We have demonstrated the release from respiratory control in membrane particles from *Micrococcus denitrificans* by carbonyl cyanide *m*-chlorophenylhydrazine and combinations of gramicidin, valinomycin, nigericin, monensin and dianemycin (John & Hamilton, 1970, 1972). From these findings it has been concluded that the increases in membrane permeability show ion specificity identical with that demonstrated in other systems (Henderson *et al.*, 1969). Tetrachlorosalicylanilide and trichlorocarbanilide have also been found to be proton-translocating uncouplers. Studies of protoplast and spheroplast stability in hyperosmotic media (Hamilton, 1970) and the decay of an acid pulse (W. A. Hamilton, unpublished work) have allowed us to extend these findings to the organisms *Bacillus megaterium*, *Micrococcus lysodeikticus*, *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa*.

The behaviour of trichlorocarbanilide is somewhat anomalous, in as much as it apparently increases both H^+ and Cl^- permeability. Accordingly this compound has been further studied with black lipid membranes formed from oxidized cholesterol or phosphatidylcholine (lecithin) + cholesterol.

In KCl media, trichlorocarbanilide caused a time-dependent increase in membrane conductance. This effect was decreased by the replacement of chloride by sulphate or acetate, but was unaffected by the replacement of KCl by NaCl. In the presence of trichlorocarbanilide, anionic diffusion potentials were observed across these membranes in concentration gradients of NaCl or K_2SO_4 . This confirms the increase in anion permeability, since untreated membranes demonstrated cation diffusion potentials under these conditions.

Where, however, the concentration gradient across the membrane was in the form of a pH gradient in an acetate-buffered medium with a low (10mM) chloride concentration, the diffusion potential measured in the presence of trichlorocarbanilide was acid-side-negative. This implies an enhanced proton permeability of the same order as that for chloride.

In these artificial membrane systems, therefore, we have been able to verify the action of trichlorocarbanilide in increasing the permeability to protons and to anions, in particular to those of small hydrated radius, such as chloride. We have found no evidence for significant effects with the alkali-metal cations.

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The Protonmotive Force in *Staphylococcus aureus*

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

Measurements have been made of the gradient of electrochemical potential of protons that exists across the cell membrane of *Staphylococcus aureus*. Acid-base titration experiments demonstrate that the membrane acts as a barrier of low proton permeability with an effective proton conductance of 10^{-6} – 10^{-7} mho/cm². This conductance is increased by classical uncouplers, such as carbonyl cyanide *m*-chlorophenylhydrazine, and by the further addition of valinomycin in a K^+ medium.

Respiration-driven proton translocation is outwardly directed and appears to be electrogenic, on the basis of the effects of valinomycin in a K^+ medium and of classical uncoupling agents.

Estimates of the transmembrane pH gradient of respiring cells in the steady state indicate a value of about 1 pH unit (inside alkaline) near pH6. This estimate gains confirmation from the measurements of pH changes in the external medium on the addition of nigericin to cells suspended in media of various K^+ concentrations.

Estimates of the transmembrane potential of respiring cells have been performed with a high concentration of valinomycin to approximate a condition where K^+ ions are in electrochemical equilibrium across the membrane. Measurements of the activity of K^+ ions externally confirms that the respiration-driven proton translocation is electrogenic, since the resulting K^+ gradient across the membrane is dissipated by classical uncouplers. The membrane potential is in excess of 120mV (inside negative).

Cells metabolizing glucose can generate an appreciable membrane potential anaerobically. This potential is observed to increase after prolonged (longer than 20min) anaerobic incubation. Such an observation is consistent with the fact that *S. aureus* is a facultative anaerobe. This anaerobic potential is also apparently not an ionic diffusion potential, and seems likely to arise via an electrogenic protonmotive adenosine triphosphatase.

The magnitude of the protonmotive force observed in *S. aureus* (in excess of 180mV) suggests that it could reasonably act as an intermediary, coupling the processes of electron transport and phosphorylation if ATP synthesis were catalysed by an adenosine triphosphatase of type II (Mitchell, 1966).

The sign and magnitude of the pH gradient also suggests that this may represent an adequate driving

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.