SHORT COMMUNICATIONS

A NOVEL MECHANISM FOR SALT AND FLUID TRANSPORT ACROSS EPITHELIA

By H. A. EDWARDS

The Zoological Laboratory, Downing Street, Cambridge CB2 3EJ, England

Some salt transporting epithelia can absorb inorganic ions from extremely low external concentrations. Salt depleted frogs, for example, can absorb sodium ions from freshwater at concentrations down to 0·01 mM (Krogh, 1939) while freshwater mosquito larvae can maintain haemolymph sodium at 30 mM in external media containing as little as $1·55 \times 10^{-3}$ mM sodium (Stobbart, 1974). The ability of the frogskin to absorb ions, into the blood, from such low external concentrations has been held to present difficulties (Hansen & Zerahn, 1964) for the classical model, which proposes a passive entry of external sodium ions into the cells and pumping at the inner epithelial membrane (Koefoed-Johnsen & Ussing, 1958). At least two current hypotheses exist to explain how net sodium transport from low external concentrations is achieved. The first postulates an involvement of ion pumps located on the outer epithelial membrane (Hansen & Zerahn, 1964; Leblanc, 1972) and the second that the electrical potential across the outer membrane is large enough to create an inwardly directed electro-chemical gradient to induce a passive entry of sodium ions from media containing as little as 1 mM sodium (Nagel, 1977). This communication proposes an alternative model for unidirectional salt transport from dilute environments which may be applicable to the uptake of cations and anions in a variety of salt absorptive epithelia.

The model proposes that ions bind passively to charged sites on a superficial mucopolysaccharide matrix. The mucopolysaccharide and associated ions is engulfed in small vesicles which coalesce to form larger vesicles where the mucopolysaccharide is broken down to release ions into the cell. The released ions are then transported across the basal membrane by specific pumps (see Fig. 1).

The apical layer of mucopolysaccharide of the model is present in many salt transporting epithelia (Wichard, Komnick & Abel, 1972; Komnick, Rhees & Abel, 1971; Holdich & Ratcliffe, 1970; Van Lennep & Komnick, 1971). Furthermore, mucopolysaccharides on the surface of transporting cells have been shown to absorb ions from hypotonic media (Van Lennep ·& Komnick, 1971; Wichard & Komnick, 1973). The expected pH sensitivity of an ion binding system has been demonstrated by Garcia-Romeu (1971) in anuran skins where changes of pH cause marked alterations in salt transport. The accumulations of mucopolysaccharide-like material on the apical surface, recorded from the anal papillae of *Aedes aegypti* when no salt
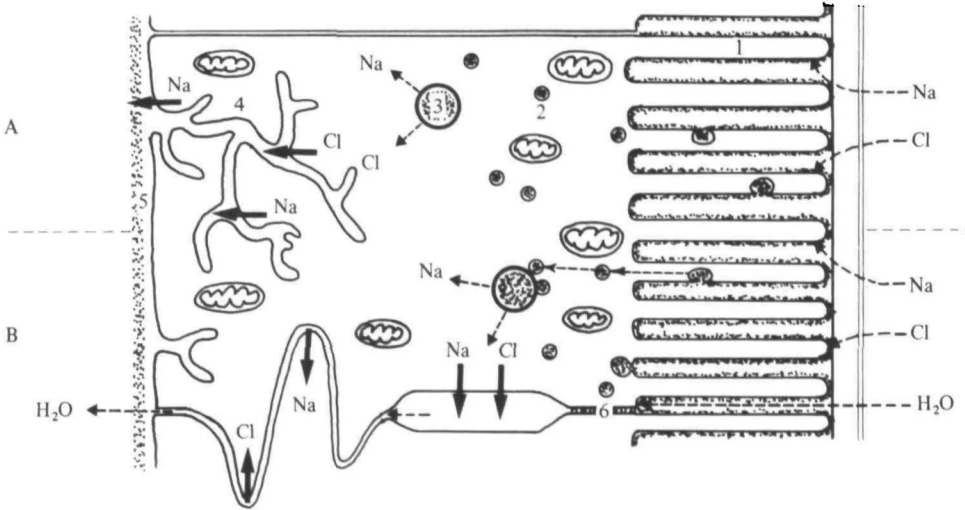
Fig. 1. Model for A salt and B fluid transport. (1) The folded apical plasma membrane covered with ion binding matrix. (2) A pinocytotic vesicle of the surface matrix. (3) A larger vesicle in which the matrix is broken down to release ions. (4) The unfolded basal. (5) The basement membrane. (6) A junction permeable to water but impermeable to ions.

transport is taking place (Edwards, 1979) and from the apical crypts of chlorine cells in the gills of *Fundulus* (Philpott & Copeland, 1963), may represent cases where salt transport is inhibited but where matrix production continues.

The demonstration of pinocytosis, vesicles and lysosomic activity in association with salt transport is also required by the model. Vesicles have been reported in most transporting epithelia, and in some cases it has been suggested that the surface matrix is engulfed pinocytotically (Holdich & Ratcliffe, 1970; Talbot, Clark & Lawrence, 1972). There is a marked increase in the number of vesicles in toad bladder, when stimulated to transport; these coalesce to form larger lysosomes in which acid phosphatase activity is demonstrable (Masur *et al.*, 1971).

The activity and localization of inorganic ion pumps should be consistent with the proposed mechanism for salt uptake. In the chloride cells of fish gills the Na^+/Ka^+ ATPase activity increases as the concentration of the external medium falls, that is, when the need for transport is increased (Karnaky, Ernst & Philpott, 1976a). Na^+/Ka^+ ATPase activity has been shown on both faces of transporting epithelia but the bulk of the activity is located in the basal canaliculi (Karnaky *et al.* 1976b).

The characteristic arrangement of cell membranes in transporting epithelia are explained in terms of the model. The apical foldings found under conditions of high salt transport act to increase the surface of exposed mucopolysaccharide and thus increase the ion binding per unit area for salt pumps and thus allow transport at a rate equal to the release into the cell. There is no increase in the osmotic gradient into the cell. When the transport rate is low these spaces become expanded forming large lacunae, and thus reducing the surface area and transport capacity of the cell.

Purely salt transporting epithelia have straight lateral borders. Fluid transporting epithelia on the other hand, have elaborate folding of the lateral plasma membranes which often include lateral spaces. Models of fluid transport have located salt pumps

the lateral borders and on the membranes bounding the lateral spaces (Wall & Oschman, 1970; Berridge & Gupta, 1968). There is also evidence that water moves around rather than through cell junctions due to the selective permeability of the lateral borders (Hill, 1978; Lane, 1979). Thus in fluid transport, inorganic ions enter the cell by the same route as in salt transport but are then pumped into the lateral space; water enters the lateral space by the paracellular route down its osmotic gradient. The hydrostatic pressure increases and fluid moves down the lateral space; salts are reabsorbed by pumps located on the extensive lateral borders.

The model takes account of the known structure and physiology of transporting cells. By binding ions to a matrix before transport they are osmotically inactive and can be gathered from very low external concentrations without creating a steep osmotic gradient. While using vesicles for transport across the apical membrane and placing salt pumps on the basal and lateral plasma membranes, pumps located on the apical membranes are not precluded. Pumps are probably necessary on all membranes for cell maintenance. Even if bulk movements of salt are in a bound state pumps may be used to alter the composition of fluid transported.

The model does not extend to reversible systems, such as the gills of fish capable of absorbing or secreting ions depending on the external conditions. However, Davis, Jones & Hagler (1979) have shown a vesicle/matrix mechanism for calcium uptake into the cells of the chick duodenum. The vesicle model may have a wider application in both vertebrate and invertebrate transporting epithelia. A critical test of the model will be the degree of ion binding to surface mucopolysaccharides and the rate at which vesicles of this material can be formed.

REFERENCES

BERRIDGE, M. J. & GUPTA, B. (1968). Fine-structural localization of adenosine triphosphatase in the rectum of *Callophora. J. Cell Sci.* **3**, 17–32.

DAVIS, W. L., JONES, R. G. & HAGLER, K. K. (1979). Calcium containing lysosomes in the normal chick duodenum: a histochemical and analytical electron microscope study. *Tissue & Cell* **11**, 127–138.

EDWARDS, H. A. (1979). *Aedes aegypti: salt, water and cells.* Ph.D. thesis, Cambridge University.

GARCIA-ROMEU, F. (1971). Anionic and cationic exchange mechanisms in the skin of anurans, with special reference to Leptodactylidae *in vivo. Phil. Trans. R. Soc. Lond.* B **262**, 163–174.

HANSEN, H. H. & ZERAHN, K. (1964). Concentration of lithium, sodium and potassium in epithelial cells of the isolated frog skin during active transport of lithium. *Acta physiol. scand.* **60**, 189–196.

HILL, A. E. (1978). Fluid transport across *Necturus* gallbladder epithelium. In: *Comparative Physiology: Water, Ions and Fluid Mechanisms* (ed. K. Schmidt-Nielsen, L. Bolis and S. H. P. Maddrell), ch. 3. Cambridge University Press.

HOLDICH, D. M. & RATCLIFFE, N. A. (1970). A light and electron microscope study of the hindgut of the herbivorous isopod, *Dynamene bidentata* (Crustacea: Peracarida). *Z. Zellforsch.* **111**, 209–227.

KARNAKY, K. J., ERNST, S. A. & PHILPOTT, C. W. (1976a). Teleost chloride cell. I. Responses of pupfish *Cyprinodon varigatus* gill Na/K ATPase and chloride cell fine structure to various high salinity environments. *J. cell Biol.* **70**, 144–156.

KARNAKY, K. J., KINTER, L. B., KINTER, W. B. & STIRLING, C. E. (1976b). Teleost chloride cell. II. Autoradiographic localization of gill Na/K ATPase in killifish *Fundulus heteroclitus* adapted to low and high salinity environments. *J. Cell Biol.* **70**, 157–177.

KOEFOED-JOHNSON, V. & USSING, H. H. (1958). The nature of the frog skin potential. *Acta physiol. scand.* **42**, 298–308.

KOMNICK, H., RHEES, R. W. & ABEL, J. H. JNR. (1972). The function of ephemerid chloride cells. Histochemical, autoradiographic and physiological studies with radioactive chloride on *Callibaetis. Cytobiologie* **5**, 65–82.

KROGH, A. (1939). *Osmotic Regulation in Aquatic Animals.* Cambridge University Press.

LANE, N. J. (1979). Tight junctions in a fluid transporting epithelium of an insect. *Science* **204**, 91–93.

Leblanc, G. (1972). The mechanism of lithium accumulation in the isolated frog skin epitheliu■ *Pflügers Arch.* **337**, 1–18.

Masur, S. K., Holtzman, E., Schwartz, I. L. & Walter, R. (1971). Correlation between pinocytosis and hydroosmosis induced by neurohypophyseal hormones mediated by adenosine 3′,5′-cyclic monophosphate. *J. Cell Biol.* **49**, 582–594.

Nagel, W. (1977). The dependence of the electrical potential across the membranes of the frog skin upon the concentration of sodium in the mucosal solution. *J. Physiol., Lond.* **269**, 777–796.

Philpott, C. & Copeland, D. (1963). Fine structure of chloride cells from three species of *Fundulus*. *J. Cell Sci.* **18**, 389–404.

Stobbart, R. H. (1974). Electrical potential differences in the larva of the mosquito *Aedes aegypti* (L.). *J. exp. Biol.* **60**, 493–533.

Talbot, P., Clark, W. H. Jnr & Lawrence, A. L. (1972). Light and electronmicroscopic studies on osmoregulatory tissue in the developing brown shrimp, *Penaeus aztecus*. *Tissue & Cell* **4**, 271–286.

Van Lennep, E. W. & Komnick, H. (1971). Histochemical demonstration of sodium and chloride in the frog epidermis. *Cytobiologie* **3**, 137–151.

Wall, B. J. & Oschman, J. L. (1970). Water and solute uptake by rectal pads of *Periplaneta americana*. *Am. J. Physiol.* **278**, 1208–1215.

Wichard, W. & Komnick, H. (1973). Fine structure and function of the abdominal chloride epithelia in Caddisfly larvae. *Z. Zellforsch.* **136**, 579–590.

Wichard, W., Komnick, H. & Abel, J. H. Jnr. (1972). Topology of ephemerid chloride cells. *Z. Zellforsch.* **132**, 533–551.