The permeability properties of septate junctions in Malpighian tubules of *Rhodnius*

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Summary

This paper describes the structural characteristics and permeability properties of the smooth septate junctions between the upper Malpighian tubule cells of a blood-sucking bug, *Rhodnius prolixus*. The permeability of the paracellular route was tested only for solutes that could be demonstrated not to cross the epithelium via the cellular route. The intercellular clefts were readily permeated by sucrose, inulin and polyethylene glycol (PEG), showing a higher permeability to molecules of smaller radius (PEG versus sucrose). Negatively charged molecules permeated the clefts more readily than positively charged ones. The effects of pH, urea and luminal flow rate on permeability were studied. The results are discussed in relation to the physiological tightness of the Malpighian tubules to certain solutes and to its function as an excretory epithelium.

Key words: *Rhodnius*, Malpighian tubules, septate junctions, permeability.

Introduction

Substances in the haemolymph of insects pass into the primary excretory fluid by crossing the epithelium of the Malpighian tubule. This single-layered sheet of cells can be crossed either through the cell membranes and through the cells (transcellular movement) and/or by moving through the intercellular spaces (paracellular movement).

We have recently shown that many substances not able to enter the cells can nevertheless penetrate some insect epithelia by the paracellular route (O'Donnell et al. 1984). The significance of this is that the cells in these invertebrate epithelia are joined laterally by septate junctions, which have been thought to constitute impenetrable permeability barriers (Noirot-Timothee & Noirot, 1980).

In the present paper we describe the permeability properties of the paracellular pathway in the upper Malpighian tubules of *Rhodnius*. We have used substances differing in molecular size, shape and charge, and have subjected the epithelium to a variety of conditions that might be expected to affect the junctions mechanically or chemically.

Materials and methods

The insects used were 5th instars of *Rhodnius prolixus* Stal from a laboratory culture maintained at 28°C. The insects were taken 7-21 days after their moult from the 4th instar. Lengths of the upper, fluid-secreting parts of the Malpighian tubules were dissected from the insects and isolated into drops of saline under *in vitro* conditions (Maddrell, 1969). The standard saline contained (mM): NaCl, 129; KCl, 8.6; MgCl₂, 8.5; CaCl₂, 2.0; NaHCO₃, 10.2; NaH₂PO₄, 4.3; glucose, 34.0. The tubules were then either stimulated to secrete fluid by the inclusion of 10⁻⁵ M-5-hydroxytryptamine (5-HT) in the saline (Maddrell et al. 1971) or were cannulated and perfused with saline (Maddrell et al. 1974). In either case, a substance under test was included in the bathing saline at a concentration of 0.1-2.0 mM, with radioactive molecules of the same solute giving a radioactive content of 20,000-100,000 cts min⁻¹ µl⁻¹. The fluid emerging from the lumen of the tubules was collected and its radioactive content determined by standard scintillation counting techniques. Where it was necessary to check that a tracer had not been metabolized or otherwise altered in crossing the tubule wall, a sample of the luminal fluid was subjected to chromatographic procedures listed on the batch analysis sheet supplied by Amersham International for the tracer.
Verifying that a penetrant solute follows a paracellular route

To investigate the permeability of the septate junctions one has to be sure that the particular solute used crosses the Malpighian tubule entirely by the paracellular route. The appropriate criteria were described by O'Donnell et al. (1984). Briefly, if a solute crosses by a cellular route, it will tend to reach an appreciable concentration within the cells. We have, therefore, not used any solute that labels the cells of the epithelial wall. A further possibility is that a substance could be actively transported through the cells and yet not be found there at significant levels because it is rapidly pumped from the cells into the lumen. To rule out this possibility, we have not used any solute that showed saturation kinetics in its transport into the lumen or any solute whose passage into the lumen was diminished by inhibitors of active transport of compounds similar to the test molecule.

To avoid the possibility of measuring spuriously high permeabilities from tubules damaged in dissection, we have used only isolated tubules that had low permeabilities to sucrose (permeabilities of less than 0·02 n1 ml cumulative area). For freeze-fracture, Malpighian tubules were fixed briefly and following washing were immersed in 25% glycerol in buffer with 6% sucrose for 10–30 min. They were mounted in yeast paste on Balzer's gold specimen planchettes, frozen in melting Freon 22 and stored in liquid nitrogen. They were fractured at −100°C under a vacuum of ≈2×10⁻⁶ Torr in a Balzer's 360M apparatus, shadowed with a tungsten/tantalum mixture and backed with carbon. The replicas were coated with a collodion film to stabilize them during cleaning with dilute (1–10%) bleach solutions. The collodion coat was removed by soaking in amyl acetate after the replicas had been mounted on EM grids. All specimens were examined in a Philips 300 EM operated at 60 kV.

Results

Organization of the epithelium

The upper two-thirds of the Malpighian tubules of *Rhodnius* are composed of a single type of cell; large, flattened and binucleate, and arranged in a regular pattern around the lumen (Figs 1, 2). Opened out, the cells of the tubule can be represented as a series of hexagons with 50–60 μm sides, separated by an intercellular space approximately 17 nm wide. The ratio of surface areas presented to the haemolymph by the basal side of the cell and the intercellular cleft can be calculated from these dimensions, giving a value of 3000:1. However, both the basal and apical membranes of the upper tubule cells are highly convoluted (Fig. 2). The amplification of the basal surface of the cell is approximately 40-fold (Maddrell, 1980). Thus the relative surface areas of the basal cell membranes and the intercellular clefts are in fact in the ratio of about 120 000:1 (10⁵:1).

Organization of the septate junctions

The 17 nm intercellular clefts are occupied by smooth septate junctions for the apical two thirds of their length. The lateral borders of the cells are in general straight-sided, although occasionally the apical end of the junction may be thrown into folds where the cells interdigitate. These junctions have the appearance characteristic of smooth septate junctions. Transverse thin sections show a regular intercellular space spanned by septa, which, however, are frequently obscured by electron-dense material (Fig. 3). Tangential sections of tissue infiltrated with colloidal lanthanum as a negative stain reveal intermembrane septal ribbons (Fig. 4) and freeze-fracture replicas show the intramembrane particle rows and grooves (Fig. 5) that are associated with them. These features are typical of arthropod smooth septate junctions (Lane & Skaer, 1980; Noirot-Timothée & Noirot, 1980).

The septal ribbons revealed by lanthanum infiltration frequently appear discontinuous (Fig. 4) (Filshie & Flower, 1977). However, examination of sections tilted using a goniometer stage or of serial sections...
Fig. 1. Part of the upper Malpighian tubule viewed with Nomarski optics (A); and a diagram (B) to point out the main features. The binucleate cells are arranged as a single layer around the lumen. A, ×500.

Fig. 2. Low-power electron micrograph of an upper Malpighian tubule cell showing its flattened shape and the elaboration of both the apical and basal cell membrane. Arrows indicate the junctions between cells. l, lumen; h, haemolymph. ×3100.
reveals these breaks to be artefactual, resulting simply from structures leaving the plane of focus or the realm of a section (Fig. 6). It seems likely therefore that the intercellular space in the region of the junction is occupied by ranks of unbroken septa running around the cells.

Measurements of tracer penetration
Unstained sections of tubules incubated for 30 min in 1 mM, 10 mM or 27 mM solutions of ionic lanthanum and then fixed in a solution designed to precipitate the lanthanum in situ showed that the ion had penetrated right through to the apical end of the junction (Fig. 7). Differing concentrations of lanthanum applied to the haemolymph side of the tubule for 30 min made no difference to the degree of penetration (Figs 7, 8A). X-ray microanalysis of the electron-dense deposits found in the junctions confirmed the presence of lanthanum (see Fig. 8B).

Permeability of the tubule wall to sucrose
In our earlier paper on solute passage across the tubule wall (O'Donnell et al. 1984), we provided evidence that sucrose crossed via the paracellular route and reached its steady-state concentration in the lumen within a few seconds of its introduction into the bathing medium. We showed that radioactive sucrose does not label the walls of Malpighian tubules (O'Donnell et al. 1984), nor does its passage across the walls show saturation kinetics. It seemed reasonable to suggest that sucrose penetrates through the intercellular clefts. However, two further possible alternative explanations have occurred to us. It is possible that the failure to label the walls might result from active transport in which sucrose is pumped rapidly out of the cells so that its intracellular concentration is always maintained at a low level. Alternatively, it is conceivable that sucrose (and other substances) might be actively taken through the cells in vesicles and so again not label the main body of the cytoplasm. To test these possibilities, we investigated the effects of 1 mM-KCN on sucrose penetration through the wall. Cyanide at this level rapidly and reversibly stops active fluid transport completely. In 20 tubules, treatment with 1 mM-CN⁻ for periods of up to 60 min caused no detectable changes in the rate of
penetration of sucrose. This result effectively rules out the possibility that sucrose is actively transported across the tubule wall. We conclude that sucrose can rapidly penetrate the intercellular clefts through the septate junctions.

**Penetration by solutes of differing characteristics**

**Effects of molecular shape.** Sucrose is a compound of glucose and fructose, both of which are in the ring form, so although its relative molecular mass ($M_r$) at 342 is relatively low, its minimum cross-sectional area is not small. By comparison, polyethylene glycol (PEG) of $M_r$ 4000 is a large and long molecule but has a considerably lower minimum cross-sectional area than sucrose. Using molecular models, it was possible to pass the PEG structure through a ring representing a pore of diameter 0.38 nm, while the sucrose model required an aperture representing a pore of diameter 0.69 nm.

Experiments comparing the permeability of Malpighian tubules to sucrose and PEG appeared to show that PEG penetrates consistently faster than does sucrose (Fig. 9); for sucrose the permeability was $0.012 \pm 0.001$ nl min$^{-1}$ mm$^{-2}$ ($n = 6$), while for PEG the permeability of the same tubules was $0.056 \pm 0.007$ nl min$^{-1}$ mm$^{-2}$ ($n = 6$). The ability of PEG to penetrate the junctions so rapidly may be due in part to its capacity to associate with membranes. However, chromatograms developed in butan-2-one:water, 1:1 ($v/v$), showed that the PEG in the secreted fluid behaved differently from the PEG in the bathing solution (Fig. 10). It seems likely that the PEG supplied contains a small proportion of short-chain fragments of PEG and that these can penetrate the tubule walls very readily. Since in 15 cases the tubule cells did not become significantly radioactively labelled even when bathed in the PEG solution for 40 min, the radioactive material appearing in the lumen is likely to have crossed paracellularly. If so, the higher permeability of the tubule to PEG than to sucrose shown in Fig. 9 is attributable to PEG of short chain length, comprising perhaps only 5% of the radioactivity in the bath. This indicates that the junctions are some two orders of magnitude more permeable to these short-chain PEG molecules, which are linear, than to sucrose molecules, which are globular.

**Effects of charge.** To investigate the effects of charge on solute penetration, we compared the rate of penetration of sucrose, an essentially uncharged substance, with orotic acid ($C_5H_4N_2O_4$, $M_r$ 156.1), which, like sucrose, is a non-linear molecule but carries a negative charge. Typical results of the initial experiments are shown in Fig. 11; orotic acid penetrates the tubule wall considerably faster than does sucrose. The measured

![Fig. 5. Freeze-fracture preparation of the upper Malpighian tubule cells showing the septate junction in a convoluted region of the intercellular border. The junction has fractured in several regions, revealing the intramembranous organization of discontinuous rows of particles (EF) and grooves (PF) also frequently overlaid with particles. ×37 400.](image-url)
Fig. 6. A–C. Tilt series of a single section of a septate junction between two upper Malpighian tubule cells infiltrated with colloidal lanthanum. D–E. A serial section tilted as in A–B above. Comparison shows that septa may appear to be blind-ending (arrows) either because they leave the plane of focus (cf. A,B,C, or D,E) or because they leave the plane of section (cf. A,D). A–C, ×64 400; D,E, ×57 250.
Fig. 7. Unstained section of the upper Malpighian tubule, which was incubated for 30 min in a 27 mM solution of ionic lanthanum prior to fixation. The tracer has stained the basement membrane and can be seen in the basal folds. It has also penetrated through the junction to its apical end (arrow, inset). ×11400; inset, ×31900.

Fig. 8. Edax microprobe analysis of the dark deposits at the apical end of the septate junction of a sample incubated in a 1 mM solution of ionic lanthanum for 30 min before fixation (A). The spectrum shows that there is lanthanum present in the deposits. The fingerprint for La is shown by the vertical bars (B). A, ×32200.

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The different permeabilities shown by a Malpighian tubule to sucrose and to polyethylene glycol (PEG), during exposure first to sucrose, then to PEG and finally to sucrose again. The tracer-containing solutions were changed at times indicated by the vertical lines.

permeability to orotate in five tubules was 0.21 ± 0.03 nl min⁻¹ mm⁻², a value 10–20 times higher than that typical for sucrose permeability. Chromatography using two different solvent systems (thin-layer plates, PEI cellulose, 0.3 M LiCl as solvent and paper with n-butanol: water: acetic acid, 12:5:3 (by vol.), as solvent) showed that the penetrant radioactivity ran identically to the orotic acid as supplied.

Before it can be claimed that the intercellular clefts are more permeable to orotic acid than sucrose, however, the possibility must be excluded that significant amounts of orotic acid cross through the cells. To test this possibility, tubules were immersed in saline containing radioactive orotic acid for different periods. If orotic acid were to cross passively through the cells, the tubules would accumulate counts rapidly to begin with as the cells fill and then more slowly as counts in the lumen increase. The pattern of accumulation of radioactive orotic acid in tubules is shown in Fig. 12. For comparison, the ways in which radioactive mannitol (known to cross via the cells; O'Donnell et al. 1984) and sucrose enter Malpighian tubules are also shown in Fig. 12. Sucrose and orotic acid label the tubules in a comparable fashion (though at different rates, reflecting the difference in permeability) and show no sign of loading more than one compartment. The behaviour of mannitol is clearly different. Orotic acid then appears not to cross the tubule wall passively through the cells.

To examine the possibility that orotic acid is actively transported through the cells, we investigated the effect on the rate of penetration of radioactive orotate of adding 6.8 mM non-radioactive orotate to the bathing solution. In five experiments this had no discernible effect on the rate of penetration of orotate. It follows either that orotate is actively transported by an active transport process that shows no signs of saturation at 6.8 mM or that orotate penetrates passively through the intercellular junctions. The former possibility is not unlikely, since active transport by the lower Malpighian tubules of urate, which has some similarities in structure to orotate, shows no saturation at 2 mM (O'Donnell et al. 1983). So, as a further test, we measured the penetration of orotate through the Malpighian tubules of 5th-stage Rhodnius fed 6–8 days earlier. Feeding not only induces very much faster urate transport by the lower Malpighian tubules of urate, which has some similarities in structure to orotate, shows no saturation at 2 mM (O'Donnell et al. 1983). So, as a further test, we measured the penetration of orotate through the Malpighian tubules of 5th-stage Rhodnius fed 6–8 days earlier. Feeding not only induces very much faster urate transport by the lower tubules and this reaches a peak by 6–8 days (O'Donnell et al. 1983), but it also evokes faster transport of p-aminohippuric acid by the upper tubules with a similar time course (Maddrell & Gardiner, 1975). The results showed that the permeability to orotate of the walls of tubules from the fed insects was not significantly higher than in control unfed insects, and so is not transported by a process stimulated by feeding.
Fig. 11. The different permeabilities shown by a Malpighian tubule to sucrose and to orotic acid, during exposure first to sucrose, then to orotic acid and finally to sucrose again. The tracer-containing solutions were changed at times indicated by the vertical lines.

Fig. 12. The rates of accumulation of radioactive sucrose, mannitol or orotic acid from the bathing solution by Malpighian tubules. The points represent the mean values and the vertical lines attached to them represent ±S.E.

As a final further check, we measured the rate of penetration of orotate through the walls of tubules before, during, and after exposure to cyanide. In 11 tubules, treatment with 1 mM-cyanide for periods of up to 60 min caused no change in the rate of penetration of orotic acid. This result provides strong evidence that orotic acid is not actively transported across the walls of the Malpighian tubules. We conclude that orotic acid penetrates to the lumen by the paracellular pathway.

Effects of pH
Changes in pH affect the three-dimensional structure of many large molecules and so might alter the paracellular permeability of the Malpighian tubules. To investigate this, we measured the sucrose permeability of cannulated and perfused tubules exposed alternately to solutions of pH 6.56 and 8.20. As Fig. 13 shows, sucrose penetrates considerably faster at the more acid pH. Other tubules secreted fluid at normal rates in 5-HT-containing solutions at these pH values, suggesting that they are not adversely affected by these relatively extreme pH values.

Effects of exposure to La³⁺ on sucrose permeability
Lanthanum ions (La³⁺), which have a high charge density, have been shown to penetrate the intercellular space (Figs 7, 8). Because of their high charge density, they might be expected to affect the charge and, therefore, the configuration of proteins in the intercellular cleft. This in turn might affect the paracellular permeability to other solutes such as sucrose. Fig. 14 shows the penetration of sucrose in the absence and then in the presence of lanthanum ions. Clearly, at this concentration (2.4 mM), lanthanum ions do not affect the permeability of the intercellular space to sucrose.

Effects of exposure to urea
Urea at concentrations of 1 M and above disrupts the structure of isolated insect smooth septate junctions (Green et al. 1983). We exposed 10 cannulated and perfused tubules to saline containing 1 M-urea for periods of between 2 and 5 min and followed the effects
Fig. 14. The effect of exposure to 2.4 mM-lanthanum ions on the sucrose permeability of a Malpighian tubule. The bathing solution was changed for the lanthanum-containing one at the time indicated by the vertical line.

Fig. 15. The effect on the sucrose permeability of a Malpighian tubule of exposure to 1 M-urea. The tubule was in the urea solution for the period indicated by the vertical lines.

this had on sucrose permeability. In all cases sucrose permeability increased 3–10 times; Fig. 15 shows a typical result. It might be argued that 1 M-urea might exert its effect osmotically. However, urea is a rapidly penetrating solute and tubules appear not to shrink very much when immersed in saline containing 1 M-urea. It therefore seems likely that the increase in permeability resulted from disruption of the septa.

Effects of intraluminal pressure on permeability

When fast fluid secretion by Rhodnius Malpighian tubules is stimulated, the lumen of the tubule distends. The intraluminal pressure developed must subject the lateral cell junctions to tension in the plane of the epithelial wall and so might affect their permeability. Some preliminary results have strongly suggested such a possibility (O'Donnell et al. 1984). For the present experiments, we have varied fluid flow rates along the lumina of Malpighian tubules either by stimulation with 5-HT or by changing the rate of fluid perfused through the tubule from a cannula. In either case, the results clearly show a strong correlation between the rate of fluid secretion and/or fluid perfusion and the rate of penetration of sucrose into the lumen (Figs 16, 17). The fact that variation in the rate of fluid perfusion changes sucrose entry in a similar fashion to that produced by 5-HT stimulation suggests that the effect does not depend on fluid transport across the tubule wall. The effect, however, might be due to changes in the concentration gradient between bathing solution and the lumen; faster fluid flows might thin the boundary layer (unstirred layer of fluid) next to the luminal surface of the cells. This possibility can reasonably be excluded, since penetration through the wall by sorbitol is unaffected by changes in fluid perfusion rate (Fig. 18). Sorbitol is known to penetrate...
Fig. 17. The effects of changing the rate at which fluid was perfused through a Malpighian tubule on its permeability to sucrose. The rate of perfusion was changed at the times shown by the vertical lines. The upper trace shows the sucrose permeability of the tubule. The lower trace shows the rate of fluid emerging from the cut end of the tubule.

Fig. 18. The lack of effect of changing the rate at which fluid was perfused through a Malpighian tubule on its permeability to sorbitol. The rate of perfusion was changed at the times shown by the vertical lines. The upper trace shows the rate of fluid emerging from the cut end of the tubule and the lower trace shows the permeability of the tubule to sorbitol.

Effects of osmotic concentration on permeability

The septate junctions would be expected to be stressed mechanically if the cells were altered in volume osmotically. To test the effect this might have on sucrose permeability, we treated tubules for 5 min with normal saline supplemented with 0.34 M-sucrose (a doubling of osmotic concentration to 682 mosmol⁻¹), for 5 min with 1 M-sucrose solution (1 mosmol⁻¹), or for 5 min with standard saline containing 1.1 M-sucrose (1.44 mosmol⁻¹). The saline of 682 mosmol⁻¹ had no effect on sucrose permeability (6 tubules), but...
treatment with 1 M-sucrose caused a 2–10 times increase in sucrose permeability (8 tubules) and treatment with 1·44 osmoll⁻¹ a 10–100 times increase in sucrose permeability (4 tubules). These results show, perhaps not surprisingly, that shrinking the cells osmotically, which presumably stresses the junctions mechanically, increases their permeability.

**Loss of injected solutes through the excretory system in vivo**

When *Rhodnius* are fed, fluid crosses the gut wall into the haemolymph and then is rapidly excreted by the Malpighian tubules (Buxton, 1930; Wigglesworth, 1931; Maddrell, 1963). Urine is formed by fluid secretion in the upper tubules and then passes out through the lower tubules and rectum. From our results, we therefore expect that solutes injected into the haemolymph of fed insects should rapidly appear in the urine. We have tested this by injecting freshly fed 5th-stage insects with saline containing one of the following radioactive substances: sucrose, PEG, orotic acid or lanthanum. We then followed the appearance of the solutes in the urine for periods up to 40 min, before taking a sample of haemolymph to determine the solute concentration there.

If it is assumed that the major site of loss from the haemolymph is the upper Malpighian tubules, the permeability of these regions to the solutes can be calculated. Such calculations gave the following results: for sucrose, 0·012 ± 0·004 nl min⁻¹ mm⁻² (n = 19); for orotate, 0·24 ± 0·04 nl min⁻¹ mm⁻² (n = 6); for PEG, 0·061 ± 0·010 nl min⁻¹ mm⁻² (n = 5); and for lanthanum, 0·0034 ± 0·0014 nl min⁻¹ mm⁻² (n = 4). The values for sucrose, PEG and orotate are all close to those found in vitro; we have not tested the permeability of the tubules to lanthanum in vitro.

How likely is it that these results apply to the upper Malpighian tubules? The question arises because, of course, these solutes could have entered the urine across any or all of the epithelia separating the urine from the haemolymph, that is the upper Malpighian tubules, the lower tubules and the rectum. The planar surface areas (i.e. not taking membrane folding into account) exposed by each to the haemolymph can be calculated from their dimensions to be 36 mm² for the upper tubules, 22 mm² for lower tubules and 6 mm² for the rectum. Strictly on this basis then, the upper tubules would be the most likely site by which solutes could enter the urine by a paracellular route. Clearly, however, one cannot claim more for the results than that they are compatible with a paracellular entry into the urine across the walls of the upper tubules with a permeability similar to that seen in vitro. What fraction of the solutes tested actually entered the urine through the walls of the lower tubules and/or the rectum cannot be determined. However, the intercellular junctions of both these epithelia are also septate (Lane & Skaer, 1980).

**Discussion**

The results in this paper show that the smooth septate junctions of the upper Malpighian tubules of *Rhodnius* are readily permeable to a wide variety of substances. Even lanthanum ions (La³⁺), which, because of their high charge density are extensively hydrated and therefore large, can penetrate all the way through the junctions. The permeability of the junctions to different solutes is affected by the shape and charge of the molecules. Molecules of polyethylene glycol, straight chains of low cross-sectional area, penetrate much more rapidly than does sucrose, which is ring-structured. A negatively charged, ring-shaped molecule, orotic acid, penetrates faster than does the more neutrally charged sucrose, which suggests that the material in the intercellular clefts may have an excess of fixed positive charges. However, orotic acid, although it is of similar minimum cross-sectional area to sucrose, is only half its relative molecular mass (156 as opposed to 342) and it is arguably this that allows it to penetrate more rapidly. On the other hand, sucrose penetrates junctions faster at low pH, suggesting that the clefts may indeed contain material with fixed positive charges; the material would tend to expand as the number of positive charges increases and negative charges are suppressed by protons.

It is widely held that septate junctions act as transepithelial permeability barriers (see, for example, Flishie & Flower, 1977; Szollosi & Marcaillu, 1977; Noirot-Timothée & Noiriot, 1980; Green & Bergquist, 1982; Flower, 1986; Keil & Steinbrecht, 1987; and review by Lane & Skaer, 1980), some regarding them as homologous with vertebrate tight junctions (Noirot-Timothée & Noiriot, 1980; Green & Bergquist, 1982; Miranda & Cavicchia, 1986; Flower, 1986). Evidence to support these claims derives from their distribution (they are found predominantly in epithelial tissues) and positioning (they encircle the apical portion of the cell), and from their reported ability to restrict the paracellular movement of tracers and ions. Evidence based on the parallel distribution and position of septate junctions and vertebrate tight junctions, however, can be no more than circumstantial and hypothetical without parallel physiological studies. Evidence from tracers is clouded in a number of studies where the tracer was administered during fixation and yet its penetration was taken as a physiologically relevant measure of permeability. Where such tracers have been applied in physiological saline and incubation completed before fixation, the majority of studies demonstrate complete penetration of the junction (e.g. see Lane, 1979; Leslie, 1975; McLaughlin, 1974; Ryder & Bowen, 1980).

It is our contention that, in the Malpighian tubules of Rhodnius, the septate junctions are readily permeable to a range of substances. How far might a similar conclusion apply to the septate junctions found in other insect epithelia? Since the paracellular pathway is occluded by tight junctions in at least some cases where a physiological barrier has been shown (see, for example, Lane et al. 1977; Lane & Treherne, 1972), it might well be supposed that septate junctions alone cannot act as occluding junctions. Against this view it has been argued that the septate junctions of the rectal chloride epithelium of the dragonfly, for example, do constitute a permeability barrier (Kukulies & Komnick, 1983). These authors were led to their conclusion by their observation (1) that lanthanum ions did not appear in electron microscope (EM) sections to penetrate all the way through the entire length of the septate junctions; and (2) that lanthanum ions did not appear in appreciable quantities in the haemolymph of dragonfly larvae that had approximately 45–80 mM solutions of lanthanum salts in contact with the rectal epithelium for up to 8 days. They concluded that the primary function of the septate junctions is to act as “impermeable obstacles on the paracellular route”. In fact their interpretation may not be so far removed from our own conclusion as it may seem. They suggest that the relative tightness of septate junctions might be attributable not to an intrinsic impermeability of the material of the junction but to a reduction of net diffusion caused by “the collective detour effect of the intercellular septa and the convolution of the junction”, in other words to an increase in the diffusional distance to be traversed. They calculate that the diffusional pathway may exceed the apparent length of the junction by a factor of about 70 and may amount to 1500 μm. Such an increase would of course greatly slow down the diffusional passage of molecules through the junction but it is important to note that it would not in itself stop it. Indeed it is easy to show that, even with a pathway of this length, 50% of the eventual steady-state net flux even of such a large ion as lanthanum would be achieved in only 10 min. So why did Kukulies & Komnick not observe complete penetration of lanthanum through the junction? At steady-state, there will be a concentration gradient of lanthanum through the junction, so the concentration at the basal end will be very low, too low perhaps to be revealed in EM sections of the tissue. The situation in the Malpighian tubules of Rhodnius is different because it appears that the apical end of the junction, the end furthest from the applied lanthanum, has an affinity for lanthanum. Indeed we occasionally observe an apical plug of lanthanum deposit with no visible lanthanum upstream from it (Fig. 8A). This may explain how it is that we find lanthanum that has penetrated the entire length of the junction.

Kukulies & Komnick (1983) also found that appreciable quantities of lanthanum ions did not appear in the haemolymph of insects where the rectal lumen was exposed to 45–80 mM-lanthanum even for 8 days. Two factors may act to explain this. The first is that the Malpighian tubules will steadily remove any lanthanum that appears in the haemolymph by the usual excretory mechanism. The second is that the rate of entry of lanthanum through the rectal epithelium depends on the total area of intercellular pathway as well as the permeability of the septate junctions. If the area is low, then the rate of entry will also be low. In Malpighian tubules, inulin, which can readily penetrate the septate junctions, appears in the secreted fluid at concentrations about 500–1000 times lower than in the bathing solution (O'Donnell et al. 1984). This low concentration depends partly on the high rate of fluid secretion but also on the low total area of the septate junctions.

Malpighian tubules, like many other epithelia, support an electrical potential difference between the lumen and the haemolymph. The existence of such potentials has been taken as direct evidence of the barrier function of septate junctions (Loewenstein & Kanno, 1964; Hand & Gobel, 1972; Noirot-Timothee & Noirot, 1980). However, the development of a transepithelial potential depends not directly on a paracellular seal but on the relationship between the rate of ion transport across the cells and the transepithelial resistance. In turn, the resistance of the epithelium depends not only on the tightness of the paracellular pathway, but also on the resistance of the cell membranes. In Rhodnius Malpighian tubules, where the ratio of the area of the cell membranes to the area of the paracellular pathway is in the region of 120,000:1, the resistance of the cell membrane assumes virtually total significance.

Filshie & Flower (1977) calculated the reduction in the cross-sectional area of the paracellular pathway that would be produced by the presence of septa in the intercellular clefts in the septate junctions of Hydra to be 300-fold. This calculation assumed that the apical ranks of septa were punctuated every 5 μm by gaps 15 nm by 15 nm. Our evidence (Fig. 6) indicates that in Rhodnius Malpighian tubules there may well be no visible punctuations in the septal ribbons. Examination of tilted specimens and of serial sections indicates that the septa are unbroken. In spite of this, the junctions are readily permeable to a variety of molecules. Two possible explanations for this permeability can be suggested. First, as has been suggested before (e.g. see Hand & Gobel, 1972; Staehelin, 1974), the septa are not solid structures spanning the intercellular space but are fenestrated, permitting the passage of molecules
Fig. 20. Diagram of a single upper Malpighian tubule cell showing the septa of the junction tilted at an angle to the horizontal. The effect of even quite a shallow tilt is a very considerable reduction in the paracellular pathlength (arrows).

through them. Second, the septa might be arranged so as to reduce the path length. For example, if, instead of running horizontally round the cells, the septa are pitched at an angle to the horizontal plane, then the paracellular path length would be very considerably reduced (G. E. Palade, personal communication; see Fig. 20). Tricellular junctions, which occur when three cells meet, if they are organized as has been suggested by Fristrom (1982), Graf et al. (1982), Noirot-Timothée et al. (1982), might also shorten this direct pathway. Evidence concerning the tilt of the septa relative to the horizontal axis of the cell cannot be obtained easily, either by freeze-fracture or by tangential sectioning of lanthanum-stained material. The intercellular borders are too convoluted to permit the extended view of the junctional membrane needed to make the necessary measurements.

The evidence presented in this paper suggests that the septate junctions of *Rhodnius* Malpighian tubules are freely permeable to uncharged and negatively charged molecules of relatively small cross-sectional diameter. However, even larger, globular molecules and those carrying a high positive charge penetrate into the lumen more slowly. There is thus some selectivity in permeability, possibly conferred by a positively charged intercellular matrix trapped between the septa (Staehelin, 1974; Oschman, 1978; Skaer et al. 1979).

Although the junctions themselves are permeable, the overall permeability of the Malpighian tubule epithelium is low. For the functional design of the Malpighian tubule this appears to present a paradox. In spite of their low passive permeability to haemolymph solutes, the tubules clear toxic compounds from the haemolymph. The solution to this paradox lies in the restriction of the area for passive permeability (the paracellular clefts) rather than in restricting the permeability of these sites. In this way, compounds, for which there is no active transport mechanism diffuse slowly through the intercellular clefts into the lumen and are excreted, provided there is no uptake mechanism for their recovery. Useful components of the haemolymph also move only slowly into the lumen and can be recovered from the excretory fluid by active transport. Thus the paramount requirement of an excretory system (to be able automatically to remove toxins not encountered before; Ramsay, 1958) can be met without excessive loss of valuable haemolymph solutes (Maddrell, 1981). If the septate junctions act as occluding junctions, the haemolymph components might be more effectively retained, but novel toxins would present an insuperable problem to the insect, as they then could not pass passively into the tubule lumen and would not be cleared from the haemolymph.

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References


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