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Fermentation of Pyruvic Acid by Bacteria of the Colon-Aerogenes Group.*

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In view of the intermediary rôle assigned to pyruvic acid, an understanding of its behavior is of importance in formulating schemes of dissimilation for bacteria of the colon-aerogenes group. The anaerobic dissimilation of pyruvic acid by organisms of this group is discussed in the present work.

Aubel[1] and de Graff and Le Fevre[2] report the products of the fermentation of pyruvic acid by *Escherichia coli* to be acetic and formic acids, carbon dioxide and hydrogen. Acetaldehyde is reported to be an intermediate.

Reynolds,[3] fermenting glucose with *Esch. coli* under conditions of forced aeration, recovered much of the carbon as pyruvic acid with corresponding decreases in lactic acid. The rise and fall of pyruvic acid during the fermentation of glucose by *Citrobacter freundii* further substantiates its position as an intermediate.

Cells were prepared by centrifuging 24-hour cultures, washing once with physiological salt solution, and resuspending in enough solution to make a paste. A medium consisting of 10 gm. glucose, 8 gm. peptone, 5 gm. disodium phosphate per liter gave good growth. The medium was sterilized at 20 pounds for 30 minutes. Phosphate buffer was sterilized separately and added to the glucose and peptone after autoclaving. Incubation took place at 30°C. The cultures were identified strains used for several years in this laboratory: *Aerobacter indologenes,* strain 23B, isolated and described by Burkey[4]; *Esch. coli,* American Type Culture Collection No. 26; and *C. freundii,* strain described by Werkman and Gillen.[5]

The dissimilations were conducted anaerobically in 300 ml. of 0.1M phosphate buffer at pH 6.2. The flask and absorption-train were flushed with oxygen-free nitrogen before inoculation.

Carbon dioxide was absorbed in Bowen potash bulbs and weighed.

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1 Aubel, E., *Bull. de la Soc. Chim. Biol.*, 1924, **6**, 288.

2 de Graff, W. C., and Le Fevre, A. J., *Biochem. Z.*, 1925, **155**, 313.

3 Reynolds, H., unpublished thesis, Iowa State College, 1935.

4 Burkey, L. A., *Iowa State Coll. J. Sci.*, 1928, **3**, 57.

5 Werkman, C. H., and Gillen, F. G., *J. Bact.*, 1932, **23**, 167.

An aliquot of the fermentation-liquor was acidified to congo red with 25% H_2SO_4, and residual carbon dioxide was determined by heating and aerating under a reflux condenser for an hour. Hydrogen was determined with the Hempel buret.

Before analysis of the liquor, the cells were removed by adding 1 ml. of phosphotungstic acid (20% in 5% H_2SO_4) per 20 ml. of medium and centrifuging. Acetic and formic acids were determined according to Osburn, Wood, and Werkman[6] on the steam distillate of an aliquot. Lactic acid was determined by the method of Friedemann and Kendall[7] and 2,3-butylene glycol according to Brockmann and Werkman.[8]

Purity of the cultures was established by examination of gram-stained smears before and after fermentation.

Controls of cells and buffer only were run and the small yields of products from cells were subtracted.

TABLE I.
Dissimilation of Pyruvic Acid by *Esch. coli.**

	Pyruvic	Acetic	Lactic	CO_2	H_2
Initial	65.46				
Final	0	51.26	15.60	47.46	34.41

*Products expressed as millimoles per liter.

The results indicate (Table I) pyruvic acid to be a precursor of lactic acid. The difference in yield of carbon dioxide and hydrogen is equivalent to that used in reducing the pyruvic acid to lactic acid. The results suggest the following breakdown:

(1) $CH_3COCOOH + HOH \rightarrow CH_3.C.(OH)_2.COOH \rightarrow CH_3COOH + HCOOH$
(2) $HCOOH \rightarrow H_2 + CO_2$

Although de Graff and Le Fevre[2] accept acetaldehyde as an intermediate in the decomposition of pyruvic acid by organisms of the colon-typhoid group, subsequently they report the products as acetic and formic acids, carbon dioxide and hydrogen. Whetham[9] presents evidence for the same products. *Esch. coli* possesses an active formic acid-splitting enzyme. Hydrogen thus liberated serves to reduce suitable acceptors such as pyruvic acid. *Esch. coli* lacks the enzymic equipment necessary to use the hydrogen to reduce 2 mole-

[6] Osburn, O. L., Wood, H. G., and Werkman, C. H., *Ind. and Eng. Chem.* (Anal. Ed.), 1933, **5**, 247

[7] Friedemann, T. E., and Kendall, A. I., *J. Biol. Chem.*, 1929, **82**, 23.

[8] Brockmann, M. C., and Werkman, C. H., *Ind. and Eng. Chem.* (Anal. Ed.), 1933, **5**, 206.

[9] Whetham, M. D., *Aust. J. Exp. Biol. and Med. Sc.*, 1927, **4**, 35.

cules of acetic acid to 2,3-butylene glycol through acetylmethylcarbinol. The case is different with *Aerobacter indologenes.*

TABLE II.
Dissimilation of Pyruvic Acid by *Citrobacter freundii.**

	Pyruvic	Acetic	Lactic	Formic	CO_2	H_2
Initial	48.16					
Final	0	39.89	6.00	14.51	32.09	28.71

*Millimoles per liter.

The results (Table II) with *C. freundii* suggest the same mechanism of breakdown as for *Esch. coli.* In this fermentation, however, not all of the formic acid was decomposed. Six millimoles of pyruvic acid have been reduced to lactic acid; the remainder oxidized to acetic acid, and formic acid which is split into CO_2 and H_2. *C. freundii* is unable to bring about the reduction of acetic acid to 2,3-butylene glycol.

TABLE III.
Dissimilation of Pyruvic Acid by *Aerobacter indologenes.**

	Pyruvic	Acetic	2,3-B.G.	AMC	CO_2	H_2
Initial	50.60					
Final	0	28.8	9.4	—	48.54	24.85

*Millimoles per liter.

The differentiating characteristic of *Aerobacter indologenes* is its property of condensing and reducing acetic acid to 2,3-butylene-glycol through acetylmethylcarbinol. This utilization of hydrogen results in the ratio of $CO_2:H_2 = 2$ instead of 1 which is characteristic of *Esch. coli.* Thus it is apparent that the gas-ratio and the Voges-Proskauer test (for amc.) so widely used in differentiating the genera, are expressions of the same basic mechanism of dissimilation.

In Table III, 18.8 mM of acetic acid (or a precursor) have been reduced to 2,3-butylene-glycol requiring 28.2 mM hydrogen.