

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

before glucose had disappeared from the system, although in the beginning they were present in the ratio of two molecules of the salt to one of glucose. This observation was unexpected, in view of the fact that under most known chemical conditions butyric acid is much more difficult to oxidize than glucose.

The rate at which the peroxide disappeared in the two experiments in each pair was about the same. That is, the peroxide was used up only a little faster in the experiment containing glucose and butyric acid than in that containing butyric acid alone.

### Experimental Part

**Potassium Phosphate.**—The experiments were set up as follows: (A) 30 g. of dipotassium phosphate, 500 cc. of 2.4% hydrogen peroxide, 25 cc. of butyric acid solution (0.25 g. in 5 cc.), 25 cc. of potassium hydroxide solution (5 cc.  $\equiv$  0.25 g. of butyric acid); (B) the same as (A) except that 1.25 g. of glucose was added.

These mixtures were allowed to stand for 20 days at room temperature after which they were analyzed by the methods previously described.<sup>1</sup>

**Analysis of (A).**—A 110cc. portion, equivalent to 0.25 g. of butyric acid, gave 0.0595 g. or a 35.2% yield of acetone and not more than a few per cent. of unchanged butyric acid. The volatile acid even at this stage was almost pure acetic acid; 0.1188 g. of carbon dioxide was obtained. The unchanged hydrogen peroxide was 1.088% (originally 2.18%).

Fifteen days later, 0.0442 g. or 26.8% of acetone, 0.0576 g. or 33.9% of acetic acid, 0.162 g. or 32.4% of carbon dioxide, no unchanged butyric acid and 0.633% of unchanged hydrogen peroxide were found.

The acetic acid formed was identified qualitatively by the ethyl acetate test and by its typical volatility curve.<sup>2</sup> A portion of a distillate was fractionated from a total volume of 225 cc. into four fractions of 50 cc. each and the 0.1 *N* sodium hydroxide required for titration of these fractions was as follows: (1) 0.95 cc., (2) 1.15 cc., (3) 1.25 cc., (4) 1.50, (residue) 2.06 cc., respectively. No indications of the formation of propionic acid were obtained.

**Analysis of (B).**—A 110cc. portion gave 0.0558 g. or 34.0% of acetone, some unchanged butyric acid mixed with acetic acid, 0.136 g. of carbon dioxide and 1.014% of unchanged hydrogen peroxide (2.18% originally present).

Fifteen days later, 0.0549 g. or 35.5% of acetone, no unchanged butyric acid, but 0.0754 g. or 44.4% of acetic acid, 0.146 g. of carbon dioxide and 0.442% of unchanged hydrogen peroxide were obtained.

Fifty cc. of this solution titrated for total oxidizable organic matter

<sup>1</sup> Witzemann, *J. Biol. Chem.*, **49**, 123 (1921).

<sup>2</sup> Witzemann, *THIS JOURNAL*, **41**, 1946 (1919).

by the method previously used<sup>3</sup> consumed 4.081 g. of potassium permanganate for the entire solution. This when calculated to glucose corresponds to 0.968 g. of glucose (1.25 g. of glucose used). Of course this is quite incorrect because the acetone and other oxidizable intermediates from butyric acid also reduce large amounts of permanganate. Qualitatively the solution gave a distinct test for sugar with Fehling's solution. By the Benedict-Osterberg quantitative sugar method<sup>4</sup> 0.02% or 0.110 g. of glucose was still present. This latter result shows that although there were about two molecules of butyric acid present originally to each molecule of glucose used, the glucose was still partly unoxidized after the butyric acid was all reacted.

**Sodium Phosphate.**—The experiments described above were repeated using 60 g. of disodium phosphate dodecahydrate instead of the potassium phosphate. After the mixtures had stood for 18 days at room temperature (A) on analysis gave 0.0307 g. or 18.7% of acetone, 0.145 g. or 58.0% of unchanged butyric acid and 0.995% of unchanged peroxide (2.13% originally present).

Mixture B on analysis gave 0.0258 g. or 15.7% of acetone, 0.148 g. or 59.4% of unchanged butyric acid and 0.986% of hydrogen peroxide. (B) contained 0.076% or 0.418 g. of glucose as determined by the Benedict-Osterberg method.

Besides proving that the effect of potassium phosphate is not specific, this experiment shows conclusively that the glucose and butyric acid are really burned simultaneously. It should be stated that the amount of unchanged butyric acid appears to be greater than it really is, owing to the fact that the small amount of acetic acid volatilized with the first fraction is necessarily calculated as butyric acid and may constitute as much as 15% of the acidity calculated as butyric acid in this experiment.

### Summary

It was found, when glucose and potassium butyrate are oxidized simultaneously with hydrogen peroxide in the presence of phosphates, that the oxidation of potassium butyrate is not influenced by the presence of glucose, that it is more quickly oxidized than glucose and that the glucose exercises no antiketogenic effect like that observed in the organism.

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<sup>3</sup> Witzemann, *J. Biol. Chem.*, **45**, 1 (1920).

<sup>4</sup> Benedict and Osterberg, *ibid.*, **34**, 195 (1918).