FRUCTOSE UTILIZATION BY PSEUDOMONAS PUTREFACIENS[1]

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In a previous communication concerning the mutation of *Pseudomonas putrefaciens* to glucose utilization, it was reported that neither wild type nor glucose-utilizing mutant cells were capable of oxidizing fructose. Furthermore, the hexokinase of the glucose-oxidizing mutant was found to be specific for glucose; fructose could not serve as a phosphate acceptor. As a result of these observations, and because repeated attempts to isolate a fructose mutant proved unsuccessful, it was concluded that "free fructose is completely unavailable to *P. putrefaciens*" (Klein and Doudoroff: J. Bact., **59**, 739, 1950).

Subsequent experiments, however, have revealed that fructose can be metabolized by this organism if this sugar is present in high concentrations. At concentrations below 0.005 M, there is no observable oxidation of fructose, and even at a concentration of 0.01 M the rate of oxidation is less than twice that of the endogenous respiration. As the substrate concentration is increased, however, there is observed a strict proportionality between the concentration of this hexose and the rate of its oxidation. This relationship is not found in the oxidation of other sugars tested (sucrose, glucose, and maltose). With all the other sugars whose oxidation by *P. putrefaciens* has been studied, the rate of oxidation is independent of substrate concentration until this concentration reaches extremely small values (of the order of 0.0001 to 0.0005 M).

Fructose oxidation also differs from the oxidation of the other sugars studied by its nonadaptive nature. Irrespective of whether cells are grown in sucrose or in nutrient broth, fructose oxidation proceeds at a steady rate from the point of substrate addition.

That fructose, and not some impurity, is oxidized under these conditions has been chemically verified by observing fructose disappearance during its oxidation by resting cell suspensions.

These results pointed to the possibility of growing *P. putrefaciens* in a synthetic medium provided with high concentrations of fructose. Accordingly, *P. putrefaciens* was inoculated into the synthetic medium used in the previous study. Cultures containing less than 1 per cent fructose showed no increase in turbidity over a period of incubation of 7 days, but with the highest fructose concentration tested (3 per cent), there was a tenfold increase in turbidity during this time interval.

It is apparent, therefore, from these observations that fructose, when pro-

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vided at high concentrations, is metabolized by *P. putrefaciens*. The pathways involved in the degradation of fructose by this organism have not been elucidated. Determinations of the respiratory quotient for the oxidation of fructose by resting cell suspensions disclosed a value of 1.0 for the process, thereby eliminating from consideration any incomplete oxidative pathway for fructose. We have been unable to demonstrate any hexokinase activity, using lyophilized or dried cell preparations, even at fructose concentrations as high as 1 M. The possibility of the formation of a labile fructose ester (e.g., fructose-1-phosphate) rather than fructose-6-phosphate was also investigated, and no evidence for its formation could be obtained.