text{CO}_2\text{H} + \text{O} \rightarrow \text{CH}_3\text{COCH}_3 + \text{CO}_2 + \text{H}_2\text{O} \), then the carbon dioxide found corresponds to a yield of 18.8 per cent for this reaction.

The acetone obtained (20.6 per cent) and the butyric acid recovered (52.4 per cent) account for 73.0 per cent of the butyric acid used. The results indicate that much butyric acid was oxidized in the presence of ammonia.

When (15), containing potassium hydroxide instead of ammonium hydroxide, was analyzed in exactly the same way 0.00396 gm. of acetone or a 2.4 per cent yield was obtained. 0.018 gm. of carbon dioxide as barium carbonate was obtained, which corresponds to 1.8 per cent complete oxidation to carbon dioxide. With nine-elevenths of the distillate the Volatility Curve V, Fig. 1 was obtained which corresponds to a recovery of 0.242 gm. or 96.8 per cent of the butyric acid used. A small amount of lower acids probably acetic was also present as indicated by the slight deviations for Fractions 3 and 4.

The total recovery of butyric acid is 96.8 + 2.4 = 99.3 per cent. The results indicate that almost no butyric acid was oxidized in the presence of potassium hydroxide.

From the two preceding experiments, one must conclude that ammonium butyrate is nearly 50 per cent oxidized under those conditions, while potassium butyrate is not appreciably attacked.

Influence of Ammonium Hydroxide in the Presence of Potassium Hydroxide.—As an interpretation of the results described above it might be suggested that in such dilute solutions the ammonium butyrate is largely hydrolyzed in comparison with the potassium butyrate and that this fact may give the key to the interpretation. In order to determine if this may be true some experi-
Experiments were done to test the influence of the simultaneous presence of potassium hydroxide. On this basis the amount of oxidation occurring could be expected to diminish as more potassium hydroxide was added.

Experiment 16 was repeated as follows:

(17). The same as Experiment 16 except that 5 cc. of potassium hydroxide (≡ 0.25 gm. of butyric acid) were added.

(18). The same as Experiment 16 except that 10 cc. of the same potassium hydroxide solution were added. This is double the amount of potassium hydroxide necessary to completely neutralize the butyric acid used.

The amount of ammonium hydroxide used was the same in both cases as in No. 16.

The solutions contained 1.50 per cent hydrogen peroxide at the beginning. After 92 hours No. 17 contained not over 0.06 per cent and No. 18 about 0.19 per cent hydrogen peroxide. Experiment 17 on analysis in the usual way gave 0.0239 gm. or a 14.6 per cent yield of acetone. Carbon dioxide corresponding to 0.052 gm. or a 5.2 per cent complete combustion to carbon dioxide was obtained. The fractionation of nine-elevenths of the total distillate gave the data for the unchanged butyric acid in Curve VI in Fig. 1, which corresponds to 0.152 gm. or a 60.8 per cent recovery of the butyric acid used.

No. 18 analyzed in the usual way gave 0.012 gm. of acetone or a 7.3 per cent yield. No carbon dioxide was evolved during the first distillation as with No. 20 or ammonium hydroxide experiments, but about a 6.0 per cent yield of carbon dioxide was obtained during the phosphoric acid distillation. The fractionation of four-fifths of the total distillate gave the data for the butyric acid in Curve VII, Fig. 1, which corresponds to 0.216 gm. or 86.4 per cent of the butyric acid recovered unchanged.

<table>
<thead>
<tr>
<th>No.</th>
<th>Acetone found.</th>
<th>Butyric acid recovered</th>
<th>KOH used.</th>
<th>NH₄OH used.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>per cent</td>
<td>per cent</td>
<td>cc.</td>
<td>cc.</td>
</tr>
<tr>
<td>15</td>
<td>2.4</td>
<td>96.8</td>
<td>20</td>
<td>0</td>
</tr>
<tr>
<td>18</td>
<td>7.3</td>
<td>86.4</td>
<td>10</td>
<td>20</td>
</tr>
<tr>
<td>17</td>
<td>14.6</td>
<td>60.8</td>
<td>5</td>
<td>20</td>
</tr>
<tr>
<td>16</td>
<td>20.0</td>
<td>52.4</td>
<td>0</td>
<td>20</td>
</tr>
</tbody>
</table>
These results both for acetone found and for butyric acid recovered are intermediate between those of Nos. 15 and 16 as shown in the tabulated data. They show conclusively that the amount of oxidation taking place is definitely related to the amount of potassium hydroxide present when the amount of ammonium hydroxide is considerable and constant.

**Influence of NaHCO₃ on the NH₄OH Effect.**—The question as to whether ammonium butyrate plays a special rôle in this reaction may be tested in another way also. In other experiments that will not be described here, it was found that the bicarbonates of potassium and sodium catalytically decompose hydrogen peroxide much faster than the hydroxides do, but not so fast as do the carbonates and ammonium hydroxide. If the effect of ammonium hydroxide is essentially due to this catalytic effect on peroxide, then considerable oxidation should take place even when the ammonium hydroxide in (16) is partly replaced with sodium bicarbonate. Three experiments were done.

(19). 5 cc. (0.25 gm.) butyric acid solution. 5 cc. (0.25 gm. butyric acid) ammonium hydroxide solution. 5 cc. sodium bicarbonate (0.028 gm. per cc. of solution). 50 cc. 3 per cent hydrogen peroxide.

(20). Same as (19) except that 10 cc. of sodium bicarbonate solution were added.

(21). Same as (19) except that no bicarbonate was used.

The results obtained on analyzing the mixtures 45 hours later are tabulated below.

<table>
<thead>
<tr>
<th>No.</th>
<th>H₂O₂ remaining</th>
<th>Acetone yield</th>
<th>Butyric acid recovered</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>gm.</td>
<td>per cent</td>
</tr>
<tr>
<td>19</td>
<td>0.16</td>
<td>0.0078</td>
<td>4.7</td>
</tr>
<tr>
<td>20</td>
<td>0.28</td>
<td>0.0078</td>
<td>4.7</td>
</tr>
<tr>
<td>21</td>
<td>1.52</td>
<td>0.018</td>
<td>11.1</td>
</tr>
</tbody>
</table>

These results show that although the peroxide is nearly completely decomposed in (19) and (20) there is even less oxidation than in (21) in which there was no excess of ammonium hydroxide nor any other compound catalytically active toward peroxide. Although only 58 per cent of the amount of bicarbonate, equivalent to the ammonia and butyric acid, was used in (19), peroxide
decomposition was nearly complete, but oxidation was very slight. This shows that the strong catalytic effect of ammonium hydroxide on peroxide is probably not the only characteristic of this compound necessary for catalyzing the oxidation of butyric acid. In this respect the behavior of ammonia seems to be specific in the same way that that of phosphate is in the case of the oxidation of glucose to carbon dioxide with hydrogen peroxide.8

There is, however, one difference in the results as they stand and that is that the effect of ammonia can be increased in suitable mixtures with potassium hydroxide as shown in the next section, while nothing was tried that stimulates the phosphate effect similarly.

The Combined Effect of Ammonium and Potassium Hydroxide.—The interpretative experiments in the above section are defective as far as suggestiveness for biological conditions is concerned, because too much of these bases was used. The experiments described below were done in order to extend the above experiments to smaller amounts of the bases when used simultaneously. In these experiments 0.25 gm. butyric acid was used and enough potassium hydroxide to neutralize this acid (i.e., 0.159 gm. of potassium hydroxide). In all cases 50 cc. of 2.3 per cent hydrogen peroxide were used. The total volume was 80 cc. The results upon analysis after 96 hours at room temperature are tabulated below.

<table>
<thead>
<tr>
<th>No.</th>
<th>Acetone found.</th>
<th>Butyric acid recovered.</th>
<th>KOH used.</th>
<th>NH₄OH used.</th>
<th>H₂O₂ left.*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>per cent</td>
<td>per cent</td>
<td>cc.</td>
<td>cc.</td>
<td>per cent</td>
</tr>
<tr>
<td>22</td>
<td>4.0</td>
<td>88.0</td>
<td>5.0</td>
<td>0.0</td>
<td>0.908</td>
</tr>
<tr>
<td>23</td>
<td>17.0</td>
<td>57.6</td>
<td>5.0</td>
<td>5.0</td>
<td>0.153</td>
</tr>
<tr>
<td>24</td>
<td>15.0</td>
<td>61.6</td>
<td>5.0</td>
<td>10.0</td>
<td>0.151</td>
</tr>
<tr>
<td>25</td>
<td>11.8</td>
<td>68.4</td>
<td>5.0</td>
<td>20.0</td>
<td>0.187</td>
</tr>
</tbody>
</table>

* All contained 1.45 per cent H₂O₂ at the beginning.

More carbon dioxide was obtained in No. 23 than in any of the other experiments but the amount was small in all cases. The above experiments were repeated using somewhat different amounts of alkali. Otherwise the conditions were the same.

Because No. 27 contained more available alkali than the others the above experiments were repeated as follows and the results given below were obtained.

<table>
<thead>
<tr>
<th>No.</th>
<th>Acetone found.</th>
<th>Butyric acid recovered</th>
<th>KOH used.</th>
<th>NH₄OH used.</th>
<th>H₂O used.</th>
<th>H₂O₂ left.*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>per cent</td>
<td>per cent</td>
<td>cc.</td>
<td>cc.</td>
<td>cc.</td>
<td>per cent</td>
</tr>
<tr>
<td>26</td>
<td>3.2</td>
<td>97.0</td>
<td>5.0</td>
<td>0.0</td>
<td>5.0</td>
<td>1.760</td>
</tr>
<tr>
<td>27</td>
<td>11.3</td>
<td>68.4</td>
<td>5.0</td>
<td>5.0</td>
<td>0.0</td>
<td>0.646</td>
</tr>
<tr>
<td>28</td>
<td>7.9</td>
<td>87.2</td>
<td>0.0</td>
<td>5.0</td>
<td>5.0</td>
<td>1.085</td>
</tr>
</tbody>
</table>

* All contained 1.77 per cent H₂O₂ at the beginning.

The results of the three sets of experiments show: (1) That the loss in peroxide from the solution is greatest in those cases in which the most butyric acid disappeared. (2) That the presence of one equivalent of ammonium hydroxide is most favorable for oxidation when one equivalent of potassium hydroxide is also present. (3) That the presence of two equivalents of either base is not so favorable for oxidation or for peroxide decomposition as the presence of one equivalent of each. (4) That the effect of these bases is not proportional to the available alkali or the hydroxyl ion concentration.

**The Relative Influence of Sodium Hydroxide upon Butyric Acid Oxidation.**—In the above experiments potassium hydroxide came to be used as the alkali instead of sodium hydroxide without any reason or intention of discrimination. Normally, sodium salts are probably more abundant in biological conditions. Accordingly, it seemed desirable to do a few more experiments on the influence of sodium hydroxide upon this oxidation. In the six experiments given below 0.25 gm. of butyric acid, 50 cc. of 3.2 per cent hydrogen peroxide, and the alkali given in the table were allowed to react in a total volume of 100 cc. for 5 days at room temperature. The results are summarized in the table.
Comparison of the results in (32), (33), and (34) shows that the sodium hydroxide is somewhat less active than the potassium hydroxide when used in conjunction with ammonium hydroxide. This is confirmed by the results in (36) and (37). Other unpublished experiments on the influence of these two bases on the spontaneous decomposition of peroxide show about the same degree of difference in their action, when used in the absence of ammonia.

The results in (34) in which one equivalent of all three bases was used stand between (36) and (37), which is to be expected if the small difference in their behavior is correct.

Biologically these results are of interest because they show that if under any conditions fatty acid oxidation in the organism resembles these oxidations either the potassium or sodium salt of mixtures would be attacked with about the same ease in the presence of ammonia.

The results in this section are of especial interest in another way because they show that the catalytic influence of ammonium, sodium, and potassium as the bases is just the reverse of their influence as the phosphates, upon the peroxide oxidation of a number of simple organic compounds. Further experiments upon mixtures will be done in developing these unpublished results on phosphates.

**Partial Interpretation.**—The experiments in the preceding section bring out a number of facts concerning the interpretation. (a) The ammonium and potassium butyrates here under consideration are the salts of a weak and a strong base, respectively, with a weak acid. At these dilutions there would be considerably more free butyric acid produced by hydrolysis when
ammonia only is used. That this free acid is not the only significant factor in the catalysis is shown by the fact that the simultaneous presence of both bases promotes the greatest oxidation.  

(b) That the mere presence of many butyrate ions does not determine the oxidation, is shown by the fact that in the presence of ammonium hydroxide alone, in which, of all the experiments, there is the smallest concentration of butyrate ions, the oxidation proceeds much faster than when potassium hydroxide only is used where the butyrate ion concentration is greatest. (c) That the butyrate molecules do not determine the oxidation is shown by the fact that oxidation is slowest where the concentration of these is greatest; namely, in the solutions containing potassium hydroxide alone. (d) That the capacity to decompose peroxide spontaneously may be a factor is suggested by the fact that potassium decomposes peroxide much more slowly than ammonium hydroxide. That this is not the sole factor is shown by the experiments with sodium bicarbonate which decomposes peroxide well but does not facilitate these oxidations. (e) The concentration of the hydroxyl ion has no definite relation to the velocity of the oxidation as observed with these three bases.

The known facts about these oxidations are not yet sufficient for a satisfactory interpretation. The statements given in the next two paragraphs suggest a partial interpretation.

The oxidation of butyric acid in the presence of ammonia described above may belong to the so called "coupled" or induced oxidations. The formation of a peroxide of ammonia may represent one phase of this coupling. That such a peroxide of ammonia may be obtained under suitable conditions was shown by Melikoff and Pissarjewski. If this peroxide is the intermediate concerned it is much more unstable than the peroxide of potassium since when equal concentrations of the two bases are placed in solutions of hydrogen peroxide the ammonium hydroxide decomposes the peroxide much more rapidly than the potassium hydroxide does. The conceptions of an ideal catalyst and the theory of dislocation of Böseken offer a concrete partial interpretation of this part of the effect of ammonia. According to this

view of catalysis that catalyst will be best which has the greatest tendency to form the least stable complex with the substrate.

If it is granted that the data justify this much of an interpretation for the interaction of ammonia and peroxide they fail to indicate how such a complex activates the oxidation of butyric acid. Sweeping hypotheses on this subject would be undesirable, especially in view of the fact that equally anomalous data are already in hand showing that when these bases are used as phosphates the effects on the oxidation of butyric acid are quite different.

Possible Biological Significance of the Ammonia Effect. — The experiments showed that butyric acid, whether free or combined with sodium or potassium, is peculiarly susceptible to oxidation by peroxide when ammonia is also present, and that under these conditions relatively little carbon dioxide but much acetone is obtained. This high degree of acetone formation depends upon the simultaneous presence and the interaction of ammonia, peroxide, and butyric acid. Without entering into details it would seem that these three components play an active rôle in metabolism, particularly in the hepatic metabolism, and that acetone as acetoacetic acid is also formed in this organ. It will be recalled, for instance, that Embden and Kalberlah found that the liver is the only organ that, on perfusion in the surviving state with normal blood, gives acetoacetic acid. Moreover there is in the liver an active traffic in ammonia equivalents involved in its important deaminizing and urea-forming functions. That it can actually supply this component of this reaction system better than other organs has recently been brought out by experiments of Keeton of this laboratory, who found, in harmony with old observations, that when an inorganic acid is administered by stomach to dogs it is partially excreted as the ammonium salt, and causes an increase in the urinary ammonia nitrogen, both relative and absolute. When, however, the acid is injected into a peripheral vein, although it may cause some absolute increase of the urinary ammonia, it causes little or no increase in the quan-

tity of ammonia nitrogen relative to the total nitrogen. This difference in the behavior of acid administered by the portal and the peripheral routes, respectively, is interpreted as due to the fact that ammonia is an available base in the liver and enters into the neutralization of acid when the acid is neutralized in the liver, but plays little part in the neutralization of acids elsewhere in the body.

It may not seem amiss to suggest, since the same type of oxidation as that observed in the above described test-tube experiments is known to occur in the liver in a high degree, that this oxidation in the liver may possibly be favored as in the test-tube by ammonia.

The question has long been discussed as to whether the formation of acetoacetic and β-hydroxybutyric acids in the body represents a purely abnormal type of fatty acid oxidation; that only occurs under certain conditions (as in diabetic acidosis, etc.) or whether it is a step in the normal oxidative breakdown of the fatty acids, having an even number of carbon atoms, which is obscured in health because of the rapid occurrence of subsequent events. The first view would make acetoacetic acid an abnormal product, the latter would make it a normal product, which in acidosis of the diabetic type fails to break down further. Neubauer\(^9\) in his well known study of the breakdown of the fatty and amino-acids favored the view that the formation of acetoacetic acid is a normal step.

The above experiments and discussion would tend to favor the view that the formation of acetoacetic acid may be considered as a normal step in the oxidation of fatty acids, in harmony with Neubauer's conception, and particularly in media in which ammonia is available. Moreover the special occurrence of this type of oxidation in the liver might possibly be brought into relationship with the special availability of ammonia in this organ, it being less characteristic of the oxidation of fatty acids elsewhere in the body, where relatively less ammonia is available. According to this line of thought a certain special physiological function of the body, namely its power to form acetoacetic acid, would be associated with a special characteristic of ammonia.

\(^{13}\) Neubauer, O., *Deutsch. Arch. klin. Med.*, 1908-09, xciv, 211.
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or its equivalent and the suggestion would follow that where acetoacetic acid is formed in the body ammonia may be present and acting in this characteristic way. The problem of why in health the evidence of acetoacetic acid formation is suppressed, i.e. the problem of "antiketogenesis" (or "ketolysis") is clearly another matter.

SUMMARY.

Experiments were performed with the object of determining the reason for the difference in the results obtained in the oxidation of butyric acid with hydrogen peroxide in the presence of different alkaline substances.

In the presence of potassium hydroxide in amounts varying from 0.20 to 4.0 equivalents no appreciable oxidation of butyric acid took place, as was proved by the almost quantitative recovery of the unchanged acid.

In the presence of ammonium hydroxide in amounts varying from 0.20 to 10.0 equivalents much oxidation took place.

The amount of oxidation in the presence of ammonia was found to increase with increase in the ammonium hydroxide, other things being equal, until more than 4.0 equivalents of ammonium hydroxide were present, after which it decreased somewhat.

This decrease with large excess of ammonium hydroxide was due to the spontaneous liberation of oxygen by the action of ammonium hydroxide on the hydrogen peroxide, before it could be utilized in oxidation.

If one equivalent of both ammonium hydroxide and potassium hydroxide is used more oxidation takes place than if two equivalents of either of these bases are added.

The type of oxidation observed in these experiments was mainly of the β type or the conversion of butyric acid into acetone and 1 molecule of carbon dioxide. Consequently the statement in the above paragraph is equivalent to saying that the presence of ammonia in a solution containing potassium butyrate and hydrogen peroxide catalyzes the oxidation of butyric acid to acetone and carbon dioxide.

The additive effect of ammonium and potassium here described in favoring oxidation constitutes a chemical analogy to the biological effects of certain mixed salts.
The experiments on the additive effects of sodium and ammonium show that this pair acts nearly the same as potassium and ammonium. There is just enough difference in the behavior of potassium and sodium to constitute a confirmation of unpublished observations of specific effects of these bases as phosphates.

A few suggestions for a partial interpretation were made. It is clear, however, in this case, as in the phosphate effect on glucose previously reported, that the rôle of alkalinity and alkali is not primary in this oxidation. A satisfactory interpretation is particularly desirable in view of the possible biological significance of the results.

Finally in discussing the possible biological significance of these results it is suggested that the ammonia effect here described may be the agency by which the normal oxidation of fatty acids is brought about in the liver. This suggestion rests upon the fact that in the liver the substances required for this effect are all available and that this organ also normally shows the greatest tendency to form acetoacetic acid.
THE CATALYTIC EFFECT OF AMMONIA ON THE OXIDATION OF BUTYRIC ACID WITH HYDROGEN PEROXIDE

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