force for phosphate uptake in this organism (Mitchell, 1954).

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The Mechanism of Energy Coupling in the Active Transport of Amino Acids by *Staphylococcus aureus*

By D. F. NIVEN and W. A. HAMILTON (*Department of Biochemistry, University of Aberdeen, Aberdeen AB9 1AS, U.K.*)

In an attempt to elucidate the mechanism of energy coupling to the transport of amino acids by *Staphylococcus aureus*, a non-kinetic approach has been employed.

The cells were preincubated for 3 h in phosphate buffer to diminish endogenous metabolism and the amino acid pool to low and constant values. This minimized errors due to exchange or isotope dilution in the pool. The intracellular pool volume was calculated by balancing H^+ and K^+ concentrations in the presence of nigericin to obtain a measure of the intracellular K^+ concentration, and by using flame photometry to obtain the K^+ content of the cells. A value of 1.55 ± 0.05 ml/g dry wt. of bacteria was obtained, which was in good agreement with the value given by Mitchell (1954). The uptake of L-[^{14}C]isoleucine into the cold-trichloroacetic acid-soluble pool was measured after a predetermined incubation period, and the results were expressed as intracellular concentration.

In the absence of added glucose as an energy source, facilitated diffusion caused the equilibration of extracellular and intracellular concentrations over the range 5–100 μM . The addition of glucose resulted in active transport with accumulation ratios of up to 20.

The effect of uncouplers and ion-specific antibiotics on this system has been studied. Carbonyl cyanide *m*-chlorophenylhydrazone, gramicidin, tetrachlorosalicylanilide and trichlorocarbanilide, which are all known to increase the permeability of bacterial membranes to protons (Hamilton & Jeacocke, 1972), inhibit the cells' ability to accumulate isoleucine by active transport without affecting the mechanism of facilitated diffusion. With valinomycin maximal activity against active transport was dependent on the presence of K^+ .

Preliminary results indicate that the same pattern of sensitivity to these agents is found with cells accumulating isoleucine under anaerobic conditions.

These results are consistent with the hypothesis that the driving force for the active transport of

amino acids in *S. aureus* is in equilibrium with the oxidoreduction energy of the electron-transport chain and the hydro-dehydration energy of the membrane adenosine triphosphatase, and that the protonmotive force demonstrated in this organism (Jeacocke *et al.*, 1972) is the intermediate energy state linking electron transport, ATP synthesis and active transport.

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Properties of *Escherichia coli* Mutants with Alterations in Glucose Uptake

By H. L. KORNBERG and R. E. REEVES (*Department of Biochemistry, School of Biological Sciences, University of Leicester, Leicester LE1 7RH, U.K.*)

The uptake of fructose by fructose-grown *Escherichia coli* is strongly inhibited by glucose (Kornberg, 1972); in many strains, non-catabolizable analogues of glucose such as 2-deoxyglucose and α -methyl glucoside exert a similar effect and hence act as powerful inhibitors of growth on fructose. A mutant K2.1.22a was selected by spreading strain K2.1t (Brice & Kornberg, 1967) on plates containing 5 mm-fructose and 5 mm-2-deoxyglucose as carbon source: unlike its parent organism, this mutant grew very poorly on glucose, although its growth on glucose 6-phosphate, fructose or gluconeogenic substrates was unimpaired; it also failed to take up ^{14}C -labelled α -methyl glucoside. Genetic analysis (Kornberg, 1972) indicates that it lacks that component of the phosphoenolpyruvate-dependent phosphotransferase system (Kundig *et al.*, 1964) that specifies the uptake of α -methyl glucoside and of a major fraction of the total quantity of glucose taken up by wild-type organisms. Secondary mutants and revertants were selected by incubating cultures of mutant K2.1.22a on plates containing 10 mm-glucose as sole carbon source. One of these further mutants, designated mutant R5s, was chosen for further study, as it still lacked the ability to take up ^{14}C -labelled α -methyl glucoside, and differed from both its parent and its grandparent organisms in that its growth on glucose exhibited a strong concentration-dependence: whereas mutant K2.1t grew on 0.5 mm-glucose nearly as rapidly as it did on 25 mm-glucose, mutant R5s required at least 5 mm-glucose to achieve half-maximal growth rate. This behaviour was reflected also in the amounts and properties of the glucose phosphotransferase system(s) present in these organisms. These system(s) were assayed spectrophotometrically.

metrically in whole cells decyptified with toluene (Gachelin, 1969), by linking the phosphoenolpyruvate-dependent phosphorylation of glucose to the reduction of NADP⁺ in the presence of glucose 6-phosphate dehydrogenase, or by linking the release of pyruvate, consequent on the phosphoenolpyruvate-dependent phosphorylation of glucose or of glucose analogues, to the oxidation of NADH in the presence of lactate dehydrogenase. Both procedures gave similar rates of reaction and, when added glucose was limiting, both assay systems reflected equivalent nucleotide changes on a molar basis.

It was found that mutant K2.1t contained a highly active phosphotransferase system for glucose with K_m below 25 μ M, the activity of which was not significantly affected by changes in glucose concentration between 0.1 and 50 mM. This system was present but in greatly decreased amounts in both mutant K2.1.22a and mutant R5s. However, only mutant R5s contained a second, inducible, phosphotransferase system for glucose, with K_m in the region of 10 mM. Since this value is close to the glucose concentration at which mutant R5s grows at half-maximal rate, it is probable that this mutant, which still lacks the system that effects the uptake of α -methyl glucoside, contains a component of fraction II of a phosphotransferase system (Kundig & Roseman, 1971) sufficiently altered in its sugar specificity to permit the uptake of glucose when this hexose is present at high concentrations.

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The Control of Glycerol Utilization by Glucose Metabolism

By W. EDGAR, I. S. FORREST, W. H. HOLMS and B. JASANI (*Department of Biochemistry, University of Glasgow, Glasgow W.2, U.K.*)

Escherichia coli strain ML308 shows diauxic growth on a mixture of glucose and glycerol in simple defined media under aerobic conditions. When cells have been trained to glycerol and are growing on an excess of this substrate, the addition of even a small amount of glucose inhibits almost immediately

synthesis of glycerokinase. Glycerol utilization falls by about 67% at the time of glucose challenge and continues at this low rate for about 50 min, when it ceases altogether, and it does not start again until glucose is exhausted. If the amount of glucose added is sufficiently large, a diauxic lag can be seen before growth on glycerol recommences. After glucose challenge the glycerol carbon continues to enter protein, nucleic acid and lipid with a slight increase in the proportion entering the last. The delay before glycerol utilization is completely shut off suggests that this process requires protein synthesis. This is supported by the observation that addition of glucose to washed-cell suspensions metabolizing glycerol depresses the rate of glycerol metabolism but never abolishes it.

These phenomena are a clear example of catabolite inhibition (McGinnis & Paigen, 1969). In this case glycerol metabolism could be depressed by fructose 1,6-diphosphate inhibition of glycerokinase (Lin & Zwaig, 1968). However, fructose 1,6-diphosphate at 10 mM and above inhibits glycerokinase of this strain to a maximum of 93%. In cultures growing on glucose, glycerol or both, direct measurement shows the intracellular concentration to be about 3 mM in all cases. It would seem that the total inhibition of glycerol metabolism cannot stem from glycerokinase inhibition by fructose 1,6-diphosphate, but possibly involves antagonism of glycerol permeation across the bacterial membrane.

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Effect of *n*-Alkanes on the Transport of Glucose in *Candida* sp. Strain 107

By C. O. GILL and C. RATLEDGE (*Department of Biochemistry, University of Hull, Hull HU6 7RX, U.K.*)

Candida sp. strain 107 is a hydrocarbon-utilizing yeast that has a propensity for producing large amounts of intracellular lipid (Ratledge, 1970). Addition of *n*-alkanes (C₁₃ to C₁₅ in chain length) to this yeast growing exponentially on glucose resulted in the immediate formation of oxidation products of the alkanes and a decrease in glucose consumption (Ratledge, 1968). The constitutive nature of the *n*-alkane-oxidizing system has been confirmed by use of an oxygen electrode: washed suspensions of the yeast grown on either glucose or *n*-alkanes both immediately oxidized all individual alkanes from C₁₀ to C₁₈.

We have subsequently investigated the effect that *n*-alkanes have on glucose utilization. Both alkane- and glucose-grown cells immediately and rapidly