

and sulphuric acid, at room temperature for 15 days, penta-acetylglucose was obtained in 25 per cent yield.

The production of cellulose from ethylene glycol by *A. acetigenum* is of particular interest in view of a suggestion of Kluyver that in certain cases glycollic aldehyde may be polymerized to sugar by bacteria; compare also the suggestion of Hibbert and Perry<sup>6</sup> that glycol may be the precursor of carbohydrates in Nature. Wirth and Nord<sup>7</sup> detected pyruvic acid in cultures of a *Fusarium* species on pentoses. They assumed the other fission product of the pentose molecule to be glycollic aldehyde, but they did not succeed in isolating the latter. During this bacterial synthesis of cellulose from glycol, it was found possible to trap glycollic aldehyde and to characterize it as the 2:4-dinitrophenylosazone, m.p. above 310°. The bacterial conversion of glycerol to cellulose involved the formation of dihydroxyacetone. This was characterized as the phenylosazone, m.p. and mixed m.p. 132°, and also as the 2:4-dinitrophenylosazone, m.p. and mixed m.p. 284°.

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<sup>1</sup> Brown, *J. Chem. Soc.*, 49, 432 (1886).

<sup>2</sup> Hibbert and Barsha, *Can. J. Res.*, 5, 580 (1931).

<sup>3</sup> Tarr and Hibbert, *Can. J. Res.*, 4, 372 (1931).

<sup>4</sup> Barsha and Hibbert, *Can. J. Res.*, 10, 170 (1934).

<sup>5</sup> Khouvine, *C.R. Acad. Sci. Paris*, 196, 1144 (1933).

<sup>6</sup> Hibbert and Perry, *Can. J. Res.*, 8, 102 (1933).

<sup>7</sup> Wirth and Nord, *Arch. Biochem.*, 1, 143 (1942).

### Detection of Acetic Acid in *A. niger* Growing on a Glucose Medium

MANY different views have been expressed concerning the chemical steps by which certain species of *Aspergillus* and of *Penicillium* convert glucose into citric acid. Some of the earlier hypotheses are now only of historical interest, and those modern views for which a reasonable amount of experimental support can be adduced have one feature in common, namely, the presumption that acetic acid is an essential intermediate. Up to the present time, however, the occurrence of this acid in cultures of *A. niger* on sugar does not appear to have been demonstrated. We have made many attempts to detect its presence in the metabolism solution, but without success, an outcome not entirely unexpected in view of the ease with which *A. niger* metabolizes acetic acid in the presence of sugar (compare Pfeffer<sup>1</sup>).

We succeeded, however, in demonstrating the presence of this acid in the mycelial mat of young cultures at the time when formation of citric acid had just commenced. Acetic acid was separated from the mycelium after the cells had been ruptured by grinding them with sand. Using three strains of *A. niger*, we obtained in each case only about 0.3 gm. of the acid from mycelial mats grown, in the separate experiments, on 5 litres of culture liquid. The latter consisted initially of a 12 per cent solution of glucose plus the requisite inorganic salts. A fourth strain of *A. niger* gave much higher yields, and on two occasions similar experiments afforded 2.35 gm. and 2.60 gm.,

respectively, of acetic acid. It was characterized as the silver salt and also as the *p*-nitrobenzyl ester, m.p. and mixed m.p. 78°.

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### Utilization of Citric Acid by some So-called Citrate-non-Utilizing Bacteria

KOSER<sup>1</sup> showed that all types of *B. coli* are capable of growth in a simple medium in which ammonium phosphate constitutes the source of nitrogen, and a fermentable carbohydrate such as glucose or lactose is the source of carbon; when citric acid is substituted for the carbohydrates, growth occurs only with the sub-group known as *Aerobacter-Cloacae*. Growth in such medium, called Koser's test, has since become one of the basic criteria in differentiating the Koser-negative *E. coli* from the Koser-positive members of the *Escherichia* tribe.

Certain laboratory and clinical facts led us to surmise that in presence of nitrogen compounds, other than ammonium phosphate, citric acid might be utilized even by Koser-negative organisms. In order to test this hypothesis, a typical *E. coli* and a typical *E. aerobacter* were cultured in a variety of media to all of which citric acid (30 mgm. per 100 c.c.) was added. Citric acid was estimated by a modification of the pentabromacetone method of Pucher, Sherman and Vickery<sup>2</sup>; and a decrease in citric acid content of the culture flasks (as compared with a sterile medium control) was taken as the index of utilization of citric acid. Cultures and controls were incubated both at room and incubator temperatures for a period varying from 1 to 4 days.

It was first found that, despite good growth, *E. coli* does not utilize appreciable amounts of citric acid in a glucose-ammonium phosphate medium (0.1 per cent ammonium phosphate with 0.2 per cent glucose); thus the presence of a readily available source of carbon does not induce *E. coli* to utilize citric acid. Moreover, it was found that in this medium *E. aerobacter*, which utilizes citric acid in the absence of glucose, does not attack citric acid until practically all the glucose has been broken down. In a basic medium of 0.1 per cent asparagine in saline (*plus* citric acid) growth of *E. coli* is not much heavier than in the glucose-ammonium phosphate medium; however, it is found that in 48 hours, 15 per cent of the citric acid has been used by *E. coli* and 94 per cent by *E. aerobacter*. Identical figures were obtained when the asparagine content was raised to 1 per cent. On the other hand, in 2 per cent peptone water (B.D.H. peptone) 94 per cent of citric acid was used up by *E. coli* but only 60 per cent by *E. aerobacter*. Under similar conditions a strain of *Staphylococcus aureus* did not utilize citric acid.

It is thus clear that the failure of *E. coli* to grow in Koser's medium does not result from a fundamental incapacity of the organism to use citric acid either as a sole or additional source of carbon, since *E. coli* has been proved to be capable of utilizing citric acid under suitable conditions. It is thought that such conditions are provided by an appropriate