

NOTES

INHIBITION OF THE ANAEROBIC PYRUVATE DISSIMILATION IN *ESCHERICHIA COLI* BY DIHYDROSTREPTOMYCIN¹

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Received for publication December 1, 1950

The inhibition by streptomycin (and by dihydrostreptomycin) of the terminal aerobic respiration system in *Escherichia coli* has been studied by Oginsky, Smith, and Umbreit (J. Bact., **58**, 747, 1949). The present note concerns the effect of dihydrostreptomycin (DSM) on the metabolism in a typical strain of *E. coli* obtained from the departmental stock culture collection. As observed by Oginsky *et al.*, DSM inhibited the terminal steps in aerobic carbohydrate oxidation. In addition, DSM also inhibited the anaerobic dissimilation of pyruvate.

The cells were grown for 18 to 20 hours in a glucose synthetic medium (Anderson: Proc. Natl. Acad. Sci. U.S., **32**, 120, 1946) and harvested by centrifugation. They were washed in distilled water and kept in the refrigerator for 1 to 5 days before use. The CO₂ production from glucose and from pyruvate was determined manometrically under anaerobic conditions in bicarbonate buffer at pH 7.4 in the absence and presence of DSM. One mg of Difco yeast extract per ml did not increase the low endogenous respiration but greatly accelerated the fermentation of pyruvate, and was therefore added to all samples. Approximately 2 moles of CO₂ were produced per mole of glucose. DSM in a concentration of 250 μg per ml inhibited only slightly the CO₂ production from glucose. The CO₂ production from pyruvate, however, was always inhibited from 75 to 90 per cent by 100 μg DSM per ml. In the absence of DSM approximately 1 mole of CO₂ was produced per mole of pyruvate in bicarbonate buffer, but only about 0.25 moles of CO₂ were formed in phosphate buffer at the same pH.

Analysis for end products of pyruvate fermentation indicated that for each mole of pyruvate utilized, 0.75 moles were converted to acetate and formate, and 0.25 moles were decarboxylated to a nonacidic end product. No H₂ and only a trace of lactate was produced. Thus the main reaction appears to be the phosphoroclastic splitting of pyruvate to formate and acetate (Kalnitsky and Werkman: Arch. Biochem., **2**, 113, 1943). In the presence of 100 μg DSM per ml, the formation of acetate and formate was inhibited approximately 75 per cent, and the formation of metabolic CO₂ was inhibited by more than 90 per cent.

The CO₂ production from glucose and from pyruvate in bicarbonate buffer by a streptomycin-resistant and a streptomycin-dependent variant of the same strain of *E. coli* was also studied manometrically. Both variants produced CO₂

¹ This work was done on a U. S. Public Health Service Fellowship in the laboratories of Drs. J. W. Moulder and C. P. Miller. It was aided in part by the Office of Naval Research.

from glucose in the absence and presence of 100 μg DSM per ml at about the same rate as the parent strain, but they fermented pyruvate very slowly. Although the parent strain produced approximately 9 μM CO_2 from 10 μM pyruvate in 60 minutes, the variants produced less than 3 μM in the same time. DSM in a concentration of 100 μg per ml had no appreciable effect on the CO_2 production by the mutant strains.

Experiments on the growth of the parent strain *E. coli* demonstrated that, although DSM did not inhibit the anaerobic CO_2 production from glucose to any extent, the cells could not grow anaerobically in glucose synthetic medium in the presence of DSM. The dependent variant did not require DSM for the anaerobic dissimilation of glucose, but required the drug for growth in glucose synthetic medium. Viable counts of the resistant strain showed that it grows equally well with and without DSM in glucose synthetic medium.

It appears that DSM inhibits some terminal step in both aerobic and anaerobic carbohydrate dissimilation, but no conclusions can be made at present as to the primary inhibitory effect of DSM.

THE POSITION OF BACILLUS KANDIENSIS VAR. KANDIENSOIDES (CASTELLANI)

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Received for publication December 11, 1950

In the last edition of *Bergey's Manual* a number of organisms are listed as belonging to the former genus *Eberthella*, which is no longer regarded as a valid genus. It might therefore be of interest to the taxonomist to compare organisms of this group in order to determine their position in the system of classification now being used in various parts of the world.

Recently, through the courtesy of Prof. A. Castellani, Instituto de Medicina Tropical, Lisbon, the writer obtained an authentic strain of *Bacillus kandiensis* var. *kandiensoides* (Castellani) from Prof. Castellani's culture collection in London. The organism was considered a variant of *B. kandiensis*, isolated from cases of diarrhea in Ceylon by Castellani in 1912 and named after Kandy, the place of the first isolation (*Zentr. Bakt. Parasitenk.*, I, Orig., **65**, 262, 1912). The species later was placed in the genus *Eberthella* (Castellani and Chalmers: *Manual of tropical medicine*, 3d ed., 1919).

The culture obtained revealed the following cultural and biochemical characteristics. After plating on Endo's medium two kinds of colonies were seen: (a) colorless, round, glistening colonies, 1.5 to 2 mm in diameter, turning pinkish after prolonged incubation at room temperature; and (b) more reddish

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