- Schultz, S. G. & Curran, P. F. (1970) *Physiol. Rev.* **50**, 637
 Stock, J. & Roseman, S. (1971) *Biochem. Biophys. Res. Commun.* **44**, 132
 Thompson, J. & MacLeod, R. A. (1971) *J. Biol. Chem.* **246**, 4066
 West, I. C. (1970) *Biochem. Biophys. Res. Commun.* **41**, 655

Sodium Ion and Potassium Ion Transport and Permeability: a Comparison between Squid Giant Axons and *Ascaris* Muscle Fibres

By P. C. CALDWELL (Department of Zoology, University of Bristol, Bristol BS8 1UG, U.K.)

Although squid giant axons and the muscle fibres of the pig roundworm *Ascaris lumbricoides* are both invertebrate systems, the ion-transport phenomena associated with them illustrate many points that are relevant to transport in mammalian systems and micro-organisms. Na⁺ and K⁺ transport in squid axons takes place at the same temperature as that in many micro-organisms, and shows many similarities to that in mammalian erythrocytes. Na⁺ and K⁺ transport in *Ascaris* muscle on the other hand takes place at the same temperature as that in mammalian systems, but shows many unusual features.

Na⁺ transport in squid axons is diminished by removal of extracellular K⁺ (Hodgkin & Keynes, 1955) and by inhibitors of the sodium-pump enzyme system described by Skou (1957), e.g. ouabain (Caldwell & Keynes, 1959). It is also diminished by metabolic inhibitors (Hodgkin & Keynes, 1955). Many features of this transport can be interpreted in terms of models for the coupled transport of Na⁺ and K⁺ that also hold for mammalian erythrocytes (e.g. see Caldwell, 1968; Stone, 1968).

Electrochemical considerations suggest that Na⁺ transport must also take place from *Ascaris* muscle, but the system involved is very different since it is unaffected by the removal of extracellular K⁺ and the action of inhibitors such as ouabain (Caldwell & Ellory, 1968). The permeability properties of the *Ascaris* muscle membrane are also unusual. Although the membrane shows an appreciable permeability to radioactive Cl⁻ and K⁺, the resting membrane potential is not very sensitive to changes in the external concentration of these ions (Brading & Caldwell, 1964; del Castillo *et al.*, 1964). This is in contrast with squid giant axons, the resting membrane of which shows a high permeability towards K⁺ and the resting potential a high sensitivity to changes in extracellular K⁺. Some of the unusual behaviour of the *Ascaris* muscle membrane can be explained in terms of electrogenic sodium-pump mechanisms in which the outward movement of Na⁺ is not coupled to the movement of another ion.

- Brading, A. F. & Caldwell, P. C. (1964) *J. Physiol. (London)* **173**, 36P
 Caldwell, P. C. (1968) *Physiol. Rev.* **48**, 1
 Caldwell, P. C. & Ellory, J. C. (1968) *J. Physiol. (London)* **197**, 75P
 Caldwell, P. C. & Keynes, R. D. (1959) *J. Physiol. (London)* **148**, 8P
 del Castillo, J., de Mello, W. C. & Morales, T. (1964) *J. Gen. Physiol.* **48**, 129
 Hodgkin, A. L. & Keynes, R. D. (1955) *J. Physiol. (London)* **128**, 28
 Skou, J. C. (1957) *Biochim. Biophys. Acta* **23**, 394
 Stone, A. J. (1968) *Biochim. Biophys. Acta* **150**, 578

The Transport of Metabolites across the Mitochondrial Membranes

By J. D. MCGIVAN and J. B. CHAPPELL (Department of Biochemistry, University of Bristol, Bristol BS8 1TD, U.K.)

It is now fairly well accepted that at least seven anion-transporting systems are present in liver mitochondria (Chappell, 1968, 1969; Klingenberg, 1970). Lehninger (1972) has provided convincing evidence for the existence of a specific carrier for L-ornithine, which must enter the mitochondria during the process of urea synthesis. It is reasonably well established that most mitochondrial anion-transporters are exchange-diffusion carriers or antiporters (Mitchell, 1966). Thus the dicarboxylate-carrier exchanges dicarboxylate anion for dicarboxylate anion or phosphate (or arsenate), and the tricarboxylate-carrier exchanges tricarboxylate anion (e.g. citrate) for dicarboxylate anion (e.g. L-malate), and in the case of the 2-oxoglutarate carrier a similar mechanism is apparently involved, i.e. oxoglutarate is exchanged for malate. It is not known whether the phosphate carrier is an antiporter, in which case it would permit phosphate-OH⁻ exchange, or a symporter, in which case phosphate would enter in association with a proton.

The existence of an ATP/ADP-carrier, the so-called adenine nucleotide translocase, is also well documented. This carrier catalyses an obligatory 1:1 exchange between internal and external adenine nucleotides. Of the naturally occurring nucleotides only the adenine derivatives are active. The specificity of the exchange in both directions has been studied in great detail by using radiochemical methods. External ADP exchanges rapidly for internal ADP and ATP. External ATP can exchange for internal ADP under certain conditions. The ATP-ATP exchange is relatively slow, and AMP is not exchanged at a significant rate.

The adenine nucleotide translocase is inhibited by two naturally occurring compounds, namely atractyl-loside and bongkreic acid. The mechanism of

inhibition is different in each case. Atractyloside competes with adenine nucleotides for the carrier site and can displace nucleotides that are bound to the carrier. Bongkrekic acid binds the nucleotides very tightly to the carrier, so that no exchange between bound and free nucleotides is possible.

The use of atractyloside, a specific competitive inhibitor with a very low K_i (2×10^{-8} M), has permitted some progress towards the identification and characterization of the carrier sites. Experiments with radioactive nucleotides have shown that the binding has the same nucleotide-specificity as the exchange. The total number of binding sites is approximately equal to the number of cytochrome $a+a_3$ molecules in the membrane. Two types of binding sites with unequal affinities have been identified. It has been suggested that all the binding sites are identical and that the apparent higher affinity of some sites reflects their location on the inner side of the membrane, where they are rapidly saturated by endogenous nucleotides.

The control of adenine nucleotide translocation is under active investigation. The exchange of external ADP for internal ATP is rapid, whereas the exchange of external ATP for internal ADP is relatively slow, unless the membrane is made permeable to K^+ or H^+ . At about pH 7 ATP and ADP exist mainly as the species ATP^{4-} and ADP^{3-} . It was originally suggested that in respiring mitochondria the existence of a membrane potential (negative inside) might explain this discrepancy by inhibiting the inward movement of negative charge involved in the exchange of external ATP^{4-} for internal ADP^{3-} .

Recent work has shown that the position is more complicated and that the transport of adenine nucleotides is closely linked to the transport of phosphate. Thus when phosphate transport is specifically inhibited by *N*-ethylmaleimide the exchange of external ADP for internal ATP is also inhibited, whereas the ADP-ADP exchange is not. Further evidence suggests that this is due to the necessity for the external ADP^{3-} exchange for internal ATP^{4-} to be charge-compensated by the inward movement of $H_2PO_4^-$. No charge compensation is required for the homogeneous ADP^{3-} - ADP^{3-} exchange.

It appears that anions can enter mitochondria by two distinct mechanisms: (1) on a specific carrier, in exchange for another anion, as mentioned above; (2) electrophoretically or electrogenically. This means that the anion is able to cross the membrane negatively charged and without movement of a counterion. This applies, for example, to nitrate, thiocyanate and iodide in the presence of iodine (presumably I_3^- is the penetrant species). In principle it is possible that a carrier-mediated electrophoretic anion movement could occur, as has been postulated for the adenine nucleotide carrier. The difference between carrier-mediated and the electrophoretic

entry of anions is distinguished easily by following the osmotic swelling of mitochondria suspended in iso-osmotic solutions of the anion under consideration, e.g. in the case of phosphate (carrier-mediated entry) and nitrate (electrophoretic entry).

The first technique introduced for this purpose was the observation of swelling in iso-osmotic solutions of ammonium salts (Chappell & Haahrhoff, 1967). Mitochondria suspended in ammonium phosphate swell rapidly, the commonly accepted explanation being that NH_4^+ dissociates to some extent to give NH_3 , which rapidly crosses the membrane. In the matrix it takes up H^+ from water, leaving OH^- . This latter ion can then exchange with phosphate and osmotic swelling occurs. In the case of ammonium nitrate, swelling does not occur because NH_3 and NO_3^- can both penetrate, whereas H^+ cannot. However, on addition of an uncoupling agent, which permits H^+ to cross the membrane (see Henderson *et al.*, 1969), rapid swelling occurs.

Similar experiments can be performed in K^+ -containing media with valinomycin, which causes specific K^+ -permeability of a wide variety of membranes without affecting H^+ -permeability (see Henderson *et al.*, 1969). Respiration is inhibited when the mitochondria are suspended in iso-osmotic potassium phosphate, for example. Addition of valinomycin causes no swelling; this occurs only when an uncoupling agent is added. The explanation is that, although K^+ and phosphate can enter, net entry of salt does not occur because of the H^+ imbalance caused by the phosphate- OH^- exchange. Addition of an uncoupling agent permits H^+ efflux, net entry of salt and consequently swelling. With KNO_3 addition of valinomycin causes swelling, without a requirement for an uncoupling agent. This is because K^+ and NO_3^- can enter.

When similar experiments are performed with succinate, malate, 2-oxoglutarate and citrate as well as with phosphate, the results are entirely consistent with carrier-mediated antiport entry. With nitrate, thiocyanate and iodide (containing some iodine) the results indicate electrophoretic entry. With glutamate, however, anomalous results are obtained. (1) Mitochondria swell in ammonium glutamate. This finding and certain other evidence suggests that a specific glutamate carrier, possibly a glutamate- OH^- antiporter, occurs in liver mitochondria (Azzi *et al.*, 1967). (2) Mitochondria do not swell in the presence of valinomycin in potassium glutamate solutions, with or without addition of an uncoupling agent, when respiration is inhibited with antimycin. These findings are not consistent with either an antiport or electrophoretic mechanism for entry. (3) Investigation of glutamate penetration by the 'space' technique (Chappell, 1968) shows that glutamate readily penetrates the mitochondrial membrane and appears in the matrix as a mixture of glutamate and

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

This system is currently being investigated.

- Azzi, A., Chappell, J. B. & Robinson, B. H. (1967) *Biochem. Biophys. Res. Commun.* **29**, 148
 Chappell, J. B. (1968) *Brit. med. Bull.* **24**, 150
 Chappell, J. B. (1969) in *Inhibitors, Tools in Cell Research* (Bücher, T. & Seis, H., eds.), p. 335, Springer-Verlag, Berlin

- Chappell, J. B. & Haarhoff, K. N. (1967) in *Biochemistry of Mitochondria* (Slater, E. C., Kaniuga, Z. & Wojtczak, L., eds.), p. 75, Academic Press, London
 Henderson, P. J. F., McGivan, J. D. & Chappell, J. B. (1969) *Biochem. J.* **111**, 521
 Klingenberg, M. (1970) *Essays Biochem.* **6**, 119
 Lehninger, A. L. (1972) in *The Biochemistry and Biophysics of Mitochondrial Membranes* (Azzzone, G. F. & Siliprandi, N., eds.), Academic Press, New York, in the press
 Mitchell, P. (1966) *Chemiosmotic Coupling in Oxidation and Photosynthetic Phosphorylation*, Glynn Research, Bodmin

COMMUNICATIONS

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

By I. C. WEST and P. MITCHELL (*Glynn Research Laboratories, Bodmin, Cornwall, U.K.*)

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.

We thank the Science Research Council for a Special Research Grant in partial support of this work.

- Kaback, H. R. (1970) *Annu. Rev. Biochem.* **39**, 561
 Mitchell, P. (1963) *Biochem. Soc. Symp.* **22**, 142

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

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

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.