

INTRODUCTION: TRENDS IN EPITHELIAL TRANSPORT AND CONTROL

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Osmotic and ionic regulation ultimately depend on epithelial transport mechanisms and their control in most metazoans, although intracellular osmotic adjustments are probably more important in marine osmoconformers. There are a great variety of osmoregulatory organs throughout the animal kingdom and physiological information about many of these is still so incomplete that it seemed inappropriate to attempt a comprehensive review or synthesis of osmoregulation in a small discussion meeting of this type. However, given the general conservative nature of many cellular processes during the course of animal evolution, we might anticipate common protein carrier mechanisms which are organized and controlled in a limited number of ways within most epithelia. A number of such general concepts and models of epithelial transport have developed over the last ten years and these should prove useful in the future when less well studied osmoregulatory organs are investigated. With this in mind, we have included in this volume reports on some of the most intensively studied epithelia having a range of different regulatory functions. These papers will indicate (1) the types of primary and secondary transport mechanisms which are now well established and partially characterized, (2) their typical asymmetrical distribution in epithelia, and (3) cellular processes associated with their regulation. (4) Finally we consider the relative importance and characteristics of the paracellular route for ion and fluid transfer across epithelia.

Recent major advances in our understanding of these epithelia arise from the application of new techniques and experimental approaches, and these will undoubtedly be very useful in the investigation of less well studied osmoregulatory organs. The reader should find literature references to many of these methodologies. For example, the asymmetrical distribution of membrane transport mechanisms has been clarified recently by the use of isolated apical or basal membrane vesicles (see article by Murer *et al.*; also Murer & Kinne, 1980) and by use of double-barrelled ion-selective intracellular electrodes (articles by Hanrahan & Phillips, and Epstein, Stoff & Silva: see also reviews by Zeuthen, 1980). Ion selective channels have been studied by fluctuation (noise) analysis (article by Lewis; see also Miller, 1982). Characterization of transport in microscopic regions of membrane will no doubt be increasingly studied by the new technique of 'patch clamping' which is not described in this volume (see Hammill *et al.* 1981).

Another common problem facing epitheliologists is the identification of the cell type responsible for transmural movements in heterogeneous epithelia. Additionally

in a number of such systems, the direction of transport can be reversed by hormones or other stimuli: well-known examples include NaCl absorption and secretion in mammalian colon, ileum, foetal lung, trachea; teleost gill, operculum and skin; hindgut of euryhaline insect larvae; $\text{HCO}_3^-/\text{H}^+$ movements in turtle bladder. A reasonable first assumption is that different cell types are probably responsible for absorptive and secretory processes. The vibrating probe has recently been very successfully used by Foskett, Bern, Machen & Conner (see article in this volume) to demonstrate rigorously that the Cl-cell is indeed the site of NaCl secretion by integumentary structures in teleosts. It is surprising that this long standing technique has not been used more often on heterogeneous epithelia. Articles by Handler and by Cereijido, González-Mariscal & Borboa in this volume report the use of cultured single-cell lines which were isolated from epithelia and reconstituted as homogeneous and functional epithelial sheets. This method originally seemed promising as a means of unravelling functions of specific cell types from heterogeneous epithelia. However, as Handler reports, such monocultured epithelia can exhibit a wide range of transport characteristics and responses to natural stimuli depending on incubation methods. This is perhaps not surprising because a common genome in different cell types allows for expression of various potentialities depending on environmental conditions. This method therefore now seems less promising as a means of identifying specific cell function in heterogeneous epithelia. However, monocultured epithelia should continue to provide exciting new information concerning development and differentiation of epithelia; e.g. asymmetry of carrier location, appearance of hormone receptors and control processes, and intercellular junctional complexes.

Some common patterns of hormonal control in epithelia are now emerging in addition to the well documented actions of cyclic nucleotides and Ca^{2+} on protein kinases, leading to phosphorylation of membrane proteins (see article by Palfrey & Rao). In several intensively studied systems it appears that a population of membrane vesicles containing specific channels or pumps are held in reserve beneath the apical membrane. These are inserted into the apical plasma membrane in response to hormones or other stimuli by exocytosis and involving microtubules and microfilaments (see articles by Lewis and by Al-Awqati, Gluck, Reeves & Cannon). Lewis has followed the associated increase in membrane surface area by measuring membrane capacitance change when ADH stimulates Na^+ and fluid absorption. Using fluorescent probes and membrane protein markers, Al-Awqati has clearly demonstrated a rapid turnover of vesicles containing an electrogenic proton pump in turtle bladder. The vesicular membrane added to the apical plasma membrane upon stimulation is apparently spontaneously removed by pinocytosis to reform sub-apical vesicles which can be reused. This may prove to be a common type of process during hormonal control of transport in many epithelia. Agents which disrupt microtubules and microfilaments (e.g. cytochalasin B and colchicine) have commonly been used to implicate cytoskeletal elements in this and many other cellular processes. However, it was the consensus of those at this discussion meeting that these agents may have such drastic effects on cell morphology and integrity that inhibition by these agents is insufficient to implicate these organelles, i.e. in the absence of other evidence.

The coupling between cellular metabolism and epithelial transport processes is a central question for any osmoregulatory tissue. In particular, we would like to know

How many primary ion pumps (ATPases) have evolved in animal systems. This will clearly limit our expectations when considering uncharacterized epithelia. In considering this question, we did not review the ubiquitous and well-known Na,K-ATPase and Ca-ATPase which are localized in the serosal membrane of most, if not all, epithelia. Indeed similarities in the asymmetrical distribution of several carriers throughout the animal kingdom can be appreciated by comparing flatworms with mammals (see article by Podesta). Although membrane transport events in many invertebrate tissues (particularly in insects) are insensitive to ouabain, an inhibitor of the Na,K-ATPase, it has still been possible to demonstrate the presence of this enzyme in these tissues by biochemical means (reviewed in an article by Harvey, Cioffi, Dow & Wolfersberger). Reports persist of a second Na-ATPase, which is insensitive to K^+ and ouabain, is stimulated by Ca^{2+} , and is inhibited by ethacrynic acid (e.g. Del Castillo, Marin, Proverbio & Proverbio, 1982). However, given the stimulatory effect of various ions on most ATPases, detailed studies correlating this second Na-ATPase with a specific transport process measured physiologically must be completed before a role in epithelial transport can be accepted. This second Na^+ pump was originally invoked by Wittembury to explain cell volume regulation in the presence of ouabain. However, more recently, secondary transport mechanisms have been identified which can be turned on in response to cell shrinkage (e.g. electroneutral Na, K, 2Cl co-entry into the cell, discussed by Palfrey; or its functional equivalent, parallel Na^+/H^+ and Cl^-/OH^- antiporters; discussed by Murer *et al.*). Cell swelling commonly initiates KCl exit either by co-transport or by separate conductive pathways (see Ellory, Dunham, Logue & Stewart, 1982; Grinstein, Clarke & Rothstein, 1982; Hoffmann, 1982). Similar transport systems have been proposed for various epithelia and these might also be responsible for volume regulation during transmural transport.

In addition to Na,K-ATPase and Ca-ATPase (both serosal), only two other primary pump ATPases have now been rigorously demonstrated in vertebrate epithelia. One of these is the electrogenic proton-ATPase responsible for acidification in turtle bladder (article by Al-Awqati *et al.*), but widely occurring (e.g. in yeast; reviewed by Steinmetz & Andersen, 1982). The second is an electroneutral H,K-ATPase, which is described by Sachs in this volume and which is responsible for proton secretion in exchange for luminal K^+ in the stomach. Both of these ATPases are localized in the apical membrane. (For completeness sake, it should be mentioned that two secondary transport systems responsible for pH regulation in vertebrates and invertebrates are reviewed by Boron, 1980, but not discussed in this volume.) An apical K-ATPase associated with K^+ absorption in ouabain-treated mammalian colon (Gustin & Goodman, 1981; Wills & Biagi, 1982) may turn out to be identical to the H,K-ATPase described by Sachs *et al.* This introduces the question of how transepithelial transport of K^+ occurs throughout the animal kingdom.

Most plant and animal tissues generally contain more K^+ than Na^+ , therefore most non-marine and non-blood-feeding animals ingest excess K^+ which they must eliminate. It is becoming increasingly accepted that K^+ secretion in vertebrates (e.g. kidney, colon and exocrine glands) is due to the serosal Na,K-ATPase and high mucosal permeability to K^+ (e.g. Giebisch & Stanton, 1979). This is contrary to earlier suggestions of an apical K^+ pump and some dissociation of K^+ secretion from

Na^+ absorption (reviewed by Prince, 1977). There is presently no incontrovertible evidence for a mucosal K^+ pump in vertebrates which is associated with K^+ secretion. Given the relatively conservative nature of other transport systems throughout the animal kingdom (e.g. Na, K-ATPase ; Na^+/H^+ , $\text{Cl}^-/\text{HCO}_3^-$ and $\text{Ca}^{2+}/\text{Na}^+$ exchangers) it is therefore surprising to find that an apical electrogenic, ouabain-insensitive K^+ pump (actually a general cation pump) is apparently the predominant primary transport mechanism in several major secretory epithelia of insects, including most Malpighian tubules, lepidopteran midgut and integument, and dipteran salivary glands, (see articles by Harvey *et al.* and O'Donnell & Maddrell). However, despite extensive physiological evidence for this insect cation pump, the isolation of a K-ATPase which can bring about K^+ transport and ion-driven ATP synthesis when reconstituted in membrane vesicles has not yet been achieved.

Insects are not only unusual in possessing epithelia which secrete KCl -rich primary fluids, but they possess other epithelia which reabsorb primarily KCl from these fluids: e.g. locust hindgut, rectum and midgut; proximal segments of *Rhodnius* Malpighian tubules and dipteran salivary glands. The article by Hanrahan & Phillips describes an unusual K -dependent, electrogenic Cl^- pump which is the predominant active transport process in locust rectum and conceivably other insect epithelia which reabsorb from KCl -rich fluids. This again raises the long-standing controversy as to the existence in animals of a primary Cl^- (and HCO_3^-) transport process (e.g. de Pont & Bonting, 1981) involving an anion- ATPase in the plasma membrane. This topic is reviewed in the article by Gerencser & Lee.

Transmural transport of Cl^- is widespread in vertebrates and at present can be satisfactorily explained by variations of three basic epithelial mechanisms (see Frizzell, Field & Schultz, 1979; reviewed by Hanrahan & Phillips, 1983): (1) Na (or Na, K)-coupled Cl^- secretion, which is often electrogenic (see articles by Epstein *et al.* and Foskett *et al.*); (2) Na (or Na, K)-coupled Cl^- absorption; and (3) $\text{Cl}^-/\text{HCO}_3^-$ exchange. All three of these processes involve secondary transport of Cl^- by co-transport mechanisms, whereby energy for Cl^- transport is apparently provided by the electrochemical gradient of the counter-ion. Na -coupled Cl^- transport systems are characteristically inhibited by ouabain and diuretics such as furosemide, while $\text{HCO}_3^-/\text{Cl}^-$ exchange is usually inhibited by stilbene-derivatives (SITS, DIDS) and acetazolamide. NaCl co-absorption is generally found in leaky epithelia where there is not a significant transmural potential to move Cl^- by electrical coupling, which is feasible in such tight epithelia as frog skin. Absorption of NaCl from fresh water in many animal epithelia occurs by parallel $\text{HCO}_3^-/\text{Cl}^-$ and Na^+/H^+ (NH_4^+) exchangers in the apical membrane.

Since Frömter first demonstrated that most of the transepithelial current flow across gallbladder occurred between the cells, there has been extensive investigation of the properties of junctional complexes and paracellular routes in 'leaky' vs 'tight' epithelia (reviewed by Powell, 1981). The articles in this volume by Kottra & Frömter and by Cereijido *et al.* provide an update on more recent advances in this area. The somewhat artificial division of epithelia into 'tight' and 'leaky', with a different set of electrical and transport characteristics, is probably not appropriate for many invertebrate epithelia. The article by Podesta describes a flatworm epithelium which has many of the characteristics of a 'leaky' vertebrate epithelium but consists of a syncytium lacking

Paracellular pathways. Additionally, O'Donnell & Maddrell report that *Rhodnius* Malpighian tubules (M.T.) have several properties of tight epithelia (e.g. low ion movement paracellularly, large transmural potentials and ion gradients, e.g. 30-fold for K^+) but, like vertebrate leaky epithelia, transport a near isosmotic fluid at exceptionally high rates. The same situation is reported in vertebrates for the salivary gland (see review by Powell, 1981). As exemplified by detailed studies on locust rectum (article by Hanrahan & Phillips) and lepidopteran midgut (Harvey *et al.*), those insect epithelia investigated to date have in common most of the physiological properties associated with vertebrate tight epithelia; however, they also have relatively low transcellular resistances ($50\text{--}200 \Omega\text{cm}^2$), very high rates of ion transport, and in some cases (e.g. M.T. and salivary glands) isosmotic fluid transport.

Views on the route and mechanism of isosmotic fluid transport across epithelia have undergone a rapid evolution over the past 25 years. Recent work reported in the articles by Spring and by O'Donnell & Maddrell provide new evidence for a transcellular route, involving simple osmosis and very small osmotic concentration differences across apical and basolateral cell borders. The new optical approaches used by Spring to measure changes in cell volume over short time intervals should prove useful in the study of fluid transport and volume regulation in many epithelia. Apparently views on fluid transport have come full circle, but now rest on firmer ground.

This discussion meeting emphasized cellular processes, but we felt it was necessary to end this meeting by reminding ourselves that an appreciation of osmoregulation in any animal ultimately requires an understanding of how the activities of various regulatory organs are inter-related and integrated. Articles by Foskett *et al.* and Pang represent examples of what has been achieved respectively concerning the integrated control of osmoregulation in fish and the evolution of control of renal function.

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