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PARACELLULAR AND TRANSCELLULAR ROUTES FOR WATER AND SOLUTE MOVEMENTS ACROSS INSECT EPITHELIA

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SUMMARY

Because the frontal area of the intercellular clefts in Malpighian tubules is small, and the osmotic permeability of the cell membranes is large, the route for transepithelial water movement during fluid secretion is transcellular. Water movements appear to be a passive response to osmotic gradients of a few mosmol l^{-1} produced in the cells and in the lumen by active ion transport.

The excretory functions of Malpighian tubules are discussed in relation to recent analyses of the routes of passive permeation for non-electrolytes. Uncharged molecules smaller than a disaccharide appear to move at significant rates through the cells whereas molecules as large as inulin traverse the epithelium by a paracellular path. In addition there are specific active transport mechanisms for a variety of organic molecules.

The routes and mechanisms proposed for water and solute movements are discussed in relation to comparable studies in other epithelia.

INTRODUCTION

One of the most dramatic properties of insect epithelia is the capability of tissues, such as Malpighian tubules or salivary glands, for extremely rapid fluid secretion. The blood-feeding hemipteran *Rhodnius prolixus*, for example, rapidly reduces the volume of a blood meal equivalent to 12 times its own body weight by taking up fluid from the haemolymph into the Malpighian tubules and excreting up to 1·2 μl of urine per minute. A volume of fluid equal to the volume of the tubule cells enters the lumen of each tubule every 16 s. This rate of excretion, equivalent to removing the animal's normal haemolymph volume once every 15 min, equals 3·3 $\mu l\,min^{-1}\,cm^{-2}$ of tubule wall. For comparison, the latter value is three times the fluid transport rate of the canine gallbladder (Grim & Smith, 1957). The first section of this review concerns the route by which such relatively large quantities of water cross the tubule wall. Does water move through the cells, through the spaces between the cells, or by a combination of these routes?

A wide variety of haemolymph solutes also cross into the lumen of the Malpighian

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tubules. In addition to passive diffusion of solutes, there are active ion pum[illegible] associated with fluid secretion in the upper tubule and KCl reabsorption by the lower tubule, and a variety of specific active transport mechanisms for organic solutes. Tubular fluid is discharged into the alimentary canal at the junction of the midgut and the hindgut, and useful materials are selectively reabsorbed in the rectum. The second section of this review concerns the paracellular and transcellular routes by which solutes cross insect epithelia, especially Malpighian tubules. Detailed reviews are available elsewhere concerning the insect midgut (Harvey, 1980, 1982; this volume), rectum (Phillips, 1980, 1981; Hanrahan & Phillips, this volume) and salivary glands (Berridge, 1977). Where appropriate, comparisons of tubule physiology will be made with these other epithelia and with selected vertebrate tissues.

TRANSCELLULAR MOVEMENTS OF WATER

Water can cross an epithelium by several routes, as depicted in Fig. 1. The standing gradient model for osmotic water flow in rabbit gallbladder (Diamond & Bossert, 1967), emphasized the importance of both the cells and the paracellular spaces for water movement during secretion or absorption by many vertebrate epithelia (Fig. 1C). This model extended the local osmosis theory of Curran (1960); it suggested that the lateral intercellular spaces of the rabbit gallbladder form approximately cylindrical channels, closed at one end by tight junctions, and that salt transport into the closed end of the channels produces a localized osmotic gradient. Water enters the cells across the basal membranes and moves passively across the lateral cell membranes into the channels in response to this gradient. The entry of water raises channel hydrostatic pressure and forces a volume flow out of the open end of the channel. In the steady state a standing gradient of osmotic pressures exists within the channels; the fluid is

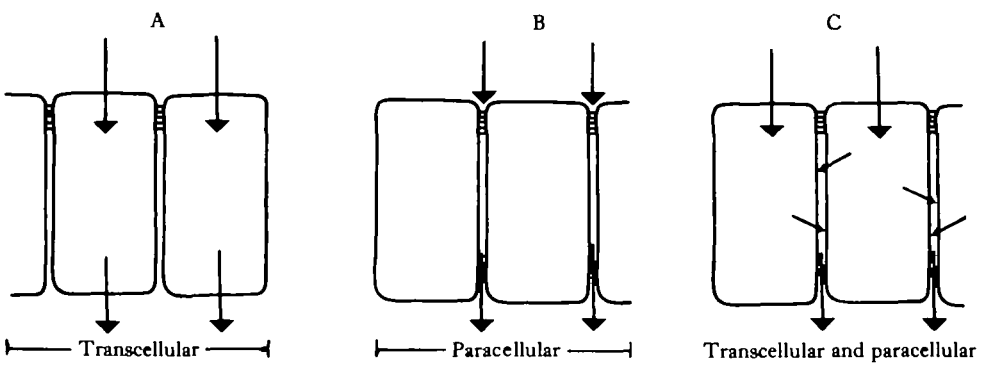

Fig. 1. Schematic diagram showing possible routes for transepithelial water flow. In each case net water flow is from top to bottom. For transcellular flow (A), water (arrows) moves in response to hypertonicity created by salt transport (not shown) across both the basal (upper) membrane into the cells, and across the apical (lower) membrane into the compartment beneath the epithelium. For strictly paracellular flow (B), water moves across the cell–cell junctions in response to salt transport into the lateral intercellular spaces. This mechanism requires that cell membrane permeability be a negligible fraction of junctional water permeability, and so there is no significant transcellular water flow. For the final case of both transcellular and paracellular water flow (C), it is assumed that the basal and lateral cell membranes are permeable to water, which therefore moves across both cell membranes and junctional complexes in response to lateral interspace hypertonicity.

most hypertonic near the closed end of the lateral intercellular space, and approaches isotonicity at the open end. Fluid absorption from the gallbladder lumen would be expected to distend the lateral intercellular spaces, as is indeed observed during normal isotonic water flow (Kaye, Wheeler, Whitlock & Lane, 1966) or during flows produced by experimentally-imposed osmotic gradients (Smulders, Tormey & Wright, 1972). Distension of the spaces produces an increase in both apparent hydraulic conductivity and the permeabilities of non-electrolytes, suggesting that the paracellular spaces are a final common pathway for the movements of both solutes and water across the epithelium (Wright, Smulders & Tormey, 1972).

In contrast, in some insect epithelia such as blowfly salivary glands, paracellular spaces between structures which are associated with secretion, such as the basal infolds and canaliculi, do not distend even when the rate of fluid secretion is increased 60-fold (Oschman & Berridge, 1970). In *Rhodnius* Malpighian tubules the significance of paracellular water movements has been calculated indirectly (Maddrell, 1980). The paracellular clefts between the tubule cells are about 17 nm in width and occupy about 0·039 % of the frontal area of the tubules. If all the fluid moved through the clefts (120 nl min^{-1}) it would pass through at 660 $\mu m\, s^{-1}$. Because each cleft is only 15 μm in length, the fluid would take only 22 ms to travel through it. Since even small ions and solutes such as urea would take 2–3 times as long to diffuse 15 μm, and larger solutes such as sucrose would take longer, a strong correlation might be expected between the rate of fluid transport and passive transepithelial movements of extracellular markers. Entrainment of solutes into a rapidly flowing fluid should accelerate transepithelial solute movement. In fact, providing intraluminal pressure is maintained constant, neither sucrose nor inulin crosses the wall of the tubule any faster during rapid fluid secretion (Maddrell, 1980). This absence of significant solute/solvent coupling in the clefts, and the small number and area of the clefts strongly suggest that water movements through a paracellular route are unlikely to be a significant contribution to total transepithelial secretion by the tubules. As well, the permeability of the paracellular path to compounds as large as inulin implies that a local osmotic gradient could not be produced by active solute transport into the clefts; any concentration gradient would be quickly dissipated by passive solute diffusion.

The other route by which fluid can cross the epithelium is through the cells (Fig. 1A). A general requirement for transcellular water flow is that the cell membranes be highly permeable to water, and the accurate measurement of osmotic permeability (P_{os}) is therefore fundamental to determining the route of water flow. The first measurements of P_{os} for Malpighian tubules suggested a value of 1 or 2×10^{-3} cm s^{-1} $osmol^{-1}$ l (Maddrell, 1980). These experiments were affected by unstirred layers, which diminish the experimental osmotic gradient and produce an underestimate of P_{os}. Unstirred layer effects can reduce the value of P_{os} by 10-fold or more; the basis for these errors has been described in detail by Diamond (1979). Nevertheless, the high permeability of the tubule wall suggested that fluid secretion by the tubules could be explained by transcellular water flow in response to slightly hypertonic conditions in the cells and in the lumen.

Recently, improved measurements of P_{os} have been obtained by rapidly perfusing both the luminal and basal surfaces of part of the tubule's length (Fig. 2) so that

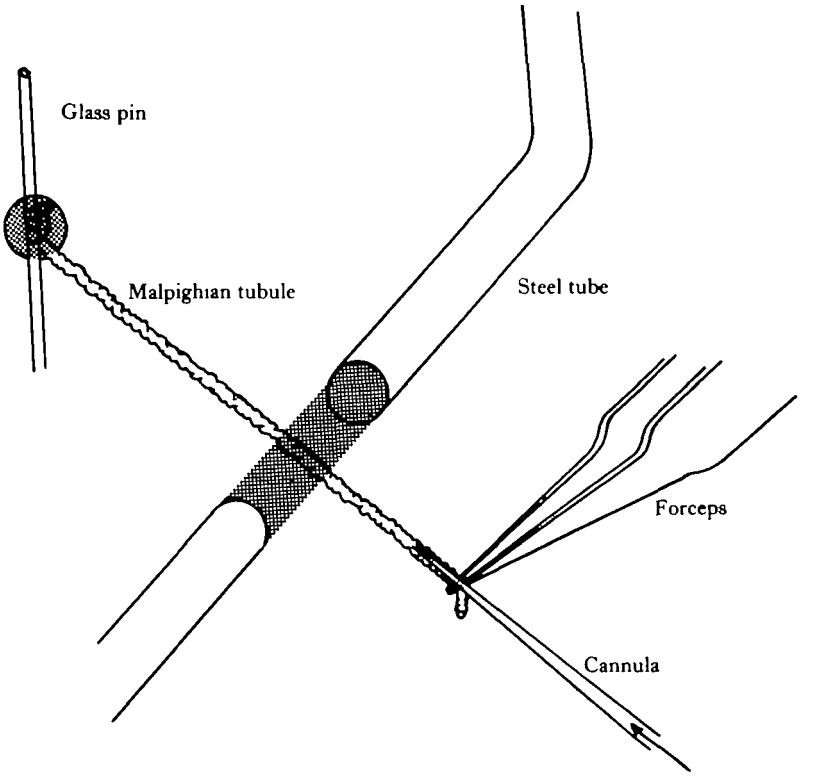

Fig. 2. Diagram of the apparatus used to perfuse both the luminal and basal surfaces of a length of Malpighian tubule. The tubule was cannulated under paraffin and the lumen perfused with normal saline (340 mosmol l^{-1}). A short (<1 mm) region of the external surface of the tubule's length was perfused by passing hypotonic saline (170 mosmol l^{-1}) between two steel tubes aligned at right angles to the tube's length. By appropriate adjustments of flow rate and pressure of the external perfusate a column of fluid of the same diameter as the inside of the steel tubes (0·9 mm) bathed the outer surface of the tubule. Water entered the tubule in response to the osmotic gradient, thereby increasing the efflux of fluid from the open end of the tubule. Rates of efflux were measured by periodically observing the diameter and calculating the volume of the droplet which flowed out of the open end of the tubule. The osmotic flux was determined by subtracting the internal perfusion rate from this value and dividing by the area of tubule wall exposed to the external perfusate. (From O'Donnell, Aldis & Maddrell, 1982.)

unstirred layers were minimized (O'Donnell, Aldis & Maddrell, 1982). An osmotic flux was produced by perfusing the lumen of the tubules with a saline which was hypertonic to the fluids bathing the tubule's external surfaces. Osmotic permeability was then calculated using the formula:

$$J_v = P_{os} (C_i - C_o)$$

where C_i and C_o are the osmolarities of the inner and outer perfusion fluids, respectively. Values for P_{os} of the upper fluid-secreting portion of the Malpighian tubule were $4{\cdot}3 \times 10^{-3}$ cm s^{-1} osmol^{-1} l. When corrected for unstirred layer effects (O'Donnell *et al.* 1982), this value increased by about 35 %, to $5{\cdot}8 \times 10^{-3}$ cm s^{-1} osmol^{-1} l.

The primary implication of the high osmotic permeability of the cell membranes, as discussed below, is that water probably moves through the cells during flu

cretion, and that water movements can be simply coupled to salt transport across the basal and apical cell membranes. In turn, a transcellular route for water flow necessitates some changes in our interpretation of the functional morphology of the transporting cells, and the influences of the cytoplasm on fluid transport. Transcellular flows also require that any regulation of osmotic permeability be accomplished through alterations of the cell membranes, and not through changes in the properties of the junctional complexes or the intercellular clefts.

Salt-water coupling during fluid secretion

In view of our improved estimate for P_{os} it is worth repeating here the calculations of Maddrell (1980) which suggested that fluid secretion in *Rhodnius* Malpighian tubules is based primarily upon transcellular fluxes of water. These calculations take account of the increase in area of the tubule wall produced by folding of the cell membranes, but do not assume any improvement in the efficiency of osmotic coupling as a result of membrane folding. In other words, it is assumed that the membranes behave as though they were planar, and that salt transport across the basal and apical cell membranes makes the cell slightly hypertonic to the bathing fluids, and the lumen slightly hypertonic to the cell. Net bath to lumen flow is viewed as a simple passive movement of water in response to these small differences in osmolarity created by active movements of salts into the cells and lumen. One test of this simple mechanism is to compare the calculated osmolarity of the luminal fluids with the osmolarity which is determined experimentally. The first step in this analysis is an estimation of membrane permeability.

The permeabilities of the basal and apical cell membranes (P_m) can be calculated from the measured transepithelial permeability (P_{os}) by accounting for the increase in surface area produced by membrane folding. According to the formula derived by Hill (1975):

$$1/P_{os} = 1/aP_m + 1/bP_m$$

where a and b are the relative increase in surface area produced by the apical microvilli and basal infoldings, respectively. Electron micrographs suggest values of 150 and 40 for a and b, respectively, and the value of P_m is therefore about $1{\cdot}8 \times 10^{-4}$ cm s^{-1} osmol^{-1} l. The concentration of the secreted fluid can be estimated from the formula (developed in Maddrell, 1980):

$$C_e = \frac{C_o + [C_o^2 + 4(N/P)]^{\frac{1}{2}}}{2}$$

where C_e, C_o are the osmolarities of the emergent and bathing fluids, respectively, and P is the osmotic permeability. N is the rate of solute transport (osmol s^{-1} cm^{-2}) per unit area of membrane and is calculated as follows. If the rate of fluid secretion is 100 nl min^{-1} and the osmolarity of the emergent fluid is 340 mosmol l^{-1}, then the rate of solute transport is 0·6 nosmol s^{-1} or 6·6 nosmol s^{-1} across each square centimeter of tubule. For the apical side of the cell, the membrane area is 150 times larger than the frontal area, and $N = 6{\cdot}6 \times 10^{-9}/150 = 45$ posmol s^{-1} cm^{-2}. Similarly, the area of the basal side of the cell is increased 40-fold by membrane infoldings, and

$N = 6{\cdot}6 \times 10^{-9}/40 = 160$ posmol s^{-1} cm^{-2}. Using the formula given earlier, and assuming that the membranes are planar, it follows that the fluid leaving the apical membrane of the tubule would be more concentrated than the intracellular fluids by 0·7 mosmol l^{-1} and the fluid transported into the cell across the basal membrane would be 2·6 mosmol l^{-1} more concentrated than the bathing fluid. It is not easy to determine the concentration of the fluid produced by both membranes acting in series. But, in the steady state, fluids of the same osmotic concentration must, of course, cross both cell membranes at the same rates. So if the fluid entering the cell is more concentrated than the bath by 0·7 mosmol l^{-1}, and the lumen is more concentrated than the cell by 2·6 mosmol l^{-1}, then the fluid secreted by the tubules will be 3·3 mosmol l^{-1} more concentrated than the bathing fluids, or within 1 % of iso-osmocity, close to the value (1·3 %) actually observed (Maddrell, 1969).

It appears, therefore, that because the tubule wall is highly permeable, osmotic gradients of only a few mosmol l^{-1} would be sufficient to drive transcellular fluid movements in *Rhodnius* Malpighian tubules. Volume flows driven by very small osmotic gradients have also been suggested for other tissues. A reduction in luminal Na concentration of 0·4–0·6 mmol l^{-1} adequately accounts for the observed volume flow in rabbit proximal tubule (Andreoli & Schafer, 1978). Most of this flow is presumed to be transcellular (Rector & Berry, 1982). In the rabbit gall bladder, Persson & Spring (1982) concluded that the normal rates of fluid absorption could be accounted for if the cell was 2·4 mosmol l^{-1} hypertonic to the lumen and the solution on the basolateral surface was 1·1 mosmol l^{-1} hypertonic to the cell. As Persson & Spring (1982) and O'Donnell *et al.* (1982) point out, very high osmotic permeabilities of cell membranes remove the need for consideration of standing osmotic gradients or specific epithelial cell geometries; in Malpighian tubules it appears that water will flow transcellularly in response to small differences in osmolarity across planar membranes.

Although water crosses *Rhodnius* Malpighian tubules by a transcellular route, in other insect epithelia there is good evidence that some of the fluid moves paracellularly, and that solute/solvent coupling occurs in an extracellular compartment. During faecal dehydration by *Periplaneta*, water is reabsorbed from the faeces by the rectal pads. Wall, Oschman & Schmidt-Nielsen (1970) sampled fluid from the extracellular spaces and found it to be, on average, 130 mosmol l^{-1} hypertonic to the rectal lumen. During secretion, the flow of water distends the spaces and causes fluid flow into the haemolymph. Paracellular fluid flow is suggested by the morphology of the rectal pads, which are comprised of tall columnar cells and long, winding intercellular spaces in which local osmosis might occur. In contrast, the morphology of Malpighian tubule cells is highly suggestive of transcellular fluid flow. The cells are squamous and the intercellular spaces are widely separated. Most importantly, the frontal area of the tubule cells is 10^5 times larger than the area of the intercellular clefts. Cell membrane area is further increased by basal infoldings and apical microvilli.

In *Calliphora* salivary glands, it has been suggested that active secretion of K^+ into the apical membrane folds and into extracellular spaces, called canaliculi, might produce local osmotic gradients which would generate iso-osmotic fluid secretion (Oschman & Berridge, 1970). Estimates of ion activities calculated from data obtained by the electron microprobe and ion-selective miroelectrodes (Gupta, Berridge, Ha[illegible]

Floreton, 1978*b*) were consistent with this proposal. It has recently been suggested (Gupta & Hall, 1981) that some of the water flow in this tissue is transcellular but that a significant proportion is paracellular. It also appears that there is a significant paracellular flow during absorption of water by the rectal pads of *Calliphora* (Gupta, Wall, Oschman & Hall, 1980; Gupta & Hall, 1981).

Where paracellular flows are suspected, direct sampling of the lateral interspace fluid by micropuncture techniques, as has been done in the cockroach (Wall *et al.* 1970), is a useful experimental tool. The presence of hyperosmotic interspace fluids suggests, but does not prove, that water moves paracellularly in response to solute transport into the spaces. Results obtained by micropuncture sampling must be interpreted with some caution however, because of the possibility of tissue damage and the mixing of fluid samples collected from small spaces (Hill, 1975). In some tissues, the spaces are so small as to preclude direct fluid collection; the alternative methods which must be employed are also open to criticism. For example, measurement of ion concentrations in the lateral spaces of *Necturus* gallbladder by ion-selective microelectrodes indicates that the interspace fluid is at most 15 mosmol l^{-1} hypertonic to the lumen (Simon, Curci, Gebler & Frömter, 1981). For technical reasons the precise degree of hypertonicity has been difficult to estimate. It was concluded, however, that although the data do not show definitely whether the space fluid is isotonic or slightly hypertonic, they definitely preclude a larger hypertonicity of the order of 100 mosmol l^{-1}, as has been suggested for the lateral spaces of the rabbit ileum on the basis of electron microprobe analysis of frozen hydrated tissue sections (Gupta, Hall & Naftalin, 1978*a*). But measurements of ion activities by ion-selective microelectrodes can also be misleading. Spring & Ericson (1982) note that in some cases changes in ion concentrations, as measured by ion-selective microelectrodes, are not the result of ionic movements, but are the consequence of changes in intracellular water content during cell volume changes. It appears, therefore, that measurements based on more than one technique (cf. Gupta *et al.* 1978*b*) are required if solute concentrations are to be estimated with confidence.

Regulation of osmotic permeability

Both fluid secretion by the upper Malpighian tubule in *Rhodnius* and reabsorption of KCl from the luminal fluids by the lower tubule are stimulated by the diuretic hormone mimic 5HT. The urine produced by the tubules is hypotonic to the haemolymph (Maddrell & Phillips, 1975). The osmotic permeability of the upper tubule does not change in response to 5HT (O'Donnell *et al.* 1982). However, one might expect P_{os} in the lower tubule to be reduced. Otherwise an osmotic flow of water would accompany the reabsorbed KCl, and a hypotonic urine could not be produced. It is physiologically advantageous, therefore, that the osmotic permeability of the lower tubule is less than that of the upper tubule (Fig. 3A). Indeed, this reduction is most apparent over the proximal 30 % of the tubule's length (Fig. 3A), the same region where most KCl reabsorption occurs (Maddrell, 1980). Moreover, values of P_{os} in the lower tubule were reduced by about 50 % in response to 5HT (Fig. 3B), which stimulates KCl reabsorption. As one would expect in a system capable of maintaining an osmotic gradient, the permeability of the lower tubules to organic solutes such as *p*-aminohippuric acid, glucose, sucrose and inulin is reduced (Maddrell & Phillips, 1975).

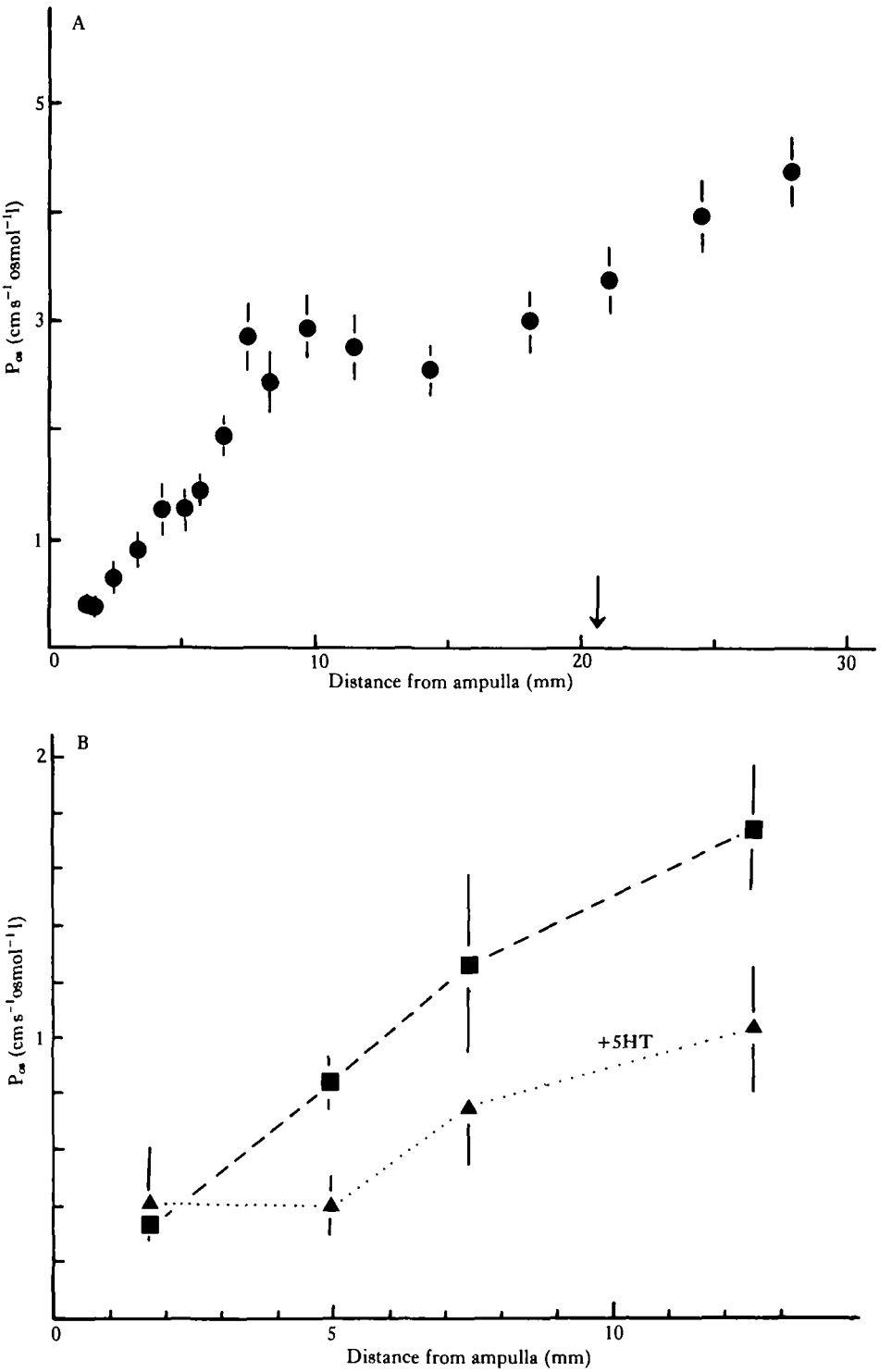

Fig. 3. Variation of osmotic permeability with distance along the lower Malpighian tubule. (A) The junction of the upper and lower tubules is indicated by the arrow on the abscissa. Bars correspond to ±1 S.E.M. (N = 16–22 tubules). (B) Effects of 5HT on the osmotic permeability of lower Malpighian tubules. Mean values are given for the same 10 tubules before (squares) and during (triangles) exposure to 5HT. (From O'Donnell, Aldis & Maddrell, 1982.)

It has been suggested before that regulation of the passive permeability within an epithelium can form the basis for control of either hyper- or hypo-osmotic secretion (Phillips, 1965). In *Periplaneta*, for example, fluid in the rectal lumen can become either hyper- or hypo-osmotic to the haemolymph, depending on the availability of water to the animal (Wall & Oschman, 1970). Similarly, the brackish water mosquito forms a hyper- or hypo-osmotic excrement depending on ambient salinity (Ramsay, 1950). In the rectal papillae of *Calliphora*, Gupta & Berridge (1966) found neurosecretory-like nerve terminals and postulated that they might release a hormone that regulates the reabsorptive process through effects upon the passive permeability of the plasma membrane, in a manner analogous with antidiuretic hormone (ADH) effects in the collecting duct of the vertebrate kidney or the toad urinary bladder.

The basis for hormonally-induced changes in permeability in the toad bladder has been the subject of some very elegant research. Freeze-fracture electron microscopy has demonstrated that ADH stimulation alters the structure of the luminal membrane of the granular cells by producing an ordered aggregation of intramembrane particles (Kachadorian, Wade & DiScala, 1975). Changes in ADH-induced osmotic water flow are explainable through changes in the frequency of aggregation sites per unit area of membrane, and not through changes in the resistances of any other flow barriers which lie in series with the luminal membrane (Levine & Kachadorian, 1981). In another tissue, the erythrocyte, similar intramembrane particles are sites which provide a preferential structural pathway for transmembrane water movements (Pinto da Silva, 1973). The increase in permeability produced by ADH in toad bladder involves both cytoplasmic microtubules (Taylor, Mamelak, Golbetz & Maffly, 1978) and bulk circulation of membranes, *via* exo- and endocytosis (Gronowicz, Masur & Holtzman, 1980). It appears that the intramembrane particles are transferred from cytoplasmic to luminal membranes; the fusion of the two types of membrane requires intact microtubules. Once fusion has occurred, microfilaments appear to be involved in the movement of particle aggregates from the intracellular membranes to the luminal membranes (Kachadorian, Ellis & Muller, 1979; Muller, Kachadorian & DiScala, 1981). It will be of great interest to see if structures comparable to the intramembrane particles in toad bladder cells are found in insect epithelia.

Morphological considerations

One of the great attractions of the standing gradient model (Diamond & Bossert, 1967) is the intuitively appealing explanation of the relationship between morphology and function in a fluid transporting epithelium. The extensive arrays of lateral intercellular spaces, sealed at one end by tight junctions, form the channels within which the standing gradients are established. In Malpighian tubules, the most striking ultrastructural features are the extensive basal infoldings and the densely-packed apical microvilli. But if water flow in the Malpighian tubules of *Rhodnius* is predominantly transcellular, as appears to be the case, what is the function of such membrane elaborations?

The correlation between shorter microvilli and slower fluid secretion in insect epithelia (Maddrell, 1971) suggests that microvilli are important to secretion. As well, the microvilli are shorter and more widely spaced in the lower Malpighian tubule of *Rhodnius* (Wigglesworth & Salpeter, 1962), and P_{os} is reduced in this part of the

tubule (O'Donnell *et al.* 1982). A mathematical analysis of the effects of membrane elaborations such as microvilli (D. L. S. McElwain, personal communication cited by Maddrell, 1980) indicates that membrane foldings improve osmotic coupling. Alternatively, it has been suggested that basal infoldings in secretory epithelia might be involved in the control of transport, as part of the apparatus involved in stimulus-secretion coupling, a function analogous to that of the transverse tubules of muscle, enabling a message at the cell surface to reach organelles deep within the cell (Oschman, 1980*a*). Yet another possibility is that the infoldings and microvilli ensure that much of the cytoplasm is in close association with membrane, and solute-solvent coupling might be altered in such a way as to facilitate fluid secretion. This suggestion is supported by studies with artificial membranes, which indicate that water molecules are ordered near membranes, and water diffusion may, therefore, be reduced (Zaccai, Blasie & Schoenborn, 1975). Similarly, bringing cell membranes closer together by folding them provides more anchoring points for the cytoskeletal apparatus, with the result that the cytoplasm is not damaged during the virtual flood of transcellular water flow.

However, one of the more plausible explanations for the function of microvilli and basal infoldings stems from the fact that such structures are regions where membranes are highly curved. This suggestion has been described before in an excellent review (Oschman, 1980*a*). Because the packing and distribution of membrane phospholipids is altered on the convex side of a membrane fold, a region of high osmotic permeability is created. Theoretical analysis of the nature of passive flows through tightly-folded membranes also suggests that, near isotonicity, such structures provide for preferential passive flows of water and that salt movements might be impeded (Richardson, Licks & Bartoli, 1973). Experiments with phospholipid vesicles also indicate that small vesicles, which are highly curved, are more permeable to water, presumably because of effects upon molecular packing (Sheetz & Chan, 1972). As well, transport properties of membranes in regions of high curvature might differ as a result of preferential adsorption of transport proteins to these bilayer regions (Sheetz & Chan, 1972). However, it must be noted that the diameters of the vesicles used in these studies (25–35 nm) were about five-fold smaller than the diameter of microvilli in insect epithelia; curvature effects may therefore be less significant in biological membranes.

Oschman (1980*a*) has also suggested that the permeability of membranes might be controlled by altering the radius of curvature of surface folds. For example, intestinal microvilli contain microfilaments composed of actin (Tilney & Mooseker, 1971). The microvilli contract in a calcium-dependent process (Mooseker, 1974) which involves changes in the cross-linking of the actin filaments (Mooseker *et al.* 1980). *In vivo*, such contraction might play a role in epithelial transport not only through alterations of membrane curvature, but also through stirring of the fluid between the microvilli (Oschman, 1980*a*).

Transcellular flows: possible influences of the cytoplasm

If water moves transcellularly during fluid secretion, one would expect that the properties of the cytoplasm might significantly affect fluid movements or solvent-solute coupling.

However, in at least one vertebrate tissue, *Necturus* gallbladder, it is unlikely that the cytoplasmic resistance to water flow is a significant contribution to epithelial hydraulic conductivity. By an elegant microscopic technique, Persson & Spring (1982) were able to observe cytoplasmic water flow as a wave which progressed across the cytoplasm in response to a sudden change in the osmolarity of one of the bathing fluids. The speed of the wave travelling across the cytoplasm was 60 times that of the maximum osmotic volume flow. In this tissue, therefore, the principal restriction to transcellular water flow is the cell membrane. It will be of great interest to know if this conclusion applies to other epithelia in which water flow is predominantly transcellular. Of great use in such studies will be new microscopic techniques, such as those developed for epithelia (Spring, 1979; Persson & Spring, 1982) and for analysis of intracellular transport in axons (Allen *et al.* 1982) and transport in extruded axoplasm (Brady, Lasek & Allen, 1982).

However, besides possible effects upon resistance to flow, the cytoplasm may affect transcellular flow through binding part of the intracellular water or salts. In the barnacle muscle fibre, 20–30 % of intracellular water is not osmotically active or available to act as a solvent for electrolytes (Hinke, 1970). Measurements of the self-diffusion coefficient of water in muscle (Caillé & Hinke, 1974) and frog eggs (Ling, Ochenfeld & Karreman, 1969) indicate that not all the water in the cell is available for diffusion. The physical basis for water binding is that polar groups, such as those on proteins, produce electric fields which orientate water molecules (Kuntz & Kauzmann, 1974). The values of the aqueous diffusion coefficient (D) in cytoplasm of various tissues has been summarized by Dick (1959), who notes that D varies inversely with the ratio of surface area to volume (S/V). In other words, small cells have a reduced value of D. The same situation may also arise in small volumes of cytoplasm confined in membranous elaborations such as microvilli. In general, it is likely that if aqueous diffusion is reduced through binding effects, then a larger osmotic gradient, and therefore a higher rate of salt transport, will be necessary to produce a given osmotic flow.

The diffusion of salts, which are required as counter-ions for intracellular polyelectrolytes, may also be restricted in cytoplasm. The self-diffusion coefficient of sodium in muscle, for example, is reduced to 50 % of the value in 1 % agar (Caillé & Hinke, 1972). Measurements with ion-selective microelectrodes also indicate that as much as 84 % of intracellular sodium is bound. Binding has important effects on transmembrane activity gradients of water and ions. The sodium concentration difference across the barnacle sarcolemma is about 3·5 times larger than the difference calculated by assuming that all the water and sodium in the cell are free (McLaughlin & Hinke, 1966).

The binding of a significant fraction of intracellular chloride has been proposed for *Calliphora* salivary glands (Gupta *et al.* 1978*b*), vertebrate muscle (Bacaner, Broadhurst, Hutchinson & Lilley, 1973) and barnacle muscle (Gayton & Hinke, 1968). Potassium, however, appears to be almost completely free, as in *Calliphora* salivary glands (Gupta *et al.* 1978*b*), or at least 80 % free, as in the midgut epithelial cells of *Hyalaphora cecropia* (Zerahn, 1977) or rabbit cardiac muscle (Lee & Fozzard, 1975). One might speculate that the effects of binding on transcellular water movement could be analysed further by experimentally shifting cell pH or ionic strength, so as to alter

polyelectrolyte conformation, and therefore affect binding by altering the number type of exposed polar groups.

PARACELLULAR AND TRANSCELLULAR PATHWAYS FOR SOLUTES

Passive movements of organic solutes

Ramsay (1958) was one of the first to point out the analogy between Malpighian tubules and the vertebrate nephron. All soluble substances of low molecular weight pass through the tubule wall into the urine and so are removed from the haemolymph. Metabolically useful substances are reabsorbed, primarily in the rectum. An advantage of this system is that any novel toxin or unwanted substance is automatically eliminated simply by not providing a specific mechanism for its reabsorption (Ramsay, 1958).

Subsequent analysis (Maddrell & Gardiner, 1974) extended Ramsay's suggestion that Malpighian tubules are permeable to a wide variety of solutes. However, the permeability to glycine (M_r 120) is only four times greater than that of inulin (M_r 5000). This observation suggests that diffusion occurs through pathways which are highly permeable but limited in number (Maddrell, 1981). The slow filtration per unit area is compensated by the large total area of the excretory system relative to the animal's body weight (Phillips, 1981). Because the colon and the rectum are relatively impermeable (Phillips & Dockrill, 1968; Maddrell & Gardiner, 1980*a*), reabsorption of useful substances must occur in the tubules or in the ileum. The low permeability of the rectum limits passive backfluxes of molecules larger than 0·5–0·6 nm in radius (Phillips & Dockrill, 1968). The rectal epithelium, which concentrates the luminal contents by reabsorption of water (Phillips, 1964), is thereby protected from many toxic molecules in the faeces.

In some instances, passive losses of useful substances through the Malpighian tubules are limited to the extent that reabsorption is unnecessary. Small or lipophilic molecules are often conjugated with proteins; the resultant molecules have a greatly increased effective radius, and passive losses are correspondingly reduced. Lipids, for example, which many insects metabolize in quantity during flight, are transported mainly as diglyceride protein complexes (Gilbert, 1967), and juvenile hormone is also circulated as a hormone-protein complex (Pratt, 1972).

Although there are analogies between Malpighian tubules and the vertebrate glomerulus, small molecules such as L-glucose, a non-metabolite, appear in fluid secreted by *Calliphora* tubules at half the concentration in the bathing fluids (Knowles, 1975), whereas glomerular fluid/plasma ratios are very close to one for compounds as large as inulin (M_r 5000) and dextran (M_r 17 000; Smith, 1951). Insects filter their haemolymph relatively slowly, presumably because they can tolerate changes in the composition of the haemolymph (Maddrell, 1981). Exchange of respiratory gases is relatively independent of haemolymph composition, because oxygen is transported by trachea, rather than haemolymph-borne proteins which might be sensitive to changes in the haemolymph. Sensitive tissues such as the eyes, testis and central nervous system, are protected by special epithelia which are characterized by large numbers of simple tight junctions (Lane & Skaer, 1980). These epithelia regulate the environment of the protected cells (Treherne, 1974). In general,

pears that insect tissues can tolerate or are protected from potentially deleterious changes in the haemolymph for long enough to allow the excretory system to correct the situation at a relatively slow rate. What is important is not the speed with which novel toxic materials are removed, but the fact that they are eventually removed.

The primary result of recent studies of passive solute movements across Malpighian tubules is that small non-electrolytes, below the size of a disaccharide, cross the epithelium primarily by a transcellular route. The permeabilities of a variety of substances are shown in Fig. 4. It is apparent that there are marked differences in the permeability of the tubule wall to charged substances, such as acetate, glycine, aspartate and tyrosine, relative to non-charged substances such as ethanol, thiourea, xylose and mannitol. Unstimulated Malpighian tubules of *Rhodnius* do not secrete Na^+ into the lumen, and Na^+ ions have a high charge density. Like the other charged species, Na^+ crosses the walls of the tubules only very slowly.

The data of Fig. 4 may be explained on the basis of the routes taken by various solutes in crossing the epithelium. Charged organic molecules, ions which are not actively secreted, and large molecules such as sucrose and inulin may be unable to cross the tubule wall except through the intercellular clefts, which, as noted above, are restricted in area. Non-charged substances of low molecular weight also move paracellularly, but in addition they may be able to penetrate the very large area of cell membrane and cross by a cellular route as well.

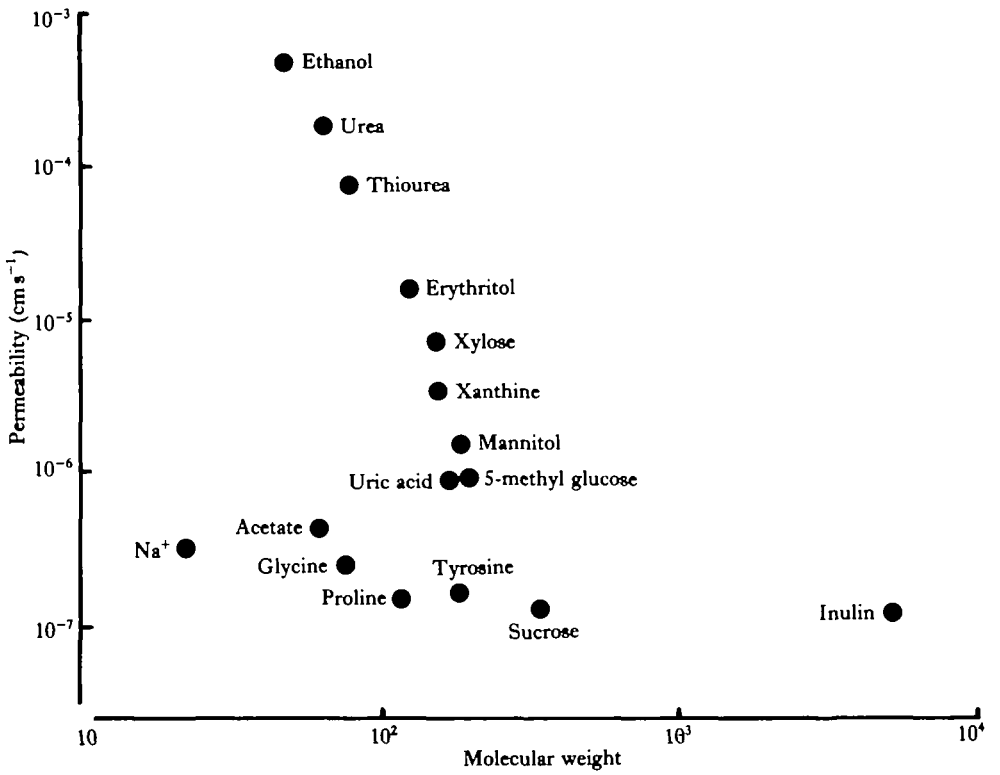

Fig. 4. Passive solute movements across Malpighian tubules. The permeabilities of a variety of substances are plotted as a function of their molecular weight. (From M. J. O'Donnell, S. H. P. Maddrell & B. O. C. Gardiner, in preparation.)

Several types of experiments have provided support for this sort of scheme. For example, it is possible to measure the steady-state cellular content of a radiolabelled solute in the tubule cells, and compare this content with the very different volumes of the two pathways, the cell and the intercellular cleft. Tubules were equilibrated in a bath of saline containing a known concentration of the labelled solute, and were perfused with the same solution (M. J. O'Donnell, S. H. P. Maddrell & B. O. C. Gardiner, in preparation). The lumen was then quickly flushed out and the external tubule surfaces washed with saline containing the same concentration of unlabelled solute. These procedures removed counts adhering in the outer wall or in luminal fluids, but the washing was sufficiently rapid as to leave counts in the tubule wall, either in the cells or the paracellular clefts. Solutes thought not to cross the tubule *via* the cells labelled only very little of the wall, whereas solutes thought to penetrate the cell membranes had about 5–10 times more counts associated with the wall, equivalent in the cases of methyl glucose, erythritol, mannitol and xylose to 50–90 nl of the bathing medium. This compares well with the volume of the tubule cells, which for the 25 mm lengths of tubule used in these studies was about 90 nl.

The time course of arrival of test substances in the lumen also provided information on the route followed by particular solutes. Substances which cross the epithelium only through the intercellular clefts will cross into the lumen at a steady rate within a short space of time (Fig. 5A). For example, inulin or sucrose will diffuse through a water-filled pathway of the length of the extracellular clefts (about 20 μm) in about 0·1–1·0 s. Similarly, substances such as thiourea, which appear to cross cell membranes very readily, will also establish a steady rate of entry into the lumen within a short time (Fig. 5B). However, for substances which traverse the cell membrane more slowly, there should be a delay before their arrival into the lumen occurs at a steady rate (Fig. 5C). This delay would be the result of the time taken to cross the basal cell membrane and raise the intracellular concentration to a sufficiently high level so that

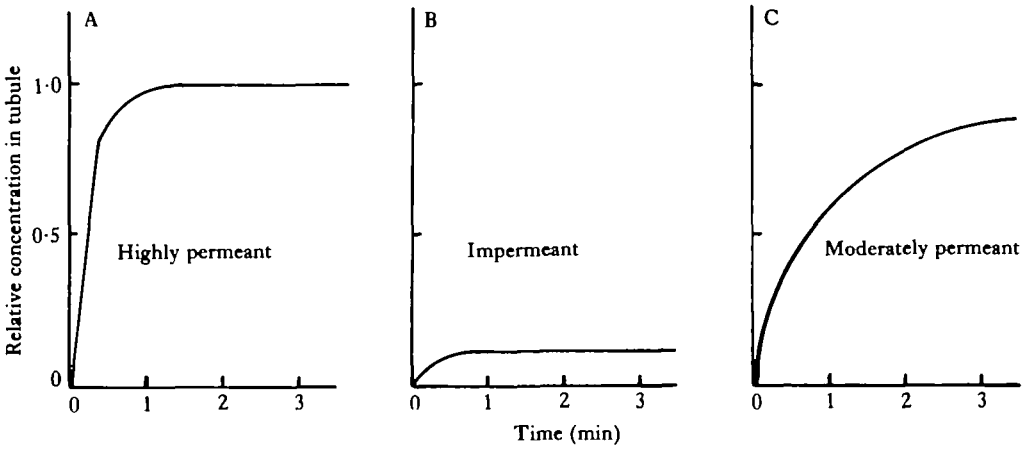

Fig. 5. Typical time courses of passive movements of solutes into the lumen of Malpighian tubules. Both highly permeant and impermeant molecules achieve a steady state concentration within a short time (A and B), although the concentrations reached in the lumen are much greater for highly permeant molecules. In contrast, moderately permeant molecules such as mannitol reach the steady state concentration very gradually (C). The basis for this effect is discussed in the text.

fusive movements across the apical cell membrane into the lumen become significant. Indeed, mannitol, thought to penetrate the cell membranes only slowly, takes nearly 3 min to approach its steady state luminal concentration. Sucrose, thought to be restricted to the paracellular route, and thiourea, which easily crosses cell membranes, both appear in the lumen at their steady state concentrations after a delay of only a few seconds. Of course, there is a great difference in the steady state concentrations of the latter two solutes; sucrose appeared in the lumen at about 0·2 % of the bath concentration whereas the steady state concentration of thiourea was 33 % of the bath concentration, a difference of 600-fold.

These results are consistent with transepithelial movements of sucrose through a paracellular route. Thiourea and mannitol follow, in addition, a transcellular route, but whereas thiourea establishes itself swiftly in this route, mannitol does so much more slowly. Further analysis of the time course of accumulation of mannitol in tubules supported the latter idea. The initial accumulation of counts upon exposure of a tubule to a bathing drop containing labelled mannitol was very rapid, consistent with the crossing of only one (the basal) cell membrane. There was a subsequent more gradual rate of increase of counts which suggested that mannitol crossed a second barrier, the apical cell membrane, into the lumen.

Furthermore, a series of experiments in which tracer substances were injected into the haemolymph of recently fed *Rhodnius* and radioactivity in the urine was measured at intervals for 30 min suggested that the permeability properties *in vivo* of the upper Malpighian tubules were not dissimilar from the properties *in vitro*.

Two aspects of these results are worth noting. First, Malpighian tubule cells are permeable to quite large molecules such as mannitol, which has been used as an indicator of extracellular space (Page, 1962). Second, that the permeability is sufficiently high that such solutes can cross the cells faster than they can penetrate between the cells. Mannitol, for example, traverses the tubule wall about ten times more rapidly than sucrose, and urea crosses nearly 1500 times faster than sucrose. These values are remarkable because the transcellular route involves the crossing of at least two cell membranes in series.

This rapid transcellular permeation by small solutes such as mannitol contrasts with the movement of small molecules in a variety of other epithelia. In the rabbit gallbladder, mannitol crosses the epithelial wall no more rapidly than sucrose, and thiourea, although it crosses much more rapidly, does not show evidence of transcellular permeation (Steward, 1982). In the rabbit ileum, mannitol permeates cell membranes only slowly, but at a rate sufficient to preclude its usefulness as an extracellular marker (Nellans & Schultz, 1976). Mannitol is also a permeant solute in the midgut epithelium of the tobacco hornworm *Manduca sexta*, but takes some 40 min to equilibrate with all of the water space (Moffett, 1979) as compared with 3 min in the upper Malpighian tubule of *Rhodnius*.

The ease with which membrane-penetrant solutes cross the walls of the Malpighian tubules appears to derive from the same membrane elaborations, the basal infoldings and apical microvilli, which increase enormously the effective surface area of the cells and provide for efficient coupling of passive solvent movements to active salt transport during fluid secretion. Because of the flattened shape of the cells, the ratio of surface a to volume is also very large, and substances which enter the cells rapidly because

of the large membrane area quickly reach high concentrations in the intracellular space. It appears, therefore, that the main factor determining the permeability of the Malpighian tubules to non-electrolytes of low molecular mass is an amplification of surface area, rather than any special arrangements or properties of the molecules which comprise the cell membranes.

What determines the facility with which a non-electrolyte crosses a cell membrane? For tissues such as the mammalian gallbladder, the patterns of selectivity for permeation by non-electrolytes resemble the patterns of a bulk lipid phase. The explanation of the pattern lies simply in differences in solute:water and solute:lipid intermolecular forces (Diamond & Wright, 1969*a*). The portions of the membrane which control non-electrolyte permeation behave like hydrocarbons with few hydrogen bonding sites (Diamond & Wright, 1969*b*). Molecular substituents of the solute (OH, ether, carbonyl, ester, amino, amide, urea, nitrile) decrease permeation in proportion to the number and strength of intermolecular hydrogen bonds which they form with water. Branched molecules are less permeant, and the inverse relationship between permeating power and molecular size is steeper for biological membranes than for permeation through a bulk lipid phase, presumably because of the organized structure of lipids in cell membranes. Importantly, small polar molecules permeate more rapidly through cell membranes than through a bulk lipid phase; it is likely that predominantly polar regions, near proteins in the membrane, provide a parallel permeation pathway which bypasses the membrane lipids (Diamond & Wright, 1969*b*). This latter type of pathway may be especially significant in Malpighian tubules; the rapid rate of fluid transport by tubules suggests that large numbers of transport proteins are probably present in the cell membranes. Although the selectivity of Malpighian tubule cell membranes appears to be qualitatively similar to the selectivity of the mammalian tissues analysed by Diamond & Wright (1969*a*,*b*), there are important quantitative differences; thiourea does not readily permeate gallbladder cell membranes (Steward, 1982), whereas thiourea and other polar molecules as large as mannitol enter Malpighian tubule cells.

As noted above, for molecules between the size of sucrose (M_r 342) and inulin (M_r 5000), the paracellular pathway in *Rhodnius* Malpighian tubules does not appear to be highly selective. Oschman (1980*b*) has reviewed many of the properties of the intercellular matrix and junctional complexes which might influence the selectivity of paracellular pathways. Recent experiments suggest that the permeability of paracellular pathways in mammalian epithelia can vary. For example, in the rabbit gallbladder, pH and the activity of divalent and polyvalent cations modulate cation conductances at the junctional complexes (Reuss, 1982), which also provided significant steric restriction to the movement of non-electrolytes (Rector & Berry, 1982).

In insect epithelia, however, extracellular solute movements can also be influenced not just by the properties of the extracellular clefts, but by acellular barriers. The basement membranes in secretory epithelia often bind ions (Gupta, Hall, Maddrell & Moreton, 1976), which might affect the supply of ions to the transporting cells. In *Calliphora* salivary glands, it has been suggested that the periluminal 'intima' (Oschman & Berridge, 1970) might provide an additional compartment in which salt-water coupling could occur (Gupta *et al.* 1978*b*). Another example is the cuticular lining in the ileum and rectum of *Schistocerca gregaria*, which is quite permeable

small neutral hydrophilic molecules (Maddrell & Gardiner, 1980*a*). However both tissues showed a reduced permeability to anions and an enhanced cation permeability. The functional significance of such properties may be that relatively large anions are actively excreted by the Malpighian tubules (Maddrell, Gardiner, Pilcher & Reynolds, 1974). Smaller molecules such as amino acids can penetrate the hindgut cuticle and specific mechanisms exist in the rectum of *Schistocerca* for their reabsorption (Balshin & Phillips, 1971).

In *Rhodnius* as well, although the haemolymph is rich in amino acids, only trace amounts are lost in the urine. Surprisingly, there is no significant reabsorption of amino acids in the excretory system of *Rhodnius* (Maddrell & Gardiner, 1980*b*). Passive losses are reduced by the low permeability of the Malpighian tubules towards amino acids, and by the dilution of the concentration of haemolymph amino acids which results from the increase in haemolymph volume after feeding. As well, the very high rate of fluid secretion by the tubules after feeding limits the time available for passive losses of amino acids into the urine.

Active transport of organic solutes

Passive movements of organic solutes across insect epithelia are supplemented by a variety of active transport mechanisms. In general, these mechanisms appear to have arisen when passive movements of a particular solute, by virtue of its charge, size or concentration, are unable to meet the required excretory or reabsorbtive needs of the animal. An important advantage of specific active pumps for such solutes is that the elimination or reabsorption of one solute can be increased with little effect on the movement of other metabolites.

Transport mechanisms for organic anions have been studied in the Malpighian tubules of several species (Maddrell *et al.* 1974). Acylamides, such as *p*-aminohippuric acid, and sulphonates, including acidic dyes such as indigo carmine and amaranth, are excreted by separate mechanisms, thereby clearing the haemolymph of conjugated compounds which are the products of detoxication of potentially toxic products of metabolism such as phenols and aromatic acids (Smith, 1962).

An important aspect of the study of organic anion transport was the observation of interactions between active transport and passive backfluxes of the excreted compounds in different species. In *Calliphora*, active dye secretion into the lumen proceeds at a high rate and a high concentration is therefore achieved in the lumen at all but high rates of fluid secretion. However, because the tubule wall is quite permeable to the dye, a large proportion of the dye diffuses passively from the lumen into the haemolymph, presumably through paracellular channels. The net amount of dye transported is therefore highly dependent upon the rate of fluid secretion. In contrast, the tubules of *Carausius* and *Rhodnius* are much less permeable, and dye secretion is not affected by the rate of fluid secretion. This example demonstrates that the contribution of passive backfluxes must be borne in mind in studies of excretory mechanisms.

Active transport mechanisms are also found for some potentially toxic components of the diet. Alkaloids, such as nicotine, atropine and morphine, are nitrogenous bases found in some plants and are believed to have evolved for protection from herbivores. Competitive inhibition studies indicate that these compounds are actively transported by the same mechanism by the Malpighian tubules of *Rhodnius*, and that this transport

is not coupled to ion transport (Maddrell & Gardiner, 1976). Nicotine is also transported by the tubules of the larvae of *Manduca sexta* and the cabbage white butterfly, *Pieris brassicae*, but not by tubules in adults of these species. In *Calliphora erythrocephala* and *Musca domestica* nicotine is removed from the bathing fluids and an unknown metabolite accumulates in the tubule fluid.

Both the upper and lower tubules excrete alkaloids in *Rhodnius*. However, the upper tubules are not involved in active excretion of uric acid, a waste product of protein metabolism, and their passive permeability to this compound is insufficient to account for more than 1–2 % of the amounts excreted (Maddrell & Gardiner, 1974; O'Donnell, Maddrell & Gardiner, 1983). Our interpretation of Fig. 4 indicates that urate is restricted to a paracellular route in the upper Malpighian tubule of *Rhodnius*. However, recent studies indicate that the lower tubules actively excrete urate, and that the mechanism differs from that of vertebrates; it is ouabain-insensitive and results in the precipitation in the lumen of free uric acid rather than urate salts (O'Donnell *et al.* 1983). Active urate transport appears to be common to insects from several orders, although in *Calliphora* tubules uric acid is converted to allantoin, which appears in the secreted fluid at concentrations 28 times greater than the bath concentration of urate. It will be of interest to investigate further the relative advantages of transporting a substance such as urate unchanged, as compared with modifying the structure of the molecule and allowing the new compound, such as allantoin, to diffuse down its concentration gradient.

In addition to actively transporting a variety of organic solutes, insect epithelia can also augment the rate of transport in response to specific physiological signals. For example, unfed *Rhodnius* do not transport *p*-aminohippuric acid at significant rates. However, the transport mechanism is rapidly activated upon ingestion of a blood meal or artificial protein-containing fluids. The induction of this transport mechanism does not appear to be controlled by a hormone released in response to abdominal distension during feeding, as is the case for diuretic hormone control of fluid secretion (Maddrell & Gardiner, 1975).

Similarly, *in vivo* experiments indicate that higher rates of urate transport are induced by the increase in haemolymph urate concentration which follows feeding (O'Donnell *et al.* 1983). *In vitro* experiments with tubules from the tsetse fly, *Glossina*, suggest that induction of urate transport may be a direct response of the tubule cells to an increase in the urate concentration in the surrounding fluids. Not all transport mechanisms for organic solutes are inducible however; alkaloid transport by *Rhodnius* persists in the absence of dietary alkaloids (Maddrell & Gardiner, 1976).

The induction of transport systems raises questions concerning the basis for alterations in the properties of cell membranes. Does the induction involve the synthesis of new enzymes, the insertion of existing enzymes into the cell membrane, or the activation of existing transport mechanisms within the cell membrane? Another aspect of changes in transport capabilities concerns the loss of the ability to transport certain materials in the adults of several species; do such changes involve degradation of membrane transport proteins, or deactivation of the transport system?

Active and passive movements of inorganic ions

If water movements during fluid secretion are predominantly transcellular, then

kely that osmotic coupling occurs within the cells and in the tubule lumen. If so, salt transport into these compartments must occur at rates sufficient to generate the necessary osmotic gradients. The transepithelial movements of ions during secretion have been described before (Maddrell, 1969, 1971, 1980), and recently, microelectrode measurements of intracellular potential have provided information on both the active or passive nature of ionic movements, and on the apical or basal location of the pumps and channels for ions (M. J. O'Donnell and S. H. P. Maddrell, in preparation). In conjunction with the data on transcellular water flows, these studies suggest that the paracellular path is not the predominant route for ion movements during fluid secretion.

Closely related to solute movements is the problem of cell volume regulation, which has been studied relatively little in insect epithelia (Machin, 1981). Especially in view of the very high rates of ion transport in tissue such as *Rhodnius* Malpighian tubules, and the apparently high osmotic permeability of the tubule cell membranes, the balance between the rate of entry of ions into the cell and the exit of ions into the lumen is of critical importance to maintenance of a stable cell volume. In vertebrate tissues, the subject of negative feedback control of sodium entry into the cells of vertebrate tissues has received considerable attention (Lewis, 1977, this volume; Taylor & Windhager, 1979; Chase & Al-Awqati, 1981). It appears likely that the increase in intracellular sodium concentration produced by an increase in sodium entry accelerates a Na^+: Ca^{2+} exchange mechanism, and that the resultant increase in cytosolic calcium lowers the permeability of the luminal membrane to sodium. Sodium entry is therefore reduced and intracellular sodium concentration declines.

ULTRASTRUCTURE AND PHYSIOLOGY

This discussion of paracellular and transcellular movements of water and solutes emphasizes the need for integration of ultrastructural and physiological studies. For example, KCl reabsorption is dramatically discontinuous over the length of the lower Malpighian tubule (Maddrell, 1978); most reabsorption occurs in the proximal third of the lower tubule. The reduction in osmotic permeability of the lower relative to the upper tubule is also most apparent in this region (O'Donnell *et al.* 1982). Yet the ultrastructure of the lower tubule shows no apparent discontinuities which would suggest these functional variations (Wigglesworth & Salpeter, 1967).

Another example arises from studies of paracellular solute movements. The paracellular cleft occupies a small fraction (0·00039) of the area of the tubule wall and the clefts are characterized by smooth septate junctions along nearly their whole length (Skaer, Harrison & Lee, 1979). It has been calculated for *Hydra* (Filshie & Flower, 1977) that the septa reduce the cross-sectional area of the clefts 300-fold, and increase the diffusive path length to hundreds of micrometers. One would therefore expect diffusion through such clefts to be slowed as much as 10 000-fold by the presence of septa. In fact, this is not the case. In the absence of septa, the expected permeability coefficient for sucrose, P_{su}, is given by $P_{su} = D_{su}A/l$, where D_{su} is the free solution diffusion coefficient for sucrose, $5{\cdot}2 \times 10^{-6}$ cm s^{-1} (Longsworth, 1953), A is the fraction of the wall occupied by the paracellular cleft, 0·00039, and l is the path length of cleft, about 20 μm. The expected value of P_{su} is therefore $1{\cdot}02 \times 10^{-6}$ cm s^{-1}. The

actual *in vitro* value is about 3×10^{-8} cm s^{-1}. It seems that diffusion through the c[redacted] is slowed by a factor of 30 because of the septa, rather than 10 000-fold. In this case, permeabilities predicted solely upon ultrastructural evidence would be grossly in error.

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REFERENCES

ALLEN, R. D., METUZALS, J., TASAKI, I., BRADY, S. T. & GILBERT, S. P. (1982). Fast axonal transport in squid giant axon. *Science, N.Y.* **218**, 1127–1129.

ANDREOLI, T. E. & SCHAFER, J. A. (1978). Volume absorption in the pars recta. III. Luminal hypotonicity as a driving force for isotonic volume absorption. *Am. J. Physiol.* **234**, F349–F355.

BACANER, M., BROADHURST, J., HUTCHINSON, T. & LILLEY, J. (1973). Scanning transmission electron microscope studies of deep-frozen unfixed muscle, correlated with spatial localization of intracellular elements by fluorescent X-ray analysis. *Proc. natn Acad. Sci. U.S.A.* **70**, 3423–3427.

BALSHIN, M. & PHILLIPS, J. E. (1971). Active absorption of amino acids in the rectum of the desert locust (*Schistocerca gregaria*). *Nature, Lond.* **233**, 53–55.

BERRIDGE, M. J. (1977). Cyclic AMP, calcium and fluid secretion. In *Transport of Ions and Water in Animals*, (eds B. L. Gupta, R. B. Moreton, J. L. Oschman & B. J. Wall), pp. 225–238. New York: Academic Press.

BRADY, S. T., LASEK, R. J. & ALLEN, R. D. (1982). Fast axonal transport in extruded axoplasm from squid giant axon. *Science, N.Y.* **218**, 1129–1131.

CAILLÉ, J. P. & HINKE, J. A. M. (1972). Evidence for Na sequestration in muscle from Na diffusion measurements. *Can. J. Physiol. Pharmacol.* **50**, 228–237.

CAILLÉ, J. P. & HINKE, J. A. M. (1974). The volume available to diffusion in the muscle fiber. *Can. J. Physiol. Pharmacol.* **52**, 822–828.

CHASE, H. S. & AL-AWQATI, Q. (1981). Regulation of the sodium permeability of the luminal border of the toad bladder by intracellular sodium and calcium. *J. gen. Physiol.* **77**, 693–712.

CURRAN, P. F. (1960). Na, Cl and water transport by rat ileum *in vitro*. *J. gen. Physiol.* **43**, 1137–1148.

DIAMOND, J. M. (1979). Osmotic water flow in leaky epithelia. *J. Membr. Biol.* **51**, 195–216.

DIAMOND, J. M. & BOSSERT, W. H. (1967). Standing gradient osmotic flow. A mechanism for coupling of water and solute transport in epithelia. *J. gen. Physiol.* **50**, 2061–2083.

DIAMOND, J. M. & WRIGHT, E. M. (1969*a*). Biological membranes: The basis of ion and non-electrolyte selectivity. *Ann. Rev. Physiol.* **31**, 581–646.

DIAMOND, J. M. & WRIGHT, E. M. (1969*b*). Molecular forces governing non-electrolyte permeation through cell membranes. *Proc. R. Soc. B* **172**, 273–316.

DICK, D. A. T. (1959). Osmotic properties of living cells. *Int. Rev. Cytol.* **8**, 387–448.

FILSHIE, B. K. & FLOWER, N. E. (1977). Junctional structures in *Hydra*. *J. Cell Sci.* **23**, 151–172.

GAYTON, D. C. & HINKE, J. A. M. (1968). The location of chloride in single striated muscle fibres of the giant barnacle. *Can. J. Physiol. Pharmacol.* **46**, 213–219.

GILBERT, L. I. (1967). Lipid metabolism and function in insects. *Adv. Insect Physiol.* **4**, 69–211.

GRIM, E. & SMITH, G. A. (1957). Water flux rates across dog gallbladder wall. *Am. J. Physiol.* **191**, 555–560.

GRONOWICZ, G., MASUR, S. K. & HOLTZMAN, E. (1980). Quantitative analysis of exocytosis and endocytosis in the hydroosmotic response of toad bladder. *J. Membr. Biol.* **52**, 221–235.

GUPTA, B. L. & BERRIDGE, M. J. (1966). Fine structural organization of the rectum in the blowfly, *Calliphora erythrocephala* (Merg.), with special reference to connective tissue, tracheae, and neurosecretory innervation in the rectal papillae. *J. Morphol.* **120**, 23–81.

GUPTA, B. L., BERRIDGE, M. J., HALL, T. A. & MORETON, R. B. (1978*b*). Electron microprobe and ion-selective microelectrode studies of fluid secretion in the salivary gland of *Calliphora*. *J. exp. Biol.* **72**, 261–284.

GUPTA, B. L. & HALL, T. A. (1981). Microprobe analysis of fluid-transporting epithelia. Evidence for local osmosis and solute recycling. In *Water Transport Across Epithelia*, (eds H. H. Ussing, N. Bindslev, N. A. Lassen & O. Sten-Knudsen), pp. 17–35. Copenhagen: Munksgaard.

GUPTA, B. L., HALL, T. A., MADDRELL, S. H. P. & MORETON, R. B. (1976). Distribution of ions in a fluid transporting epithelium determined by electron-probe X-ray microanalysis. *Nature, Lond.* **264**, 284–287.

GUPTA, B. L., HALL, T. A. & NAFTALIN, R. J. (1978*a*). Microprobe measurements of Na, K and Cl concentration profiles in epithelial cells and intercellular spaces of rabbit ileum. *Nature, Lond.* **272**, 70–73.

[illegible]TA, B. L., WALL, B. J., OSCHMAN, J. L. & HALL, T. A. (1980). Direct microprobe evidence of local concentration gradients and recycling of electrolytes during fluid absorption in the rectal papillae of *Calliphora*. *J. exp. Biol.* **88**, 21–47.

HARVEY, W. R. (1980). Water and ions in the gut. In *Insect Biology in the Future*, (eds M. Locke & D. S. Smith), pp. 105–124. New York: Academic Press.

HARVEY, W. R. (1982). Membrane physiology of insects. In *Membrane Physiology of Invertebrates*, (ed. R. P. Podesta), pp. 495–566. New York: Marcel Dekker, Inc.

HILL, A. E. (1975). Solute coupling in epithelia: a critical examination of the standing gradient osmotic flow theory. *Proc. R. Soc. B.* **190**, 99–114.

HINKE, J. A. M. (1970). Solvent water for electrolytes in the muscle fiber of the giant barnacle. *J. gen. Physiol.* **56**, 521–541.

KACHADORIAN, W. A., ELLIS, S. J. & MULLER, J. (1979). Possible roles for microtubules and microfilaments in ADH action on toad urinary bladder. *Am. J. Physiol.* **236**, F14–F20.

KACHADORIAN, W. A., WADE, J. B. & DISCALA, V. A. (1975). Vasopressin-induced structural change in toad bladder luminal membrane. *Science, N.Y.* **190**, 67–69.

KAYE, G. I., WHEELER, H. O., WHITLOCK, R. T. & LANE, N. (1966). Fluid transport in the rabbit gallbladder: A combined physiological and electron microscope study. *J. Cell Biol.* **30**, 237–268.

KNOWLES, G. (1975). The reduced glucose permeability of the isolated Malpighian tubules of the blowfly, *Calliphora vomitoria*. *J. exp. Biol.* **62**, 327–340.

KUNTZ, I. D. & KAUZMANN, W. (1974). Hydration of proteins and polypeptides. *Adv. Prot. Chem.* **28**, 239–345.

LANE, N. J. & SKAER, H. LE B. (1980). Intercellular junctions in insects. *Adv. Insect Physiol.* **15**, 35–213.

LEE, C. O. & FOZZARD, H. A. (1975). Activities of potassium and sodium ions in rabbit heart muscle. *J. gen. Physiol.* **65**, 695–708.

LEVINE, S. D. & KACHADORIAN, W. A. (1981). Barriers to water flow in vasopressin-treated toad urinary bladder. *J. Membr. Biol.* **61**, 135–139.

LEWIS, S. A. (1977). A reinvestigation of the function of the mammalian urinary bladder. *Am. J. Physiol.* **232**, F187–F195.

LING, G. N., OCHENFELD, M. M. & KARREMAN, G. (1967). Is the cell membrane a universal rate-limiting barrier to the movement of water between the living cell and its surrounding medium? *J. gen. Physiol.* **50**, 1807–1820.

LONGSWORTH, L. G. (1953). Diffusion measurements, at 25 °C, of aqueous solutions of amino acids, peptides and sugars. *J. Am. chem. Soc.* **75**, 5705–5709.

MACHIN, J. (1981). Water compartmentalization in insects. *J. exp. Zool.* **215**, 327–333.

MADDRELL, S. H. P. (1969). Secretion by the Malpighian tubules of *Rhodnius*. The movements of ions and water. *J. exp. Biol.* **51**, 71–97.

MADDRELL, S. H. P. (1971). The mechanisms of insect excretory systems. *Adv. Insect Physiol.* **8**, 199–331.

MADDRELL, S. H. P. (1978). Physiological discontinuity in an epithelium with an apparently uniform structure. *J. exp. Biol.* **75**, 133–145.

MADDRELL, S. H. P. (1980). Characteristics of epithelial transport in insect Malpighian tubules. In *Current Topics in Membranes and Transport*, Vol. 14, (eds F. Bronner & A. Kleinzeller), pp. 428–463. New York: Academic Press.

MADDRELL, S. H. P. (1981). The functional design of the insect excretory system. *J. exp. Biol.* **90**, 1–15.

MADDRELL, S. H. P. & GARDINER, B. O. C. (1974). The passive permeability of insect Malpighian tubules to organic solutes. *J. exp. Biol.* **60**, 641–652.

MADDRELL, S. H. P. & GARDINER, B. O. C. (1975). Induction of transport of organic anions in Malpighian tubules of *Rhodnius*. *J. exp. Biol.* **63**, 755–761.

MADDRELL, S. H. P. & GARDINER, B. O. C. (1976). Excretion of alkaloids by Malpighian tubules of insects. *J. exp. Biol.* **64**, 267–281.

MADDRELL, S. H. P. & GARDINER, B. O. C. (1980*a*). The permeability of the cuticular lining of the insect alimentary canal. *J. exp. Biol.* **85**, 227–237.

MADDRELL, S. H. P. & GARDINER, B. O. C. (1980*b*). The retention of amino acids in the haemolymph during diuresis in *Rhodnius*. *J. exp. Biol.* **87**, 315–329.

MADDRELL, S. H. P., GARDINER, B. O. C., PILCHER, D. E. M. & REYNOLDS, S. E. (1974). Active transport by insect Malpighian tubules of acidic dyes and of acylamides. *J. exp. Biol.* **61**, 357–377.

MADDRELL, S. H. P. & PHILLIPS, J. E. (1975). Secretion of hypo-osmotic fluid by the lower Malpighian tubules of *Rhodnius prolixus*. *J. exp. Biol.* **62**, 671–683.

MCLAUGHLIN, S. G. A. & HINKE, J. A. M. (1966). Sodium and water binding in single striated muscle fibers of the giant barnacle. *Can. J. Physiol. Pharmacol.* **44**, 837–848.

MOFFETT, D. F. (1979). Bathing solution tonicity and potassium transport by the midgut of the tobacco hornworm *Manduca sexta*. *J. exp. Biol.* **78**, 213–223.

MOOSEKER, M. S. (1974). Brush border motility: Microvillar contraction in isolated brush border models. *J. [illegible]ell Biol.* **63**, 231a.

MOOSEKER, M. S., GRAVES, T. A., WHARTON, K. A., FALCO, N. & HOWE, C. L. (1980). Regulation of microvillus structure: Calcium-dependant solation and cross-linking of actin filaments in the microvilli of intestinal epithelial cells. *J. Cell Biol.* **87**, 809–822.

MULLER, J., KACHADORIAN, W. A. & DISCALA, V. A. (1980). Evidence that ADH-stimulated intramembrane particle aggregates are transferred from cytoplasmic to luminal membranes in toad bladder epithelial cells. *J. Cell Biol.* **85**, 83–95.

NELLANS, H. N. & SCHULTZ, S. G. (1976). Relations among transepithelial sodium transport, potassium exchange and cell volume in rabbit ileum. *J. gen. Physiol.* **68**, 441–463.

O'DONNELL, M. J., ALDIS, G. K. & MADDRELL, S. H. P. (1982). Measurements of osmotic permeability in the Malpighian tubules of an insect *Rhodnius prolixus* Stal. *Proc. R. Soc. B* **216**, 267–277.

O'DONNELL, M. J., MADDRELL, S. H. P. & GARDINER, B. O. C. (1983). Transport of uric acid by the Malpighian tubules of *Rhodnius prolixus* and other insects. *J. exp. Biol.* **103**, 169–184.

OSCHMAN, J. L. (1980*a*). Morphological correlates of transport. In *Membrane Transport in Biology*, Vol. 3, (eds G. Giebisch, D. C. Tosteson & H. H. Ussing), pp. 55–93. Berlin: Springer-Verlag.

OSCHMAN, J. L. (1980*b*). Water transport, cell junctions and 'structured water'. In *Membrane Structure and Function*, Vol. 3, (ed. E. E. Bittar), pp. 141–170. New York: John Wiley & Sons.

OSCHMAN, J. L. & BERRIDGE, M. J. (1970). Structural and functional aspects of salivary fluid secretion in *Calliphora*. *Tissue and Cell* **2**, 281–310.

PAGE, E. (1962). Cat heart muscle *in vitro*. III. The extracellular space. *J. gen. Physiol.* **46**, 201–213.

PERSSON, B. E. & SPRING, K. R. (1982). Gallbladder epithelial cell hydraulic water permeability and volume regulation. *J. gen. Physiol.* **79**, 481–505.

PHILLIPS, J. E. (1964). Rectal absorption in the desert locust *Schistocerca gregaria* Forskal. I. Water. *J. exp. Biol.* **41**, 15–38.

PHILLIPS, J. E. (1965). Rectal absorption and rectal function in insects. *Trans. R. Soc. Can.* **3**, 237–254.

PHILLIPS, J. E. (1980). Epithelial transport and control in recta of terrestrial insects. In *Insect Biology in the Future*, (eds M. Locke & D. S. Smith), pp. 145–177. New York: Academic Press.

PHILLIPS, J. E. (1981). Comparative physiology of insect renal function. *Am. J. Physiol.* **241**, R241–R257.

PHILLIPS, J. E. & DOCKRILL, A. A. (1968). Molecular sieving of hydrophilic molecules by the rectal intima of the desert locust (*Schistocerca gregaria*). *J. exp. Biol.* **48**, 521–532.

PINTO DA SILVA, P. (1973). Membrane intercalated particles in human erythrocyte ghosts: Sites of preferred passage of water molecules at low temperature. *Proc. natn Acad. Sci. U.S.A.* **70**, 1339–1343.

PRATT, G. E. (1972). The transport and metabolism of juvenile hormone mimics in the locust. *J. Endocr.* **57**, *liv*. 54.

RAMSAY, J. A. (1950). Osmotic regulation in mosquito larvae. *J. exp. Biol.* **27**, 145–157.

RAMSAY, J. A. (1958). Excretion by the Malpighian tubules of the stick insect *Dixippus morosus* (Orthoptera, Phasmidae); amino acids, sugars and urea. *J. exp. Biol.* **35**, 871–891.

RECTOR, F. C., JR. & BERRY, C. A. (1982). Role of the paracellular pathway in reabsorption of solutes and water by proximal convoluted tubules of the mammalian kidney. In *The Paracellular Pathway*, (eds S. E. Bradley & E. F. Purcell), pp. 135–158. New York: Josiah Macy Jr. Foundation.

REUSS, L. (1982). The paracellular pathway in amphibian gallbladder. In *The Paracellular Pathway*, (eds S. E. Bradley & E. F. Purcell), pp. 247–269. New York: Josiah Macy Jr. Foundation.

RICHARDSON, I. W., LICKS, V. & BARTOLI, E. (1973). The nature of passive flows through tightly folded membranes. The influence of microstructure. *J. Membr. Biol.* **11**, 293–308.

SHEETZ, M. P. & CHAN, S. I. (1972). Effect of sonication on the structure of lecithin bilayers. *Biochemistry, N.Y.* **11**, 4573–4581.

SIMON, M., CURCI, S., GEBLER, B. & FRÖMTER, E. (1981). Attempts to determine the ion concentrations in the lateral spaces between the cells of *Necturus* gallbladder epithelium with microelectrodes. In *Water Transport Across Epithelia*, (eds H. H. Ussing, N. Bindslev, N. A. Lassen & O. Sten-Knudsen), pp. 52–69. Copenhagen: Munksgaard.

SMITH, H. W. (1951). *The Kidney*. New York: Oxford University Press.

SMITH, J. M. (1962). Detoxication mechanisms. *Ann. Rev. Ent.* **7**, 465–480.

SMULDERS, A. P., TORMEY, J. MCD. & WRIGHT, E. M. (1972). The effect of osmotically-induced water flows on the permeability and ultrastructure of the rabbit gallbladder. *J. Membr. Biol.* **7**, 164–197.

SKAER, H. LE B., HARRISON, J. B. & LEE, W. M. (1979). Topographical variations in the structure of the smooth septate junction. *J. Cell Sci.* **37**, 373–389.

SPRING, K. R. (1979). Optical techniques for the evaluation of epithelial transport processes. *Am. J. Physiol.* **237**, F167–F174.

SPRING, K. R. & ERICSON, A. (1982). Epithelial cell volume modulation and regulation. *J. Membr. Biol.* **69**, 167–176.

STEWARD, M. C. (1982). Paracellular non-electrolyte permeation during fluid transport across rabbit gallbladder epithelium. *J. Physiol., Lond.* **322**, 419–439.

TAYLOR, A., MAMELAK, M., GOLBETZ, H. & MAFFLY, R. (1978). Evidence for the involvement of microtubules in the action of vasopressin in toad urinary bladder. I. Functional studies on the effect of antimitotic agents on the response to vasopressin. *J. Membr. Biol.* **40**, 213–235.

[redacted]OR, A. & WINDHAGER, E. E. (1979). Possible role of cytosolic calcium and Na-Ca exchange in regulation of transepithelial sodium transport. *Am. J. Physiol.* **236**, F505–F512.

TILNEY, L. G. & MOOSEKER, M. S. (1971). Actin in the brush border of epithelial cells of the chicken intestine. *Proc. natn Acad. Sci. U.S.A.* **68**, 2611–2615.

TREHERNE, J. E. (1974). The environment and function of insect nerve cells. In *Insect Neurophysiology*, (ed. J. E. Treherne), pp. 187–244. Amsterdam: North Holland.

WALL, B. J. & OSCHMAN, J. L. (1970). Water and solute uptake by rectal pads of *Periplaneta americana*. *Am. J. Physiol.* **218**, 1208–1215.

WALL, B. J., OSCHMAN, J. L. & SCHMIDT-NIELSEN, B. (1970). Fluid transport: Concentration of the intercellular compartment. *Science, N.Y.* **167**, 1497–1498.

WIGGLESWORTH, V. B. & SALPETER, M. M. (1962). Histology of the Malpighian tubules in *Rhodnius prolixus* Stal (Hemiptera). *J. Insect Physiol.* **8**, 299–307.

WRIGHT, E. M., SMULDERS, A. P. & TORMEY, J. McD. (1972). The role of the lateral intercellular spaces and solute polarization effects in the passive flow of water across the rabbit gallbladder. *J. Membr. Biol.* **7**, 198–219.

ZACCAI, G., BLASIE, J. K. & SCHOENBORN, B. P. (1975). Neutron diffraction studies on the location of water in lecithin bilayer model membranes. *Proc. natn Acad. Sci. U.S.A.* **72**, 239–345.

ZERAHN, K. (1977). K^+ transport in insect midgut. In *Transport of Ions and Water in Animals*, (eds B. L. Gupta, R. B. Moreton, J. L. Oschman & B. J. Wall), pp. 381–401. New York: Academic Press.