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STUDIES ON THE MECHANISM OF THE PROPTONIC ACID FERMENTATION

By

B. J. BACHMANN, S. F. CARSON,
D. S. ANTHONY, M. KUNA, M. LONG

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Biology Division

OAK RIDGE NATIONAL LABORATORY
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A paper presented at the Society of American Bacteriologists, Minneapolis, Minnesota, May 11, 1948.

This document is based on work performed under Contract Number W-7405-Eng-26 for the Atomic Energy Project at Oak Ridge National Laboratory

Date Received: 9/15/48 Date Issued: SEP 29 1948

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ABSTRACT

STUDIES ON THE MECHANISM OF THE PROPIONIC ACID FERMENTATION

by

B. J. Bachmann, S. F. Carson, D. S. Anthony, M. Kuna, M. Long

The mechanism of formation of propionic, acetic and succinic acids by Propionibacterium pentosaceum is under investigation. With the use of C^{14}-labelled bicarbonate and carboxyl-labelled acetate, intermediate substances between pyruvate and propionate have been isolated, but as yet are not pure enough to be identified. From the studies on succinate formation it seems evident that this end-product arises by at least two independent paths: from CO_2 plus a three-carbon intermediate, and as well by the Thunberg-Wieland Scheme of acetate condensation; the extent of each depends almost entirely upon the redox state of the fermentation, and this can be altered.

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STUDIES ON THE MECHANISM OF THE PROPIONIC ACID FERMENTATION

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B. J. Bachmann, S. F. Carson, D. S. Anthony, M. Kuna, M. Long

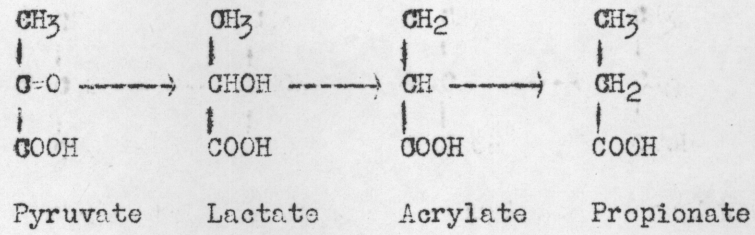
These studies were conducted on the propionic acid fermentation with Propionibacterium pentosacium using C^{14} as a tracer. The general over-all reactions of the propionic acid fermentation showing the dependence of the direction of the reaction upon the hydrogen balance, or the state of oxidation of the substrates, are on Slide I:

$$4 \text{ Glycerol} \longrightarrow 3 \text{ Propionate} + 1 \text{ Succinate} + 4H_2O$$

$$3 \text{ Lactate} \longrightarrow 2 \text{ Propionate} + 1 \text{ Acetate} + 1\ CO_2\ H_2O$$

$$3 \text{ Pyruvate} \longrightarrow 1 \text{ Propionate} + 2 \text{ Acetate} + 2\ CO_2$$

One of the older schemes proposed for the formation of propionic acid from pyruvic acid suggested lactic acid and acrylic acid as intermediates in the conversion. This scheme was discarded when it was not possible to show conversion of acrylate to propionate by the propionic acid bacteria and when it was confirmed by Barker and Lipmann that NaF inhibited the conversion of lactate to propionate but did not inhibit the conversion of pyruvate to propionate. Slide 2.



Previous tracer experiments (by Wood and Werkman, Carson and Ruben, et al.) have indicated that propionate is formed by the decarboxylation of C_4 compounds which arise by the process of CO_2 fixation or by condensation of intermediate products. Therefore it seemed desirable to set up an experiment which would show the probable equilibrium relationships between substrate-acetate-propionate and to test whether or not simple conversion of pyruvate to propionate and acetate occurs reversibly with the C_3 skeleton remaining intact between pyruvate and propionate. Using substrates at three stages of oxidation, the shift in equilibrium was traced with radioactive acetate and C^*O_2 as the amount of available hydrogen varied. Thus reactions which might appear obvious with one state of available hydrogen but not with another will be made clear by the shift in equilibrium.

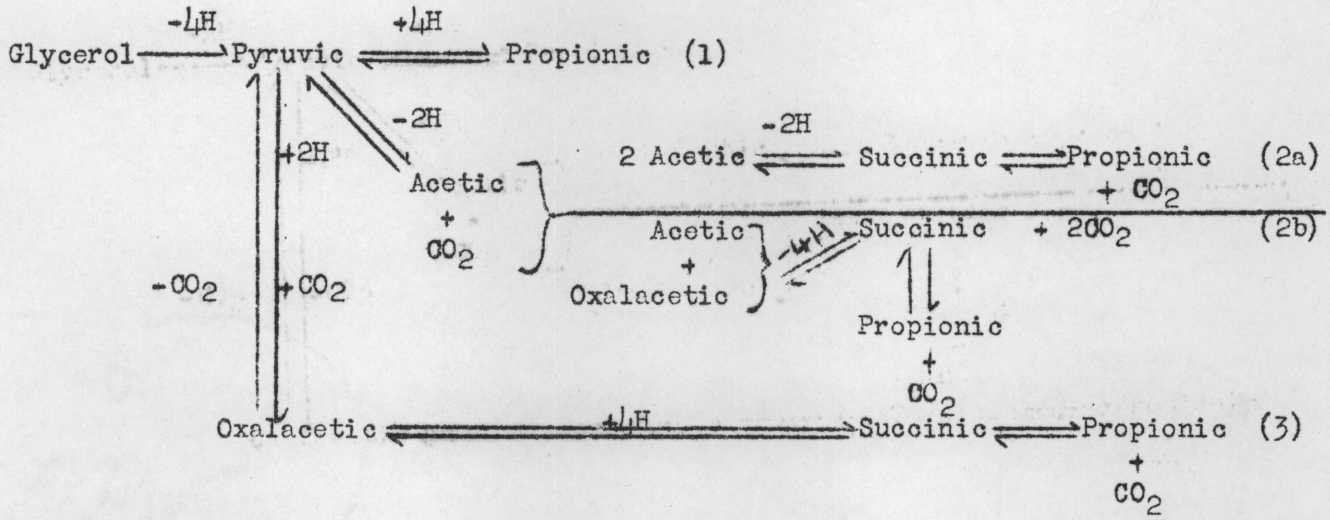
In the experiment, substrates of three states of oxidation (or available H) were used: glycerol, a highly reduced substrate; pyruvate, a highly oxidized substrate; and a 1:1 mixture of glycerol and pyruvate. Pyruvate is very probably an intermediate in the conversion of glycerol to propionate and acetate. Two fermentations were carried out with each substrate: one with carboxyl labelled acetic acid as a tracer and one with labelled CO_2 in the form of $NaHO^*O_3$ as a tracer. Slide 3.

Substrate	Glycerol		Glycerol Pyruvate		Pyruvate	
Reduction Value	14		12		10	
Tracer Added	H•Ac*	C^*O_2	H•Ac*	C^*O_2	H•Ac*	C^*O_2
% initial C* found as C^*O_2	2	66	6	73	33	58
Ratio Specific Act. $\frac{c/s}{m\,M}$ of $\frac{\text{Propionic}}{\text{Acetic}}$	$\frac{1}{100}$	$\frac{1}{3}$	$\frac{1}{33}$	$\frac{1}{1.7}$	$\frac{1}{4}$	$\frac{1}{0.5}$

With glycerol, a reduced substance, as substrate, the ratio of the specific activity of propionate formed to acetate remaining is 1:100; with a mixture of glycerol and pyruvate the proportion was 1:33, while with pyruvate as substrate the ratio was 1:4. Thus 25 times as much radioacetate was converted to radiopropionate in the presence of an oxidized substrate.

With glycerol as a substrate and C^*O_2 as a tracer, the ratio of the specific activity of propionate produced to that of acetate produced was 1:3. With a mixture of glycerol and pyruvate the ratio was 1:1.7 and with pyruvate as a substrate the ratio was 1:0.5. Thus six times as much radioactive C^*O_2 is converted to radiopropionate with an oxidized substrate. With glycerol as a substrate 2 per cent of the initial radioactivity added as HAc* was converted to C^*O_2; with a mixture of glycerol and pyruvate 6 per cent of the initial radioactivity appeared as C^*O_2; and with pyruvate as substrate 33 per cent of the original activity appeared as C^*O_2. This is an important indication that HAc* is metabolized through a condensation reaction which subsequently yeilds CO_2. Such a reaction would obviously not be apparent with glycerol as the substrate, but with the use of an oxidized substrate (pyruvate) it apparently assumes great importance in the equilibrium scheme.

On the basis of schemes suggested by earlier as well as the present tracer experiments, we have proposed a working scheme around which we can design a complete tracer experiment. Slide 4.



HAc* ⟶ Propionic*	Highest conversion with pyruvate				
HAc* ⟶ C^*O_2	"	"	"	"	
C^*O_2 ⟶ Propionic*	"	"	"	"	
C^*O_2 ⟶ Acetic*	"	"	"	"	

If reaction (1) occurs: it would yield propionate* from HAc* and propionate* from C^*O_2 only by reversal of the oxidation decarboxylation of pyruvate, therefore would <u>require</u> hydrogen, and would be expected to occur to the greatest extent with glycerol as the substrate.

However, the largest conversion of propionate* from HAc* occurred with pyruvate as the substrate, and also yielded C^*O_2. In addition, the production of propionate* from C^*O_2 was favored by pyruvate. Finally, the pyruvate remaining in these fermentations, (1/3 of the initial amount) did not contain any measurable amount of labelled C*. If reaction (3) goes, it would yield propionate* from C^*O_2 and would be favored by glycerol as a substrate. The production of propionate* from C^*O_2 was favored by pyruvate which is not what one would expect.

However, if reactions (2) and (3) were occurring simultaneously this would be possible. Reaction (2a) or (2b) would yield propionate* and C^*O_2 from HAc* and would be favored by pyruvate. Actually the largest conversion of propionate and C^*O_2 from HAc* occurred in the presence of pyruvate, indicating that reactions (2) were possible. Reaction (2) would proceed with the evolution of hydrogen which would allow reaction (3) to proceed. This would account for the greater production of propionate* from C^*O_2 in the presence of pyruvate since the hydrogen necessary to allow reaction (3) to proceed came from reaction (2).