of *d*-arabonic lactone; (b) 6.0 g. of high-melting, alcohol-insoluble brucine salt with the characteristics of brucine *d*-erythronate; from this, 0.44 g. of levorotating acids was obtained; the pure *d*-erythronic lactone was not obtained; (c) 19.8 g. of high-melting, alcohol-soluble brucine salts, from which was isolated 0.29 g. of a quinine salt having the properties of quinine *l*-glycerate; (d) 6.37 g. of hydrated calcium glycolate, plus 1.3 g. of dried calcium glycolate; (e) 4.1 g. of oxalic acid, quantitatively determined in a separate experiment. The non-volatile fraction consisted largely of unidentified hydroxy acids, the C_3 and C_4 acids occurring in such small amounts that their identification could not be sharp. The amounts of *d*-arabonic and glycolic acids actually present in the mixture were probably much greater than those here reported.

4. A comparative oxidation of *d*-fructose under the same conditions as the glucose oxidation, gave less carbon dioxide, 4.28 g. per 100, but much more oxalic acid, 9.1 g.

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[CONTRIBUTION FROM THE OTHO S. A. SPRAGUE MEMORIAL INSTITUTE, CHICAGO]

THE INFLUENCE OF PHOSPHATES ON THE OXIDATION OF BUTYRIC ACID WITH HYDROGEN PEROXIDE

BY EDGAR J. WITZEMANN

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For some time the biochemical problem of the interrelation of glucose and fatty acid oxidation has been under chemical investigation in this Laboratory. Results previously described[1] and those presented in this and two following papers represent some of the data obtained.

All of the experiments have been made using butyric acid as the fatty acid and this was oxidized with hydrogen peroxide in the presence and absence of glucose and various alkaline substances. In the first paper that we published we were laboring under the erroneous conception that the particular alkali used in such experiments was not very significant, but that the hydroxyl-ion, or the hydrogen-ion concentrations as it would generally be expressed, was the determining factor. It was some time before we directly compared the effects of sodium and potassium hydroxide upon this oxidation with that of ammonium hydroxide. The results of this investigation have been published[1b] and showed that so far as these three alkaline compounds are concerned the action of ammonia is specific and that the hydrogen-ion concentration of the solution does not determine the oxidation when these three compounds are used. Whereas Dakin[2]

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[1] Witzemann, (a) *J. Biol. Chem.*, **35**, 83 (1918); (b) **49**, 123 (1921).

[2] Dakin, *ibid.*, **4**, 77 (1908).

had found that ammonia promotes the oxidation of butyric acid with peroxide, we found that when sodium or potassium hydroxide was substituted the total oxidation was slight.

With this experience in mind the results obtained with phosphate mixtures,[1a] used originally as a means of regulating the hydrogen-ion concentration of the system, presented new possibilities and were studied in detail. The results of our study of the behavior of butyric acid alone and with glucose in the presence of hydrogen peroxide, in systems containing alkali phosphate, are briefly recorded in this and the following paper. The results obtained with glucose and hydrogen peroxide in phosphate systems have previously been reported in full.[3]

The general purpose running through this work has been three-fold.

(1) To find conditions in which butyric acid is readily oxidized (as it is in the organism) that are not too greatly different from what might exist in the living body and to determine what happens to the butyric acid when oxidized in this way.

(2) To allow glucose to be oxidized in the same system, along with butyric acid, in order to determine if and how the formation of acetone from butyric acid may be prevented, that is, whether and when the course of oxidation in (1) may be deviated to a different end result in the presence of oxidizing glucose, as it is in the living body when glucose is made to burn in an organism that was previously burning too high a proportion of fat and too low a proportion of glucose.

(3) To explore the apparently somewhat specific catalytic effects of certain compounds, that may also occur in the organism, upon the results of (1) and (2). An interesting group of these effects may be graphically described by considering them examples of a modified alkali effect. The ammonia and phosphate results cited above and the new phosphate results given here are examples of these effects.

Preliminary Experiments with Phosphate Mixtures.—Many preliminary experiments were done at various times and in various ways. Of these only the following set will be described. Each of the columns with a Roman numeral in Table I represents a separate series of experiments. Each experiment was set up as follows: 50 cc. of 3% hydrogen peroxide, 0.25 g. of butyric acid, 0.159 g. of potassium hydroxide, and mono- and dipotassium phosphates as given in Table I were made up to 100 cc. After standing at room temperature for about 45 hours the solution was placed in a long-neck distilling flask with 50 cc. of water. In most of these early experiments an excess of manganese dioxide was added to decompose unchanged peroxide before it was volatilized so that this would not interfere with the Lieben-iodoform determination of acetone. Deviations from this procedure are described below the table.

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[3] Witzemann, *J. Biol. Chem.*, **45**, 1 (1920).

TABLE I

EFFECT OF PHOSPHATE MIXTURES

K_2HPO_4, g.	KH_2PO_4, g.	Acetone found, g.			
		I	II	III	IV
6	1	0.0323[a]	++++	0.0204	0.0152
6	3	.0117	++++	.0256	.0122
6	6	.0111	++++	.0256	.0143
3	6	.0098	++	.0046	.0095
1	6	.0052	+	.0024	.0097[b]

[a] Boiled dry in distillation.

[b] Seemed to contain a trace of H_2O_2.

In Series I the distillation was conducted as usual in the presence of an excess of powdered manganese dioxide.

In Series II the distillation was conducted in the presence of precipitated calcium carbonate. On the addition of the iodine solution to the alkalinized distillate a small amount of oxygen was evolved owing to the presence of small amounts of hydrogen peroxide. The relative quantity of visible iodoform precipitated is roughly indicated by the number of plus marks. The thiosulfate titration would have had no significance in this case.

In Series III the distillation was conducted as with Series II. Manganese dioxide was then added to the distillate and a small amount of oxygen was evolved. The solutions were filtered and the usual amount of sodium hydroxide was added. After standing overnight the precipitated manganese hydroxide was filtered off and the iodoform titration was made as usual.

In Series IV a small amount of manganese dioxide was added with the calcium carbonate in distillation. Hydrogen peroxide was absent in all the distillates except possibly a trace in the last experiment of the series.

The above group of results is typical and shows a constant definite difference in the behavior of these mixtures whatever method was used. The acetone found is in all cases greater with the larger proportion of dipotassium phosphate. Without discussing these results further, the more complete results will be described.

Influence of Mono-alkali Phosphates.—The phosphate mixtures when used as regulator mixtures are composed of two phosphates one of which is slightly acid, when dissolved alone in water, and the other slightly alkaline. In the experiments with glucose[3] four molecular proportions of the alkaline phosphate were mixed with one of the acid one giving a mixture which is barely alkaline ($P_H = 7.347$). Special experiments in the case of glucose showed on the one hand that the alkaline phosphate alone produced the observed effect and that the acid phosphate alone did not produce the effect. In the following experiments on butyric acid more attention was paid to studying the influence of the individual phosphates than to the influence of the mixtures.

Monopotassium Phosphate, KH_2PO_4.—The experiment was set up with the following mixture on November 12, 1920: 30 g. of monopotassium phosphate in 250 cc. of water, 250 cc. of 3% hydrogen peroxide, 25 cc. of butyric acid solution (5 cc. = 0.25 g.), 25 cc. of potassium hydroxide solution (5 cc. ≡ 0.25 g. of butyric acid); total volume, 550 cc. After this mixture had stood in the laboratory at room temperature 18 days, 110cc. portions were analyzed on the dates given by the methods previously described. For convenience the results were calculated on the basis of the butyric acid originally present in the portion used for analysis. The results obtained are summarized in Table II.

Table II

Results with Monopotassium Phosphate

Date, Dec.	Acetone		Carbon dioxide		Butyric acid recovered	
	G.	%	G.	%	G.	%
1	0.008	4.8	...	...	0.233	93.2
2	.0098	6.0	0.031	6.2	.218	87.2
6	.0125	7.6	.035	7.0	.209	83.6
10	.0106	6.5	.042	8.4	.202	80.8

Monosodium Phosphate, $NaH_2PO_4.4H_2O$.—The experiment was set up as follows on Nov. 29, 1920: 21 g. of monosodium phosphate in 250 cc. of water, 250 cc. of 3% hydrogen peroxide, 25 cc. of butyric acid solution (5 cc. = 0.25 g.), 25 cc. of potassium hydroxide solution (5 cc. ≡ 0.25 g. of butyric acid solution); total volume, 550 cc.

The mixture was allowed to stand in the laboratory at room temperature. The analysis of 110cc. portions is summarized in Table III.

Table III

Results with $NaH_2PO_4.4H_2O$

Date	Acetone		Carbon dioxide		Butyric acid recovered	
	G.	%	G.	%	G.	%
Dec. 16	0.0118	7.2	0.042	8.2	0.202	80.8
Jan. 4	.0223	13.5	.041	8.2	.209	83.6

The above results are sufficient to indicate that the behavior of the corresponding sodium and potassium salts is similar.

Influence of Dialkali Phosphates. Dipotassium Phosphate, K_2HPO_4.—The experiment was set up as follows on November 12, 1920: 30 g. of dipotassium phosphate in 250 cc. of water, 250 cc. of 3% hydrogen peroxide,

Table IV

Results with Dipotassium Phosphate[a]

No.	Analyzed	Acetone		Carbon dioxide		Butyric acid recovered	
		G.	%	G.	%	G.	%
a	Nov. 30	0.0329	20.0	...	..	0.091	36.4
b	Dec. 1	.0352	21.4	...	..	.091	36.4
c	Dec. 3	.0438	26.7	0.131	26.2	.072	28.8
d	Dec. 6	.0441	26.9	.149	29.8	.0698	27.9

[a] Analytical data and percentages based on 0.25 g. of butyric acid.

25 cc. of butyric acid solution (5 cc. ≡ 0.25 g.), 25 cc. of potassium hydroxide solution (5 cc. ≡ 0.25 g. of butyric acid); total volume, 550 cc.

The mixture was allowed to stand at room temperature and 110cc. portions were analyzed on the dates given in Table IV.

On December 1 the peroxide concentration was 0.58% (originally, 1.05%).

The impression was obtained that butyric acid was being converted in part into acetic acid. Some end results of another experiment, which will only be given in part, proved this. Aliquot parts of a mixture like that described above were analyzed from time to time and showed after 14 weeks 0.65% of hydrogen peroxide (originally 1.36%). A portion of the distillate obtained as usual from this oxidation mixture showed the volatility curve of acetic acid[4] as follows: (1) 2.03 cc., (2) 2.07 cc., (3) 2.50 cc., (4) 3.15 cc., (residue) 4.85 cc. of 0.1 *N* sodium hydroxide solution required. This curve shows the absence of butyric acid and appreciable quantities of propionic acid and is unmistakably the volatility curve of acetic acid. Formic acid was shown not to be present and is moreover readily oxidized in this phosphate mixture as was found in other unpublished experiments. The acetic acid that would have been required to give these results, when calculated to 110 cc. of the original oxidation mixture, is 0.064 g., which corresponds to a 37.6% conversion of the butyric acid used into acetic acid.

Another experiment giving a similar final result is described in the following paper.

Disodium Phosphate, $Na_2HPO_4.12H_2O$.—The experiment was set up as follows on Nov. 29, 1920: 60 g. of disodium phosphate in 250 cc. of water, 250 cc. of 3% hydrogen peroxide, 25 cc. of butyric acid solution (5 cc. = 0.25 g.), 25 cc. of potassium hydroxide solution (5 cc. ≡ 0.25 g. of butyric acid); total volume, 550 cc.

The mixture was allowed to stand at room temperature and analyzed as usual.

TABLE V

RESULTS WITH DISODIUM PHOSPHATE[a]

Date	Acetone G.	Acetone %	Carbon dioxide G.	Carbon dioxide %	Butyric acid recovered G.	Butyric acid recovered %
Dec. 15	0.0202	12.3	0.089	17.8	0.144	57.6
Dec. 17	....	..	.093	18.6	.142	56.8
Jan. 3	.0298	18.1	.122	24.4	.126	50.4

[a] Analytical data and percentage based on 0.25 g. of butyric acid.

On January 4 there was 0.115% of hydrogen peroxide present (1.1% originally present).

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[4] For details about this method see Witzemann, THIS JOURNAL, **41**, 1946 (1919) and Ref. 3.

General Discussion of the Results

The results on the oxidation of butyric acid described in this paper are to be considered in connection with the results described in the two succeeding papers. There are, however, certain general considerations that may properly be given here.

The results here disclosed show that the dialkali phosphates are better catalysts for the oxidation of butyric acid with peroxide than the monoalkali phosphates. Moreover, the two potassium phosphates appeared to act more rapidly on the oxidation of butyric acid than the corresponding sodium phosphates. These and other observations that will not be recorded lead the author to believe that there is a group of specific effects involved here in accordance with which various substitutions in the phosphoric acid molecule tend to favor differentially the more rapid oxidation of certain compounds in comparison with other substitutions. Thus it was many times observed that the oxidation of glucose is favored more by disodium phosphate while the oxidation of butyric acid is best promoted by dipotassium phosphate. Moreover, the existing results on the influence of ammonia[1b] on butyric acid oxidation would lead one to expect such effects. More exhaustive experiments than have as yet been done will be required to determine the scope and nature of these effects.

The results in this paper taken in conjunction with those given in the two following papers show that butyric acid is completely oxidized by hydrogen peroxide in the presence of dipotassium phosphate and that acetic acid, acetone and carbon dioxide are among the products formed. Two types of oxidation were found.

$$CH_3.CH_2CH_2CO_2H + 2O \longrightarrow CH_3COCH_3 + H_2O + CO_2 \qquad (1)$$

$$CH_3CH_2CH_2CO_2H + 6O \longrightarrow CH_3COOH + 2CO_2 + 2H_2O \qquad (2)$$

If the carbon dioxide found may be considered as a basis for calculation, the carbon dioxide yield in the last analysis of the dipotassium phosphate experiment is sufficient, after subtracting the carbon dioxide required by Reaction 1 for the acetone found, to provide for a 23.1% conversion of the butyric acid used according to Reaction 3, or what is probably more nearly correct, a 46.2% conversion according to Reaction 2.

$$CH_3CH_2CH_2CO_2H + 10O \longrightarrow 4CO_2 + 4H_2O \qquad (3)$$

Any acetic acid formed would, like acetone, escape further oxidation almost completely as was shown by control experiments with these two compounds, with dialkali phosphates and peroxide, which will not be described here.

The first step in Reaction 1 is undoubtedly a β-oxidation, by which β-hydroxybutyric acid or aceto-acetic acid or both are formed. In Reaction 2 the first step is probably an α-oxidation by which α-hydroxybutyric acid or α-ketobutyric acid or both are formed as shown by experiments and arguments detailed in the last paper of this group. The amounts of acetone and acetic acid obtained are thought to indicate the relative amount of alpha and beta types of oxidation taking place.

Summary

1. Butyric acid in the presence of enough potassium hydroxide to neutralize it was oxidized with hydrogen peroxide in the presence of dialkali and mono-alkali phosphates.
2. In the presence of mono-alkali phosphate the oxidation was slower and there was perhaps a somewhat larger relative production of acetone than with the dialkali phosphates.
3. In the presence of dialkali phosphates the oxidation was much faster and acetone, acetic acid and carbon dioxide were the products isolated when the oxidation of butyric acid was complete.
4. The acetic acid obtained in the one case here described was 37% of that corresponding to complete oxidation of the butyric acid to acetic acid and carbon dioxide. For reasons developed in a following paper this formation of acetic acid is thought to be the result of oxidation of the butyric acid at the alpha carbon atom.

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THE SIMULTANEOUS OXIDATION OF GLUCOSE AND BUTYRIC ACID

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The experiments described in this paper in which potassium butyrate and glucose were oxidized together in the same solution by means of hydrogen peroxide in the presence of phosphates, were made in the hope that they might throw light on the general problem of antiketogenesis. The results show that the simultaneous oxidation of glucose and potassium butyrate in the system used did not reduce the amount of acetone formed in the oxidation of potassium butyrate below the amount obtained by oxidizing potassium butyrate alone in the same system. No effect analogous to the antiketogenetic effect of oxidizing glucose in the body was demonstrated in these systems. It was also shown that the rate of oxidation of potassium butyrate was not measurably influenced by the presence of oxidizing glucose. The two substances were apparently oxidized independently without any recognizable interaction. It was concluded that the conditions of the experiment differ in essential respects from the conditions existing in the living organism.

In these experiments the potassium butyrate was completely destroyed