2-oxoglutarate, the proportion of these two compounds depending on the initial redox state of the intramitochondrial NADP.

This system is currently being investigated.

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COMMUNICATIONS

Proton Movements Coupled to the Transport of β -Galactosides into *Escherichia coli*

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It is now accepted that β -galactosides are transported into the intracellular water space of *Escherichia coli* by a genetically determined transporter (the γ -gene product), where they can accumulate as the unmodified β -galactosides. In view of the 100-fold concentration gradients that can be established, it is clear that the inflow of β -galactoside must be coupled to another process capable of making it spontaneous, but it has not hitherto been obvious what that other process was. Phosphorylation of the β -galactosides by a phosphoenolpyruvate-dependent phosphotransferase is not observed (cf. glucosides), nor does ATP hydrolysis appear to be involved (for review see Kaback, 1970). However, evidence is given in the present communication that suggests that a flow of protons into the cell is coupled to the inflow of β -galactoside, in support of the suggestion (Mitchell, 1963) that the accumulation of β -galactosides by *E. coli* is due to the operation of a proton- β -galactoside symporter, analogous to the Na^+ -glucose and Na^+ -amino acid symporters thought to occur in animal cells.

(1) When β -galactoside is added to a suspension of non-metabolizing cells (anaerobic, and depleted of fermentable substrates), there is an alkalification of the extracellular medium, corresponding to an inflow of protons (or outflow of OH^- ions) observable with a glass electrode.

(2) Similarly, with non-metabolizing cells made permeable to K^+ ions by treatment with tris, EDTA and valinomycin, the addition of β -galactoside causes the outflow of K^+ ions, observable with a K^+ ion-sensitive glass electrode, stoichiometric with the inflow of H^+ ions, indicating that β -galactoside induces an electrogenic inflow of H^+ ions.

(3) When a transmembrane pH gradient is established in non-metabolizing cells by the injection of a small amount of O_2 (dissolved in KCl solution), the subsequent slow rise of extracellular pH towards its original value is accelerated by the presence of β -galactoside, as expected if the inflow of protons is coupled to the transport of β -galactoside.

(4) A transient diffusion potential across the cell membrane (inside negative) can be established by the addition of KSCN to non-metabolizing cell suspensions because of their greater permeability to SCN^- than to K^+ ions. The inflow of protons caused by the electrical force is increased by the presence of β -galactoside, indicating that β -galactoside participates in the electrogenic movement of H^+ ions across the cell membrane.

All these effects of β -galactosides are completely removed by inhibiting the β -galactoside porter with *N*-ethylmaleimide.

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The Ion-Specific Increases in Membrane Permeability with a Group of Membrane-Active Antibacterial Agents

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In order that the various agents known to cause uncoupling of oxidative phosphorylation in mitochondrial systems may be used as experimental tools in the study of energy-transducing mechanisms in bacteria, the specificity of the increases in ion permeability that they produce must be verified for the systems under study.

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*

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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