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Acetylmethylcarbinol Enzyme-System of *Aerobacter aërogenes.*

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It is generally accepted that in the formation of acetylmethylcarbinol from pyruvic acid by such bacteria as *Aerobacter aërogenes,* the acid must be first decarboxylated to acetaldehyde. No direct evidence, however, has been presented that *Aerobacter* can bring about such a decarboxylation. The difficulty has been due, in part, to the simultaneous activity of hydroclastic enzymes, *i. e.,* enzymes splitting pyruvic acid into formic and acetic acids (*cf.* equation 1). The formic acid is cleaved into H_2 and CO_2. The formation of CO_2 by this reaction does not involve intermediary acetaldehyde.

Employing the technic of Wiggert, *et al.,*[1] an enzyme-preparation has been extracted from cells of *Aerobacter aërogenes,* with apparently no hydroclastic activity, nevertheless, capable of converting pyruvic acid into CO_2 and acetylmethylcarbinol. Typical fermentation-balances are shown in Table I. The ratio of pyruvate utilized to CO_2 and acetylmethylcarbinol formed is approximately 2:2:1.

Attempts to increase the carbinol yield by the addition of acetaldehyde were unsuccessful. The added aldehyde was recovered unchanged. If acetaldehyde is an intermediate, only the biologically nascent form is utilized by this enzyme-preparation.

As indicated in Table II no appreciable production of CO_2 and consequently no carbinol occurs at pH levels above 6.8. There is a direct relationship between CO_2 and carbinol production.

The relationship between pH and the direct cleavage of CO_2 from

TABLE I.
Dissimilation of Pyruvic Acid by Enzyme-preparation of *A. aërogenes.*
Values in millimoles.

Initial pyruvic acid	Final pyruvic acid	Pyruvic acid utilized	CO_2	Acetylmethyl-carbinol
9.17	1.60	7.57	7.63	3.30
9.17	1.69	7.48	7.42	3.40
4.99	1.08	3.91	3.62	1.61
2.49	0.16	2.33	—	1.09

3 ml enzyme preparation-vol. 30 ml-5 ml. M/15 KH_2PO_4-atmosphere N_2-30°C.

1 Wiggert, W. P., Silverman, M., Utter, M. F., and Werkman, C. H., *Iowa State Coll. J. Sci.*, 1940, **14**, 179.

TABLE II.
Relationship Between pH and Rate of CO_2 Formation from Pyruvic Acid by Enzyme-preparation of *A. aërogenes.*

pH	3.5	5.6	6.0	6.4	6.8	7.2	7.6	8.0
mm^3 CO_2 in 15 min.	170	122	119	76	25	11	4	9
V.P. test	4+	4+	4+	4+	2+	?	—	—

.15 ml juice-vol. 2.0 ml-1.8 mg pyruvic acid-1 ml PO_4 buffer M/15-atmosphere air.

pyruvic acid by *Aerobacter* will assist in elucidating the mechanism of pyruvate-breakdown. From the investigations of Mickelson and Werkman[2] and from Table II, the carbinol is not formed at alkaline reactions and the predominating mechanism involves reaction I. Under acid conditions both reactions occur. Mickelson[3] has shown that the products of the alkaline fermentation of pyruvate by *Aerobacter* are acetic and formic acids in equimolar proportions.

$$(1)\quad CH_3COCOOH + HOH \longrightarrow HCOOH + CH_3COOH$$

$$HCOOH \searrow H_2 + CO_2$$

$$(2)\quad 2\ CH_3COCOOH \longrightarrow 2\ CO_2 + CH_3COCHOHCH_3$$

These results may be checked manometrically with cell suspensions as shown in Table III. As the system is made alkaline, more H_2 should be evolved as reaction 2 becomes inhibited. At pH 8.0 the CO_2/H_2 ratio closely approaches the theoretical value of 1.0.

The presence of inorganic phosphate has been found to be essential for pyruvate-breakdown by this enzyme-preparation.

TABLE III.
pH and the Gas Ratios of *A. aërogenes* (whole cells) on pyruvate.

pH	4.5	5.6	6.8	8.0
mm^3 H_2	164	208	335	370
mm^3 CO_2	426	449	445	398
CO_2/H_2	2.60	2.16	1.33	1.08

Vol. 2.3 ml-atmosphere N_2-cell 24 hours old-2.2 mg pyruvic acid.

2 Mickelson, Milo, and Werkman, C. H., *J. Bact.*, 1938, **36**, 67.

3 Mickelson, Milo, unpublished thesis, Iowa State College, 1939.