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Fermentation of Pyruvic Acid by *Clostridium botulinum.**

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It has been reported[1] that the fermentation of glucose by Types A and B *Clostridium botulinum* differs from the majority of bacterial fermentations in that ethyl alcohol and carbon dioxide are the main products of the fermentation, only small amounts of acetic and lactic acids together with traces of hydrogen being formed. This study has been extended to include the fermentation of pyruvic acid, which may be an intermediate compound in the fermentation of glucose and also in the degradation of amino acids such as alanine, by *Cl. botulinum.*[2]

The experiments reported below were carried out with washed suspensions of Type A (E-43) *Cl. botulinum* although studies with Type B gave essentially the same results. Glucose-broth (800 ml) containing 0.1% Difco yeast extract was inoculated with 1.0 ml of a beef-brain culture of the organism and incubated for 20 hours at 37°C in a McIntosh and Fildes anaërobic jar. The culture was then centrifuged and the cells suspended in distilled water. The suspension was diluted with an equal volume of M/7.5 phosphate buffer and placed in the central chamber of Warburg vessels. A rapid stream of O_2-free H_2 was passed through the vessels for 10 minutes and they were then equilibrated 10 minutes before tipping in the sodium pyruvate from the side-arm. CO_2 production was determined at 37°C by the Warburg technic, the initial and final bound CO_2 being determined following the addition of 10% sulfuric acid to the contents of separate Warburg vessels. In the semi-macro experiments, Warburg vessels of 40 ml capacity were employed with Clerici fluid in the manometers.

It was observed that pyruvic acid is rapidly decarboxylated by washed suspensions of *Cl. botulinum,* Q_{CO_2} values of 25-30 being observed under optimal conditions. Typical results over a pH range of 5.6 to 7.5 are reported in Fig. 1. Following correction of the results for CO_2 bound by the phosphate buffer it is apparent that the pH optimum lies near 6.0. Accordingly the majority of the fermen-

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1 Clifton, C. E., *J. Bact.*, 1940, in press.

2 Clifton, C. E., PROC. SOC. EXP. BIOL. AND MED., 1939, **40**, 338.

tations were carried out at this pH although similar results were obtained at pH 7.0. The fermentation of glucose differs from that of pyruvic acid in that the rate is much less dependent on the pH over a range of 5.6 to 7.8.

In preliminary experiments ethyl alcohol and acetic acid were identified as the main non-gaseous products of the fermentation. Alcohol was determined by oxidation with bichromate following distillation from alkaline solution and volatile acids by steam-distillation of the acidified residue. Ethyl alcohol was identified by the iodoform test and by the fact that acetic acid appears to be the only acid produced on oxidation with bichromate. This acid and that produced in the fermentation was identified as acetic by Duclaux distillations of the combined steam-distillates from several large-scale experiments. No gas other than CO_2 was produced and the CO_2 liberated by control suspensions was negligible. In general about 5% of the pyruvic acid was reduced to lactic acid during the course of the fermentation.

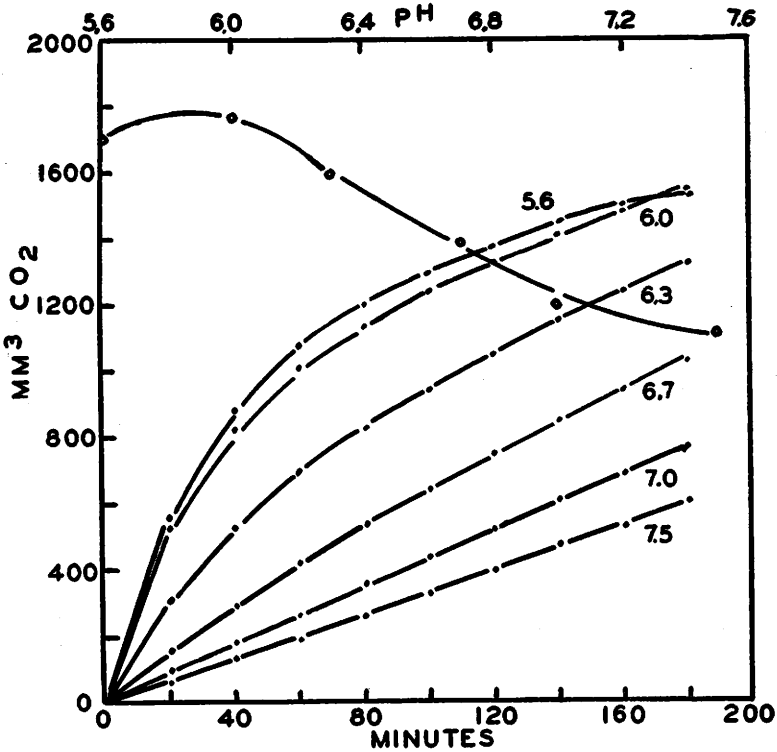

FIG. 1.

Influence of pH on the rate of CO_2 liberation during the fermentation of pyruvic acid by *Cl. botulinum*. O——O Total CO_2 produced, corrected for bound CO_2, from 0.2 ml M/2 Na pyruvate in 180 minutes.

TABLE I.

Pyruvate fermented	mg 44	Mols/ mol pyruvate	mg 44	Mols/ mol pyruvate
Products				
CO_2	19.4	.88	19.8	.90
CH_3CH_2OH	12.3	.53	13.0	.56
CH_3COOH	13.2	.44	13.8	.46
Total	44.9		46.6	

The results of typical duplicate semi-macro experiments at pH 6.0 are presented in Table I.

Similar results were obtained when the fermentation was carried out in an atmosphere of O_2-free N_2.

The results suggest that pyruvic acid is decarboxylated and the acetaldehyde produced dismuted with the production of equimolar quantities of ethyl alcohol and acetic acid. The above results are in fair agreement with this hypothesis since no correction was made for the small amounts produced by the organisms alone, the quantities being too small for accurate determination. In general the amounts of CO_2 recovered were somewhat less than the theoretical while the ethyl alcohol tended to run somewhat high. This tendency was also observed in macro experiments in which CO_2 production was determined by absorption of the liberated gas in standard $Ba(OH)_2$ solution. Possibly a small amount of CO_2 is utilized during the fermentation. It appears, therefore, that the fermentation of pyruvic acid may be represented as

$$2\ CH_3COCOOH + H_2O = 2\ CO_2 + CH_3CH_2OH + CH_3COOH$$

If the fermentation of glucose proceeds by way of pyruvic acid, dismutation of acetaldehyde may account for the small amount of acetic acid produced, the bulk of the acetaldehyde being reduced to alcohol by a H-donor other than another molecule of acetaldehyde.