PROCEEDINGS OF THE BIOCHEMICAL SOCIETY

The 522nd. Meeting of the Society was held jointly with the Society for General Microbiology at the University of Hull on Wednesday and Thursday, 5 and 6 January 1972, when the following papers were presented:

COLLOQUIUM ON 'PERMEABILITY AND TRANSPORT PHENOMENA IN MICRO-ORGANISMS AND MAMMALIAN SYSTEMS'

Ion Transport and Electrogenesis in Bacteria

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It has been known for two decades that reagents that uncouple oxidative phosphorylation from respiration inhibit active transport as well. The obvious explanation states that uncouplers interfere with production of ATP and thus secondarily with ATP-dependent transport. Plausible as this hypothesis appears, it fails to account for the paradoxical observation that uncouplers often inhibit active transport in micro-organisms even under anaerobic conditions (Windisch & Heumann, 1960).

The trail of research pursued here began with the rediscovery of this puzzling phenomenon in the bacterium *Streptococcus faecalis*. This organism lacks cytochromes and carries out no oxidative phosphorylation, but derives metabolic energy from glycolysis and from arginine degradation. Nonetheless, energy-dependent uptake of K^+ , of phosphate and of certain amino acids and sugars was abolished by low concentrations of uncouplers such as tetrachlorosalicylanilide or carbonyl cyanide *m*-chlorophenylhydrazone. The uncouplers did not interfere with glycolysis, ATP synthesis or ATP turnover, nor did they inhibit the transport carriers directly. We therefore concluded that uncouplers exert an effect on the membrane that results in the dissociation of transport from metabolism (Harold & Baarda, 1968).

It is now generally recognized that, as was originally proposed by Mitchell (1966, and earlier papers), uncouplers catalyse the passage of protons across a variety of natural and artificial membranes, including the bacterial plasma membrane (reviewed by Harold, 1970), and it is at least very probable that inhibition of transport is a consequence of proton conduction. Our attempts to understand the relationship between proton movements and energy-dependent transport of cations, anions and neutral metabolites have been guided by a generalized form of the chemiosmotic theory (Mitchell, 1966, 1970). Briefly, we propose that glycolysing cells of *S. faecalis* extrude protons, thereby generating a gradient of pH and of electrical potential across the membrane (interior alkaline and negative). This in turn supports the 'active

transport' of metabolites against the concentration gradient: K^+ uptake may occur in response to the potential gradient, whereas accumulation of anions (phosphate) and neutral metabolites may involve carriers that translocate the substrate and protons simultaneously.

Measurement of the internal pH of *S. faecalis* demonstrated that glycolysing cells do indeed maintain a cytoplasmic pH higher than that of the medium by as much as 1 pH unit. The pH gradient was abolished by proton conductors but not by valinomycin, and appears to result from the primary extrusion of protons (Harold *et al.*, 1970). To detect the generation of an electrical potential we employed the approach pioneered by Liberman & Skulachev (1970) and their associates. Glycolysing cells extrude H^+ and Na^+ , and accumulate equivalent amounts of lipid-soluble cations such as dibenzylidimethylammonium ion. All the evidence suggests that accumulation of these non-physiological cations is not the result of active transport. Instead, it appears that extrusion of Na^+ and H^+ from the cells, supported by glycolysis, generates an electrical potential across the membrane that in turn supports the uptake of dibenzylidimethylammonium ion. The potentials generated are about -150 to -200 mV, interior negative.

Experiments with cells that had been preloaded with excess of H^+ demonstrated that extrusion of protons is an electrogenic process that, at least under certain conditions, is strictly dependent on concurrent glycolysis. Proton extrusion was inhibited by dicyclohexylcarbodi-imide, a reagent known to inhibit the adenosine triphosphatase associated with the membrane of *S. faecalis*. A. Abrams, J. Smith & C. Baron (personal communication) have recently isolated a mutant of *S. faecalis* whose adenosine triphosphatase is resistant to inhibition by dicyclohexylcarbodi-imide; proton extrusion in this mutant was not inhibited by this compound. These results implicate the adenosine triphosphatase in proton extrusion, and are entirely consistent with the proposal (Mitchell, 1966, 1970) that this enzyme activity serves as a primary proton pump, translocating protons outwards to generate gradients of pH and of electrical potential. Na^+ can also be extruded against a concentration gradient, but there appears

to be no sodium pump. Instead, it appears that Na^+ efflux occurs by exchange for H^+ , which is then extruded by the proton pump. Na^+-H^+ antiport was originally reported by Mitchell & Moyle (1969) to occur in mitochondria.

What role do these gradients of pH and of electrical potential play in the energy-linked transport of nutrients? The case of K^+ is best understood. Proton-conductors, which collapse the gradients by allowing protons to flow back into the cells, block net K^+ uptake. Inhibitors of the adenosine triphosphatase likewise inhibit K^+ uptake, but reagents such as valinomycin have little effect. The inference, that K^+ uptake is dependent on the electrical potential generated by proton extrusion, is supported by the characteristics of mutants defective in K^+ uptake. One class of mutants is deficient in proton extrusion, another lacks the Na^+-H^+ antiport system. It seems clear that generation of an electrical potential is necessary for the accumulation of K^+ , though probably not sufficient.

The relationship of proton gradients to the uptake of other nutrients is less clear. Uptake of phosphate by *S. faecalis* is strictly dependent on glycolysis, and experiments with inhibitors suggest that, as in mitochondria (Palmieri *et al.*, 1969; Klingenberg, 1970), a gradient of pH is required for this process. Studies on amino acid uptake, still in a preliminary stage, point in the same direction. It now appears that substantial accumulation of threonine, glycine and related amino acids can be achieved by means of a pH gradient even in the absence of a source of metabolic energy. If this proves to be correct, it will lend strong support to the concept (Mitchell, 1970) that the circulation of protons between cytoplasm and medium provides the driving force for the active transport of many nutrients into bacterial cells.

- Harold, F. M. (1970) *Advan. Microbial Physiol.* **4**, 45
 Harold, F. M. & Baarda, J. R. (1968) *J. Bacteriol.* **96**, 2025
 Harold, F. M., Pavlasova, E. & Baarda, J. R. (1970) *Biochim. Biophys. Acta* **196**, 235
 Klingenberg, M. (1970) *Essays Biochem.* **6**, 119
 Liberman, E. A. & Skulachev, V. P. (1970) *Biochim. Biophys. Acta* **219**, 30
 Mitchell, P. (1966) *Biol. Rev. Cambridge Phil. Soc.* **41**, 445
 Mitchell, P. (1970) *Symp. Soc. Gen. Microbiol.* **20**, 121
 Mitchell, P. & Moyle, J. (1969) *Eur. J. Biochem.* **9**, 149
 Palmieri, F., Quagliariello, E. & Klingenberg, M. (1969) *Eur. J. Biochem.* **17**, 230
 Windisch, F. & Heumann, W. (1960) *Naturwissenschaften* **47**, 209

The Transport of Methyl α -Glucoside by *Pseudomonas aeruginosa*

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Glucose metabolism in *Pseudomonas aeruginosa* occurs via four pathways, namely the Entner & Doudoroff (1952) sequence, the pentose phosphate cycle, the phosphorylated oxidative route and the direct oxidative pathway leading to 2-oxogluconate. The enzymes of glucose catabolism are repressed by growth on organic acids such as succinate and citrate (Hamilton & Dawes, 1960; Lessie & Neidhardt, 1967). Experiments conducted in a chemostat, operated under conditions of nitrogen limitation, have demonstrated that citrate, or a metabolic derivative, inhibits both glucose utilization and the induction of the glucose-metabolizing enzymes by glucose when cells metabolizing citrate are presented with glucose as an alternative carbon source (Ng & Dawes, 1967; Hamlin *et al.*, 1967). Similar investigations on the competition between carbon sources for metabolism, for example the glucose-lactose diauxie of *Escherichia coli* (Loomis & Magasanik, 1967) and the succinate-glucose diauxie of *Arthrobacter crystallopoietes* (Krulwich & Ensign, 1969), have demonstrated that permeation can be a major control site. We have investigated the methyl α -glucoside transport system in *Ps. aeruginosa* in order that its role in the control of glucose utilization by organic acids may be assessed.

Uptake of ^{14}C -labelled methyl α -glucoside was measured by using a rapid membrane-filtration technique to separate the cells from the incubation medium, maintained at 37°C . The extracellular methyl α -glucoside on the filter was removed by washing. The extent of accumulation detected was critically dependent on the composition and temperature of the wash fluid employed. Washing with ice-cold phosphate buffer, supplemented with MgSO_4 , resulted in complete loss of accumulated material, whereas washing at temperatures in the range $18-22^\circ\text{C}$ resulted in a 60–80% loss. These effects could be overcome by the addition of NaCl to the basal wash fluid, the amount required increasing with decreasing temperature of the solution.

Incubation of glucose-grown cells with methyl α -glucoside results in an accumulation above the concentration in the medium. This accumulation is sensitive to the addition of NaN_3 , and extraction of the intracellular material revealed only unchanged methyl α -glucoside. Studies on the initial rate of uptake indicate that a component capable of saturation with methyl α -glucoside (K_m 2.5 mM) is involved, and exit studies with cells loaded with methyl α -glucoside indicate that exit is a first-order process (k 0.34) and is dependent on the concentration of