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*

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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

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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.