CIII. PYRUVIC ACID IN THE DISSIMILATION OF GLUCOSE BY THE PROPIONIC ACID BACTERIA¹.

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ALTHOUGH Van Niel [1928] postulated that pyruvic acid is formed during the dissimilation of glucose by the propionic acid bacteria, the keto-acid has never been identified as an intermediate product. Van Niel assumed that pyruvic acid hydrate was formed and subsequently dehydrogenated to yield acetic acid, carbon dioxide and two atoms of hydrogen, the latter serving to reduce suitable hydrogen acceptors such as methylglyoxal to propionic acid.

This assumption was necessitated by the failure to demonstrate the conversion of acetaldehyde into acetic acid or the presence of acetaldehyde itself during the fermentation of glucose by the propionic acid bacteria, although acetaldehyde would be a normal product of the decarboxylation of pyruvic acid.

By assuming a dehydrogenation of pyruvic acid hydrate, the absence of acetaldehyde is accounted for.

Van Niel based his conclusion of the presence of pyruvic acid largely upon its ease of fermentation by the propionic acid bacteria with formation of propionic acid, acetic acid and CO₂ in substantially theoretically correct proportions.

Actual proof of the intermediate formation of pyruvic acid from glucose by propionic acid bacteria would evidently lend considerable support to Van Niel's theory of dissimilation of glucose by these organisms.

In the present communication we offer such proof in the form of the isolation and identification of pyruvic acid in the fermentation of glucose by *Propionibacterium arabinosum*.

EXPERIMENTAL.

The culture of *Propionibacterium arabinosum* used in this study was kindly furnished by Dr E. R. Hitchner of the University of Maine and was described in detail by Werkman and Brown [1933].

The fermentations were carried out in a medium of Bacto yeast extract 0.5 %, glucose 2.0 %, and calcium carbonate 2 %. Either sodium bisulphite (0.5 %) or calcium sulphite (1.0 %) was included in the medium as a fixative. Constituents of the medium were sterilised separately and mixed. A number of 500 ml. flasks each containing 300 ml. of medium were prepared and the fermentations carried out anaerobically by bubbling oxygen-free nitrogen through the medium. The inoculum was a five-day culture of the organism in glucose yeast extract broth added in quantity equivalent to 5 % by volume of

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the medium. Incubation was at 30°. Growth was luxuriant and the glucose was completely utilised by the tenth day of fermentation. The cultures were proven pure by microscopic examination and colony formation in shake tubes and on agar plates.

Both the sodium bisulphite and the calcium sulphite were effective in fixing pyruvic acid. The results were substantially similar with the two fixatives. The following experiment is typical. Tests for acetaldehyde following distillation of the medium according to the method of Peterson and Fred [1920] were negative. Upon addition of 2:4-dinitrophenylhydrazine to the medium an abundant precipitate was obtained which was dissolved in sodium carbonate solution and reprecipitated by acidification. After purification of the 2:4-dinitrophenylhydrazine precipitate, the melting-point was 212·0°; a mixture with authentic pyruvic acid 2:4-dinitrophenylhydrazone (M.P. 213° [cf. Allen, 1930]) melted at 212°.

A *p*-nitrophenylhydrazone was likewise prepared from the fermentation solution; it had M.P. 218·5° alone and 218·5° when mixed with authentic pyruvic acid *p*-nitrophenylhydrazone (M.P. 219°).

DISCUSSION.

This identification of pyruvic acid in the fermentation of glucose by *Propionibacterium arabinosum* supports the scheme of glucose dissimilation proposed by Van Niel. The facts that pyruvic acid is fixed during the fermentation and that no acetaldehyde can be detected do not indicate a decarboxylation of pyruvic acid. Carboxylase is reported by Neuberg and Reinfurth [1920] to act upon the bisulphite addition compound of pyruvic acid to form the acetaldehyde bisulphite compound, so that in the presence of carboxylase, fixation of pyruvic acid by sulphite should not prevent simultaneous formation of acetaldehyde. Isolation of pyruvic acid was successful in the present case, apparently owing to the inability of *P. arabinosum* to decarboxylate the pyruvic acid bisulphite compound which is not itself subject to hydration and subsequent dehydrogenation as is pyruvic acid.

The reason for the reported failure of Virtanen [1923] to secure growth of the propionic acid bacteria in the presence of sulphite is not apparent.

The authors found no account in the literature of a similar isolation of pyruvic acid from a sulphite-containing culture of actively proliferating bacteria, although Cook [1930] identified pyruvic acid in a fermentation by resting *Bact. coli* using NaHSO₃ as a fixative. This fact may well be the result of the unusual mechanism of the dissimilation of glucose brought about by the propionic acid bacteria in which acetaldehyde does not occur as an intermediate product. This mode of formation of acetic acid and carbon dioxide from pyruvic acid explains their formation in the ratio of 1:1, a ratio that the authors have not been able to alter.

With regard to the mode of formation of the pyruvic acid from glucose, the evidence is fragmentary; possibly pyruvic acid arises from the dehydrogenation of methylglyoxal hydrate or lactic acid. The latter substance probably occurs as an intermediate only under certain conditions when it is formed as a stabilisation product. Its occurrence has been frequent in large fermentations conducted in this laboratory.

SUMMARY.

Pyruvic acid has been isolated by fixation with sulphite in the fermentation of glucose by *Propionibacterium arabinosum*.

Acetaldehyde has not been detected in these experiments by the sulphite fixation method.

The scheme proposed by Van Niel for the intermediate breakdown of pyruvic acid is shown to be in agreement with experimental results.

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