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SYNCYTIAL EPITHELIA: TRANSPORT IN THE ABSENCE OF PARACELLULAR PATHWAYS

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SUMMARY

The syncytial epithelium of parasitic flatworms offers the opportunity to examine epithelial transport physiology in the absence of paracellular pathways. The asymmetric enzymatic and permeability properties of the apical and basal membranes confirm the transepithelial transport function of the syncytial epithelium. Although the absence of a paracellular pathway has led to the suggestion that the syncytium is a 'tight' epithelium, which would be consistent with its low osmotic and diffusive water permeability, the ion transport mechanisms in the apical membrane are more consistent with those predominating in 'leaky' epithelia. Contrary to that expected of an animal covered with a 'tight' epithelium, the parasitic flatworms are not good ion regulators. The apical membrane contains a Cl^- : Na^+ co-transport system characteristic of 'leaky' epithelia. Although external acidification occurs by an active H^+ extrusion mechanism, a large part of H^+ secretion is coupled to Na^+ influx as in 'leaky' epithelia. Categorization of the syncytial epithelium as 'tight' or 'leaky' will have to await electrical potential profile determinations which are made difficult by the electrical coupling of the syncytium to the underlying nerve-muscle syncytium.

INTRODUCTION

In their epithelial physiology, the flatworms present a unique evolutionary sequence ranging from the cellular epithelial surface of free-living flatworms to syncytial epithelia of parasitic forms. The syncytial epithelia, while retaining a transepithelial transport function, are designed to evade host immune effector mechanisms (Podesta, 1980, 1982*a*,*b*). The epithelia of flatworms represent one of nature's first experiments with epithelial tissues which, in the parasitic flatworms, has become a very specialized syncytial layer (Fig. 1). The major adaptive feature of the flatworm syncytium appears to be related to the regulation of and rapid renewal of the apical plasma membrane in response to hostile environmental components (Podesta, 1982*b*).

However, the inclusion of the parasitic flatworms in the present symposium is related to the opportunity offered to examine transepithelial transport functions in the absence of the paracellular pathway present in epithelia of other animals. The objective of this communication will be, therefore, to examine and compare the transport properties of the syncytial epithelia to transport across more intensively studied

Key words: *Hymenolepis diminuta*, *Schistosoma mansoni*, epithelial transport.

cellular epithelia, the latter being the topic of the remainder of this symposium. Although not directly related to the problem of the role of paracellular pathways in epithelial physiology, such a comparison may help to decide whether or not a paracellular pathway is a prerequisite for a particular epithelial transport function.

To approach this question using syncytial epithelia, information regarding the transepithelial transport of ions, nonelectrolytes and water in two species of parasitic flatworms will be reviewed. The first species, *Hymenolepis diminuta*, inhabits the lumen of the rat small intestine and does not have a body cavity or alimentary canal so that all exchange of material with the environment must occur across the surface epithelial syncytium. The apical membrane of the syncytial epithelium of this parasite

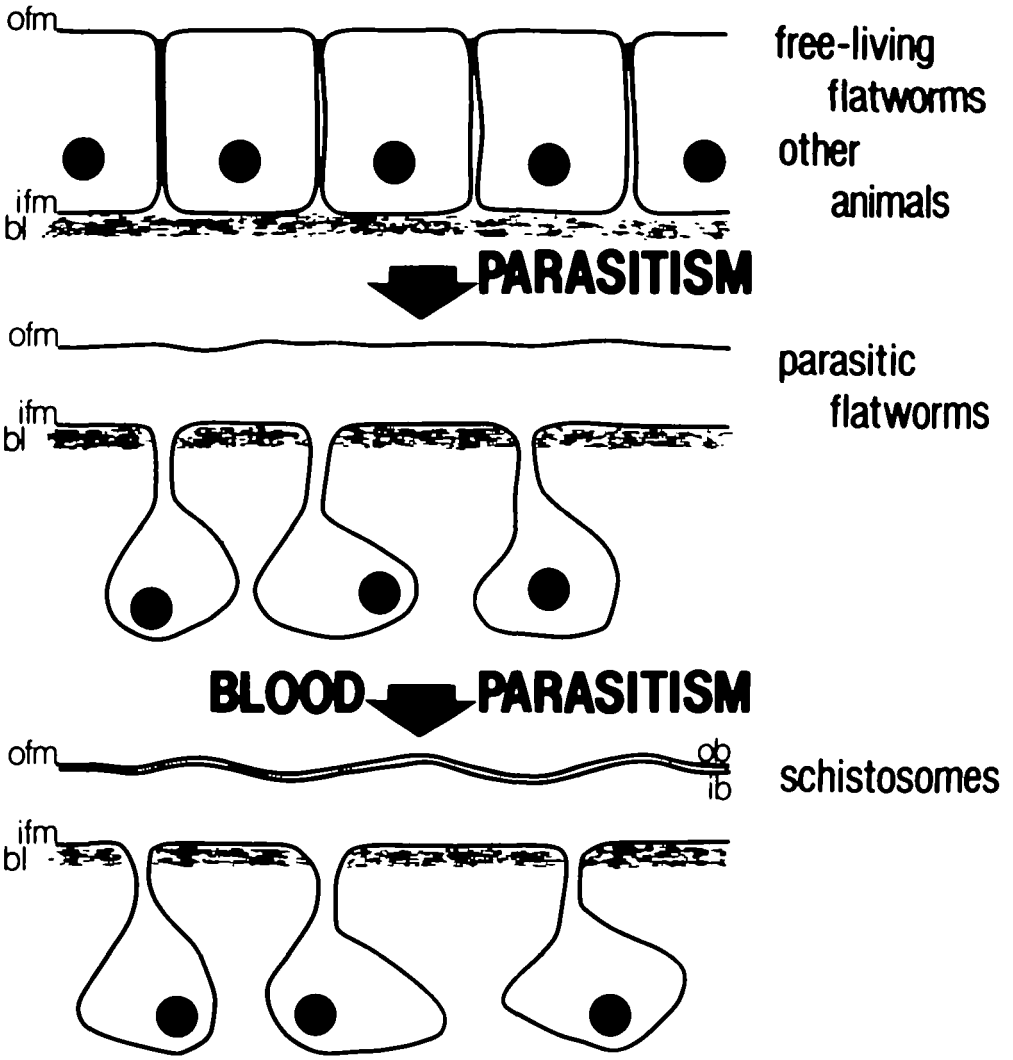

Fig. 1. Sequence of epithelia on surface of free-living flatworms, parasitic flatworms and blood-parasitic flatworms. ofm, outward-facing or apical membrane. ifm, inward-facing or basal membrane. ob, outer bilayer or envelope of schistosomes. ib, inner bilayer or apical plasma membrane. bl, basal lamina.

brush border. The second species, the human blood fluke, *Schistosoma mansoni*, has an oral opening and a sac-like gut, which poses problems in transport studies (Podesta & Dean, 1982*a*,*b*). Although the major adaptation to parasitism in the flatworms involved the function of the syncytial epithelial layer, a secondary adaptation occurred when the flatworms invaded the vertebrate bloodstream. This secondary adaptation occurs structurally as an extra bilayer or envelope overlying the apical plasma membrane of the syncytial epithelium of *S. mansoni* and other blood flukes (Fig. 1). Structurally, the envelope-plasma membrane of *S. mansoni* bears a striking similarity to the plasma membrane-envelope complex of gram negative bacteria (Podesta, 1982*b*).

TRANSPORT PHYSIOLOGY OF SYNCYTIAL EPITHELIA

Ion and water relations in parasitic flatworms

The parasitic flatworms are generally considered to be osmotic conformers with little control over the ion content of their body fluids (Wilson & Webster, 1974; Podesta, 1977*a*). The flame cell system and protonephridial canals of adult *H. diminuta* also appear to have lost the capacity for ion regulation (Wilson & Webster, 1974), although more recent evidence has suggested that the fluid in the canals is hyperosmotic relative to the body fluids and ambient medium of this parasite (Podesta, 1977*a*,*b*, 1980). With respect to total tissue fluids, *H. diminuta* contains, in mmol kg dry wt^{-1}, Na^+ 301, K^+ 383, Cl^- 326 (Podesta, Evans & Stallard, 1977*a*; Podesta *et al.* 1977*b*). Intracellular amounts were determined as Na^+ 124, K^+ 364 and Cl^- 136 (mmol kg dry wt^{-1}) while total tissue and intracellular water amounted to 3·9 and 3·23 kg/kg dry wt, respectively. Similar determinations have recently been reported for *S. mansoni*, which were approximately 25 % lower than those obtained for *H. diminuta*, being Na^+ 220 and K^+ 287 for total tissues in mmol kg dry wt^{-1} (Woldemussie & Bennett, 1982). However, the latter results may be artificially low due to the authors analysing only the supernatants of homogenized and centrifuged tissue. Hence, *H. diminuta* is approximately isosmotic with respect to the intestinal luminal environment but *S. mansoni* may maintain body fluids considerably hypotonic to a blood environment, at least with respect to Na^+ and K^+ concentrations.

When tissue or cell volume is increased in *H. diminuta* due to hypo-osmotic swelling, cell volume is regulated back toward normal (Podesta, 1977*b*), as in virtually all animal cells (Schmidt-Neilsen, 1975; Hoffman, 1977). Hypo-osmotic cell volume regulation is usually achieved by an increased membrane permeability resulting in an increased passive efflux of K^+ (but Na^+ permeability is not altered) and small organic molecules such as the nonessential amino acids (Hoffman & Hendel, 1976; Hoffman, 1977). *H. diminuta* is capable of volume regulation in hypotonic fluids and since the changes in tissue ion concentrations could not account for the dilution of tissue fluids, small organic molecules must also play a role in the regulatory volume decrease observed in this organism (Podesta, 1982*a*). It was not surprising, therefore, to find an increased efflux permeability of the brush border of *H. diminuta* to amino acids during hypotonic incubations, although it was unusual to find a similar response for galactose fluxes during hypo-osmotic stress (Podesta, 1980; Lussier, Podesta & Mettrick, 1978).

Animal cells are generally less capable of volume regulation in hypertonic me[illegible] (Schmidt-Neilsen, 1975) and again, *H. diminuta* is no exception (Podesta, 1977*a*,*b*). However, the influx and efflux permeabilities of the brush border to galactose and alanine were unusual in the sense that there is no known mechanism to account for the observed increased influx permeability and decreased efflux permeability against the direction of net osmotically induced water flow and concentration gradients (Podesta, 1980). In most cells, membrane permeability to Na^+ (but not K^+) is increased during the regulatory volume increase in hypertonic extracellular fluids (Schmidt-Neilsen, 1975). The results for *H. diminuta* were consistent with this latter observation indicating an increase in intracellular Na^+ in hypertonic fluids.

Perhaps one of the more interesting aspects of volume regulation in *H. diminuta* is the observation that the ATPase activity in the isolated apical brush border of this parasite is osmotically sensitive (Rahman, Mettrick & Podesta, 1981*a*,*b*). There occurred a reciprocal relationship between ATPase activity and the tonicity of the reaction mixture containing the brush borders such that the activity of the enzyme was maximal in fluids with the lowest osmolality. Although a similar ouabain-insensitive, ethacrynic acid-sensitive ATPase that transports Na^+ has been detected in other cell systems, and is thought to play a similar role in cell volume regulation, the above study is the first to show modulation of this enzyme by the osmolality of the incubation fluid (Podesta, 1980; Rahman *et al.* 1981*a*,*b*). An ATPase with similar properties has recently been characterized in isolated apical membranes of *S. mansoni* (Podesta & McDiarmid, 1982; McDiarmid, Dean & Podesta, 1982).

To summarize: the parasitic flatworms tend to maintain intracellular and extracellular fluids that resemble those of their host in terms of ion and osmotic concentrations. When placed in anisosmotic fluids they conform osmotically with appropriate alterations in epithelial membrane permeability bringing about predictable changes in tissue concentrations of ions and amino acids.

Membrane asymmetry in the syncytial epithelium

Asymmetric permeability and enzymatic properties of apical and basal membranes is a characteristic property of epithelial cells (Erlij, 1976). As a prerequisite to attributing an epithelial function to the syncytial epithelium of parasitic flatworms, it was necessary to test for asymmetry of the limiting membranes. With respect to membrane associated enzymes, the use of isolated membrane preparations has allowed the following conclusions to be made. The basal membrane of *S. mansoni* contains activity associated with a (Na^+, K^+), Mg^{2+}-dependent ATPase and 5′-nucleotidase (Podesta & McDiarmid, 1982). The absence of the latter enzymes and the presence of alkaline phosphatase and ouabain-insensitive Na^+, Mg^{2+}-dependent ATPase in the apical membrane of both *H. diminuta* and *S. mansoni*, is consistent with a polarized epithelium (Podesta, 1980, 1982*a*,*b*; Rahman *et al.* 1981*a*,*b*; Podesta & McDiarmid, 1982; McDiarmid *et al.* 1982).

A considerable body of information is now available which suggests that the limiting membranes of the syncytium are also asymmetrical with respect to their permeability properties. Studies involving *H. diminuta* have demonstrated that galactose transport across the apical membrane is Na^+-coupled and inhibited by ouabain whereas galactose influx and efflux across the basal membrane is no[illegible]

nsitive to Na^+, is not inhibited by ouabain and has a different stereospecificity. Similarly, Na^+ influx across the brush border was a saturable function of extracellular Na^+, was insensitive to ouabain, inhibited by amiloride and was increased as a saturable function of external galactose or glucose. However, the influx and efflux of Na^+ across the basal membrane were markedly depressed by ouabain, and were insensitive to amiloride. The ratio of radiolabelled galactose entering tissue slices *via* the brush border to radiogalactose entering *via* the exposed extracellular space indicated that under control conditions, five to six times as much galactose enters through the brush border than *via* the extracellular space. The asymmetry of influx and efflux permeabilities across the brush border is clearly indicated by their ratio in which influx is favoured by a factor of 11·9. This asymmetry is even more remarkable since, under steady state conditions, the intracellular concentration of galactose exceeds the medium concentration by a factor of approximately five (Podesta, 1977*a*,*b*,*c*, 1980). The influx permeability across the basal membrane was much less than across the brush border and the influx to efflux ratio favours influx across the brush border and efflux, although only slightly (1·1), across the basal membrane.

Electrical potential profiles in the absence of junctions

The absence of a paracellular pathway in the syncytial epithelium led to the suggestion that if this tissue behaved similarly to a transporting epithelial layer then it would do so in a manner resembling 'tight' epithelia (Podesta, 1980, 1982*a*,*b*). Although the rates of osmotic water permeability of the syncytium of *H. diminuta* and *S. mansoni* tended to support this suggestion (Brodie & Podesta, 1981), only a systematic description of ion conductance across the syncytium will allow a final conclusion to be made. Unfortunately, intracellular and transepithelial potential recordings have been difficult to obtain due to the activity of the parasites *in vitro*, the complications introduced by the cable properties of the syncytial layer, and its electrical coupling to the nerve-muscle syncytium lying beneath the epithelial layer (Koopowitz, 1975; Podesta, 1980; Brodie & Podesta, 1981; Thompson, Pax & Bennett, 1982). The potential drop which occurs across the apical brush border of *H. diminuta* amounts to 45 mV (inside negative) (Uglem & Prior, 1980) and 40–46 mV for *S. mansoni* (Thompson *et al.* 1982; Bricker, Pax & Bennett, 1982). However, it is difficult to interpret the results obtained with deeper impalements. For example, following the recording of the transapical membrane potential (with an input resistance of 0·05–4·6 MΩ and a time constant of 0·24–4·5 ms), deeper impalements give rise to a 'muscle potential' of −22 mV and having an input resistance of 10·3 MΩ with a time constant of 0·25 ms (Thompson *et al.* 1982). Both the 'tegument' and 'muscle' potentials gave rise to linear current-voltage relations. Although the authors referred to the interconnected compartments as a multidimensional syncytium, they failed to consider the complex cable properties of such an electrically coupled system in their estimations of the resistance of individual membranes. As a result, we are left with very little knowledge of the conductance pathways across the syncytial epithelium. Lack of information concerning even the magnitude and polarity of the trans-syncytial potential difference will continue to limit interpretations of data related to the transport properties of this specialized epithelial layer.

Ions and ion-coupled transport

Ion transport across the syncytial epithelium of *H. diminuta* resembles that found in epithelia of a variety of other animals. The brush border transports Na^+ by facilitated diffusion down a concentration and electrical gradient maintained by the Na^+ pump in the basal membrane (Podesta, 1980, 1982*a*,*b*). Influx across the brush border is inhibited by amiloride (Podesta, 1977*a*) and coupled to Cl^- influx (Uglem & Prior, 1980) and H^+ efflux (Podesta, 1978). Intracellular Na^+ and the syncytial transport pool of Na^+ are increased in response to ouabain, sugars, amino acids, CO_2, and decreased in response to hypotonic incubations, Na^+-free incubations and amiloride (Podesta, 1977*a*, 1980). Influx of Ca^{2+} across the apical membrane is a linear function of external Ca^{2+} concentration in *H. diminuta* (Podesta, 1980).

Acidification of the bathing medium by *H. diminuta* occurs by two independent H^+ secretion mechanisms (Podesta, 1978). Ambient-CO_2 independent secretion arises from H^+ derived from anaerobic production of organic acids while H^+ secretion that was dependent on ambient CO_2 originates from the hydration of CO_2 entering the parasite tissue. The latter process also produces HCO_3^- which accelerates anaerobic metabolism (Podesta *et al.* 1976) and the accumulation of organic acids (Podesta, 1978).

The majority of studies concerning the transport properties of flatworm surfaces have involved Na^+-coupled transport of organic molecules. Several recent reviews have dealt with this subject (Podesta, 1980, 1982*a*). More recent studies on this aspect of syncytial transport physiology (Cornford & Oldendorf, 1979; Podesta & Dean, 1982*a*,*b*) have not dealt with the problems still remaining with respect to the source of energy driving Na^+-coupled transport in flatworms (Podesta, 1982*a*; Uglem & Prior, 1980).

Water transport

The ultrastructural changes associated with volume transport in *H. diminuta* and *S. mansoni* are unusual in that the channels arising from the basal membrane into the syncytial layer remain open regardless of the direction of water flow across the syncytium (Brodie & Podesta, 1981; Podesta & Mettrick, 1975). However, the diffusive and osmotic water fluxes across the syncytial epithelia were similar to those reported for other 'tight' epithelia. Water permeability was low in these species and comparable in magnitude to water permeability in 'tight' epithelial tissues. Osmotic water permeability exceeded diffusive transport and inward flow of water exceeded the rate of outward-directed water transport (Brodie & Podesta, 1981). These results constitute the only water flux measurements made using a parasitic flatworm.

COMPARATIVE CONSIDERATIONS

As indicated in the above review, the syncytial epithelium of parasitic flatworms behaves in most respects like transporting epithelia of other animals. What is unique is that the syncytium achieves the transport functions of other epithelia, but without paracellular shunts. This section outlines areas where studies on syncytial epithelia may contribute, in a general sense, to epithelial transport physiology. However, I d

is with anxiety arising out of the relative lack of information on syncytial epithelia, particularly that relating to the electrical gradients across the epithelial layer.

Tight and leaky epithelia

The syncytial epithelium of *H. diminuta* presents an apparent contradiction in the sense that, morphologically, it is an extreme example of a 'tight' epithelial layer and yet the parasites are not able to regulate the ion concentrations of their body fluids. However, it is more realistic to assume that the selection pressures giving rise to the syncytium did not arise from a requirement for ion regulation. Since the free-living flatworms face more severe osmoregulatory problems and have retained a cellular epithelial surface, the syncytium of parasites probably arose from a need to cope with the host's immune effector mechanisms or simply to avoid being digested by the host in the case of intestinal dwelling flatworms (Podesta, 1980, 1982*b*). Nevertheless, even though the ion regulation potential of the 'tight' syncytial epithelium may have evolved secondary to a protective function, the question remains as to why the parasitic flatworms are not good ion regulators. As is the case with the rabbit submaxillary main duct epithelium (Augustus, Bijman, van Os & Slegers, 1977), the resistances of the syncytial membranes may be low. Ion regulation may be a function of the flame cells in free-living flatworms but the capacity of flame cells for ion regulation in parasitic flatworms may be lost. At present, the information available is not sufficient to explain why, given a 'tight' syncytial epithelial surface, parasitic flatworms are osmotic conformers.

Coupled transport of Na^+ and Cl^-

With few exceptions, epithelia may be categorized as those in which Na^+ and Cl^- influx are coupled electrically or are coupled through an electrically neutral co-transport system (Frizzell, Field & Schultz, 1979). The former mechanism is typical of epithelia which are able to sustain electrical gradients sufficient to pull Cl^- across the epithelial layer in response to active Na^+ extrusion across the basal membrane. This mechanism is therefore characteristic only of 'tight' epithelia. The direct co-transport mechanism is characteristic of 'leaky' epithelia which are able to sustain only small or negligible transmural potential differences.

The mechanism of Na^+ and Cl^- transport in *H. diminuta* is again an exception in that the two ions appear to traverse the brush border coupled directly in a 1:1 stoichiometry (Podesta & Mettrick, 1976; Podesta, 1977*a*; Uglem & Prior, 1980).

Acidification

Two mechanisms for H^+ secretion across the apical membrane of various epithelia are currently acknowledged – an electroneutral H^+: Na^+ exchange in 'leaky' epithelia and active, ATP-dependent H^+ extrusion in 'tight' epithelia (Steinmetz & Andersen, 1982). The situation with respect to H^+ secretion by *H. diminuta* is not as clear as in other acidifying epithelia. Although H^+ secretion in *H. diminuta* is not complicated by metabolic production of CO_2 in these anaerobic organisms, the sources of protons for secretion and the mechanism of secretion appear to combine the characteristics of secretion in both 'tight' and 'leaky' epithelia (Podesta, 1978).

In *H. diminuta*, H^+ for ambient CO_2-independent secretion (H^+_1) was derived from anaerobic generation of organic acids (lactate, succinate) or possibly from the

utilization of ATP to drive active ion transport, including H^+. H^+_1 secretion was partially inhibited (50 %) by iodoacetate and similarly inhibited by acetazolamide, the latter occurring *via* the Na^+-dependent component of H^+_1 secretion. The rate of acidification was primarily dependent upon ambient CO_2 (H^+_2) which accelerates proton secretion in two ways. First, *via* H^+ generated as a hydration product and, second, *via* HCO_3^- by virtue of its ability to accelerate metabolic production of succinate through the stimulation of phosphoenolpyruvate carboxykinase (Podesta, 1978; Podesta *et al.* 1976).

H^+ secretion was inhibited by 40–50 % using Na^+ replacement, 36 % by ouabain, and 65 % by amiloride, the latter completely inhibiting Na^+ influx across the brush border of *H. diminuta* (Podesta, 1978). Glucose, while accelerating Na^+ influx by 65–95 %, stimulated H^+ secretion by only 40 %. H^+_2 secretion was more sensitive than H^+_1 secretion to amiloride and ouabain. Addition of ATP stimulated H^+ secretion by 55 % and more than doubled H^+ secretion. These results argue for at least a partial coupling of H^+ extrusion to Na^+ influx across the brush border, combined with an active, Na^+-independent mechanism. Therefore, the apical membrane of *H. diminuta* appears to contain the $Na^+ : H^+$ exchange mechanism characteristic of 'leaky' epithelia while also having a Na^+-independent, active extrusion mechanism found predominantly in 'tight' epithelia.

Water transport

In the above discussion I have attempted to emphasize that although the absence of a shunt pathway would lead one to conclude that the syncytial epithelium is a 'tight' epithelial layer, the transport mechanisms at least in the apical membrane are more consistent with 'leaky' epithelia (coupled $Na^+ : Cl^-$ influx, $Na^+ : H^+$ exchange and absence of ion regulation). In the absence of information concerning transepithelial and transmembrane resistances, it is difficult to take a final position on these discrepancies. However, the strongest evidence presently available which supports the morphological conclusion relates to a recent study on water transport across the syncytial epithelium of both *H. diminuta* and *S. mansoni* (Brodie & Podesta, 1981). In this study, the osmotic water permeability of these epithelia was determined as $9{\cdot}6 \times 10^{-5}\,cm\,s^{-1}$, which was in the range of values expected for 'tight' but not 'leaky' epithelia. In all aspects examined, diffusive and osmotic water permeabilities resembled those found in 'tight' epithelia of other animals. An exception arose from the observation that blind-ending channels arising from the basal membrane and extending into the syncytium remained expanded regardless of the direction of osmotically induced water transport. The asymmetry in water flows (absorption > secretion) across the syncytial epithelia could not therefore be explained by collapsing channels in the secretory direction as suggested for cellular epithelia (Brodie & Podesta, 1981). Perhaps more importantly, the syncytial epithelia achieve salt-water coupling in the absence of a paracellular pathway.

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