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Fluid Secretion by the Malpighian tubules of Insects

S. H. P. Maddrell

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Fluid secretion by the Malpighian tubules of insects

By S. H. P. Maddrell
A.R.C. Unit of Invertebrate Chemistry and Physiology, Department of Zoology, Downing Street, Cambridge CB2 3EJ

[Plates 28 to 30]

Malpighian tubules of insects typically secrete an iso-osmotic fluid by a process which is thought to involve the following:

(1) Potassium ions enter the tubule cells by a process which is sodium dependent and which may be active; they are then actively pumped into the lumen by an electrogenic pump.

(2) Sodium ions cross the wall in a similar fashion to potassium ions but their entry into the cells is very restricted so that they are transported only slowly.

(3) These active cation movements create a trans-wall potential favouring the passive movements of anions from the haemolymph into the lumen.

(4) With one exception, smaller anions appear to cross the wall faster than do larger ones. The exception is that phosphate ions cross faster than any other anion in spite of their large size. The evidence suggests that this is more likely to be achieved by facilitated diffusion but active movements are not excluded.

(5) The apical and basal membranes of the tubule cells are elaborately folded. It is suggested that these foldings act to couple movement of water to the movements of ions by allowing the development of standing osmotic gradients.

(6) Such gradients will be small because the channels in which they occur are short. However, as the cell membranes probably have a high osmotic permeability water is likely to be able osmotically to equilibrate with the channel contents to produce an iso-somotic secretion.

(7) The folds in the cell membrane are such that a parallel array of channels alternately opening to the cytoplasm and to the extracellular fluid is produced. Such an arrangement leads to a steeper osmotic gradient across the cell wall and this will promote a more efficient coupling of solute and water movements.

The Malpighian tubules of insects are long slender tubes which run freely through the haemocoel in the hinder part of the insect. They run into the alimentary canal at or near the junction of the mid- and hind-gut where the secretion they produce runs into the gut lumen. Figure 1 shows in diagrammatic form the relationship of a single Malpighian tubule to the rest of the alimentary tract both from the point of view of its anatomical arrangement and its functional relationship. Broadly speaking, the Malpighian tubules secrete an iso-osmotic ion-containing solution by a mechanism, the description of which forms the theme of this paper. Other molecules of low molecular weight in the haemolymph diffuse down their concentration gradients into the tubule lumen (Ramsay 1958). This passive movement may well be enhanced by frictional interactions with the transported water and ions—what have been called solvent and solute drags respectively (Ussing 1960; Franz, Galey & Van Bruggen 1968; Biber & Curran 1968). Some other larger substances, particularly the anionic forms of aromatic acids, are actively added to the lumen fluid but by a mechanism separate from the fluid producing mechanism (Maddrell & Reynolds 1971).

As a result of these activities the Malpighian tubules pass to the hind-gut a fluid which is similar in several respects to an ultrafiltrate of the insects haemolymph. The excretion from the insect of such a fluid would be so inappropriate as to result rapidly in the insect's death. It

consists of many potentially useful molecules at fairly high concentrations; the water in the fluid produced during one hour can amount to as much as 50 % of the total blood volume. What now happens to this fluid of course is that a selective reabsorption goes on in the hind-gut of those substances that the insect requires to retain, unwanted substances remaining in the lumen, eventually to be excreted. This activity may well involve the whole of the hind-gut, but a dominant role appears to be played by the rectum and an elegant description of its activity is given in Ramsay's paper in this volume (p. 251). As he earlier pointed out (Ramsay 1958)

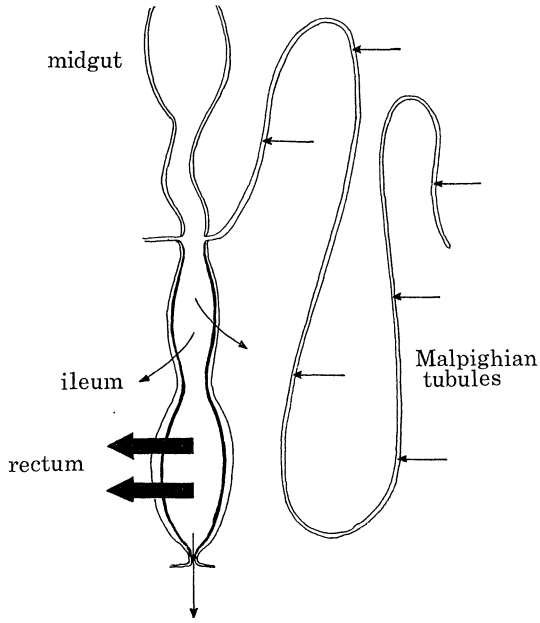
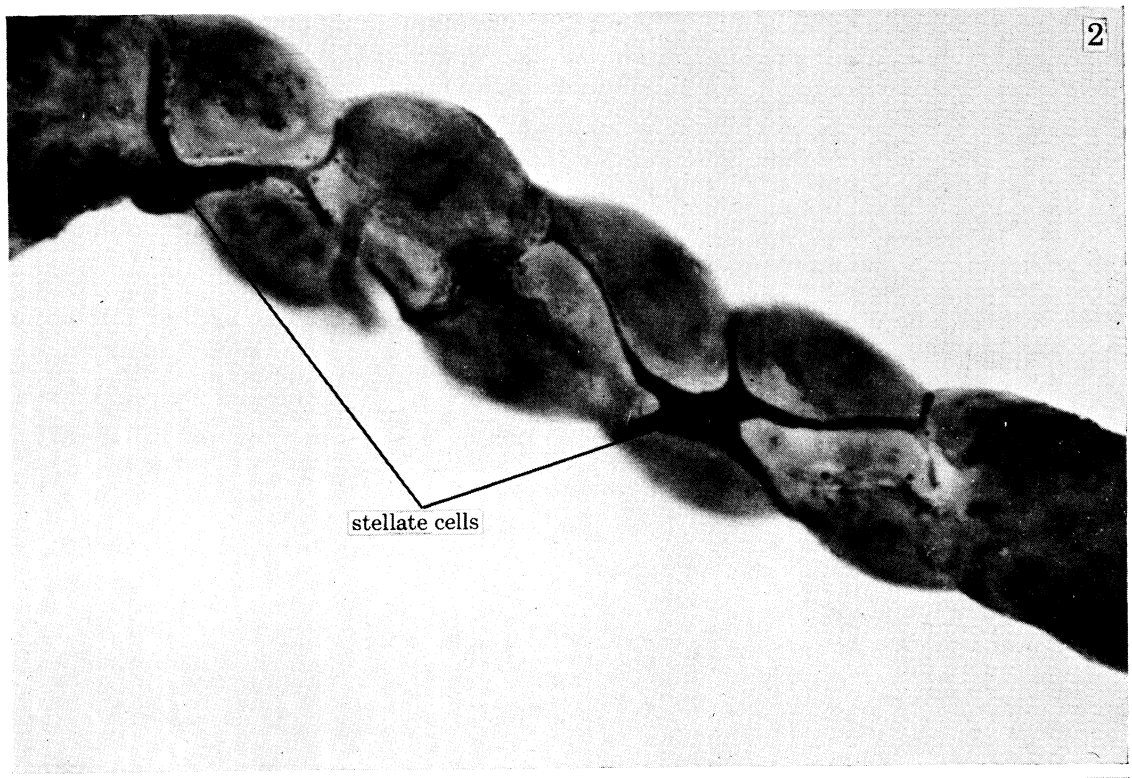


FIGURE 1. The relationship of a single Malpighian tubule (insects have between 2–150 tubules) to the mid-gut and hind-gut. The arrows indicate the directions in which and the intensities with which materials flow through the excretory system.

this committal of a mixture of useless and useful molecules to the primary excretory fluid and the later reabsorption of useful molecules is important in that it ensures the automatic excretion of all unwanted substances of low molecular weight in proportion to their concentration in the haemolymph so that the removal from the haemolymph of any new toxic or useless substance encountered by the insect or any unusual concentration of an undesirable material is automatically catered for. One might add that the relatively high rate of circulation of fluid through the system may well be adapted to promote the speedy removal of waste material from the haemolymph. A similarly high rate of circulation is characteristic of vertebrate kidneys; in man a volume of fluid equal to that of the blood is filtered every half hour (Smith 1951).

With this introduction let us return to the Malpighian tubules. Figure 1 suggests that a typical Malpighian tubule secretes fluid along its whole length. While this is true of many tubules, it must be admitted that others have sections which reabsorb ions and/or fluid and a few have lengths which produce secretions as diverse as lime and silk (Wigglesworth 1965). For the purposes of this paper we shall only refer to the lengths of tubules which form the primary excretory fluid. Figure 2, plate 28, shows the appearance of a length of Malpighian tubule while figure 3, plate 28, is of a cross-section of a tubule. They show that Malpighian tubules are thin-walled tubes, the walls of which are formed from an epithelial layer one cell thick. In some



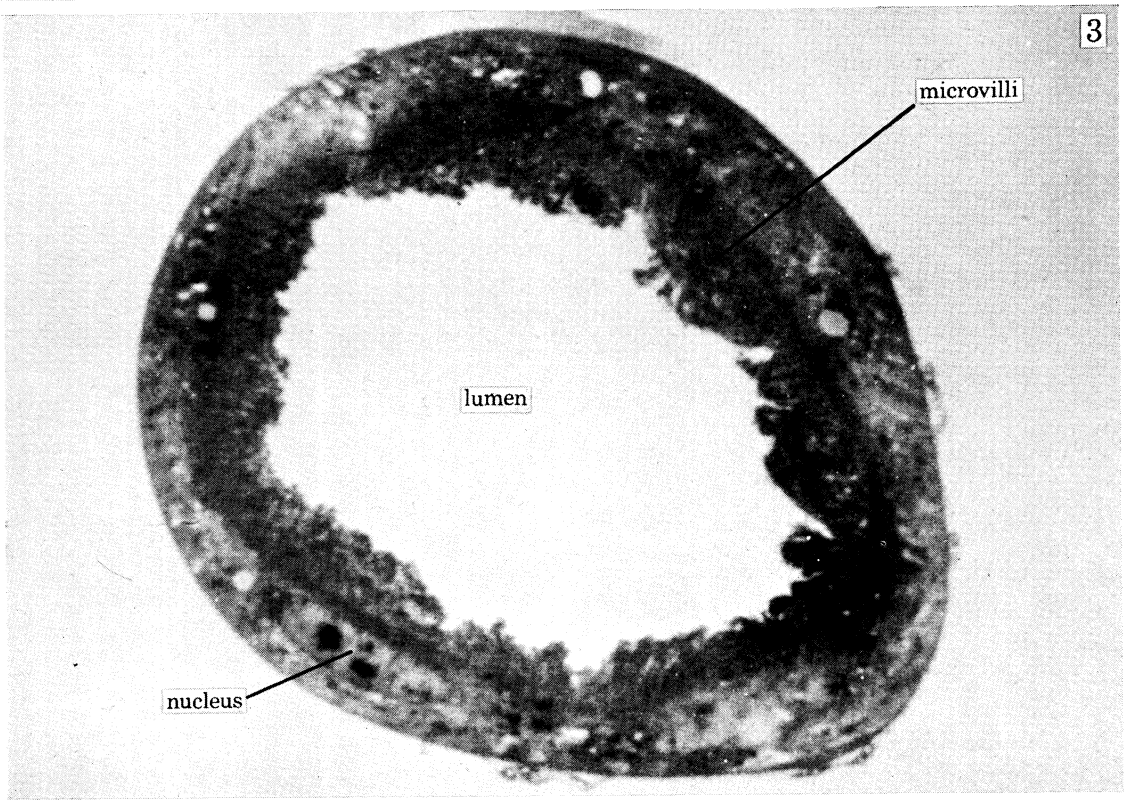


FIGURE 2. A length of Malpighian tubule from *Calliphora* fixed in glutaraldehyde and treated sequentially with lead nitrate and ammonium sulphide solutions. Note the intensely stained stellate cells. Photograph by courtesy of Dr R. C. Joyner. (Magn. × 300.)

FIGURE 3. A cross-section of a Malpighian tubule of *Rhodnius*. Note that the wall consists of a single layer of cells. (Magn. × 1000.)

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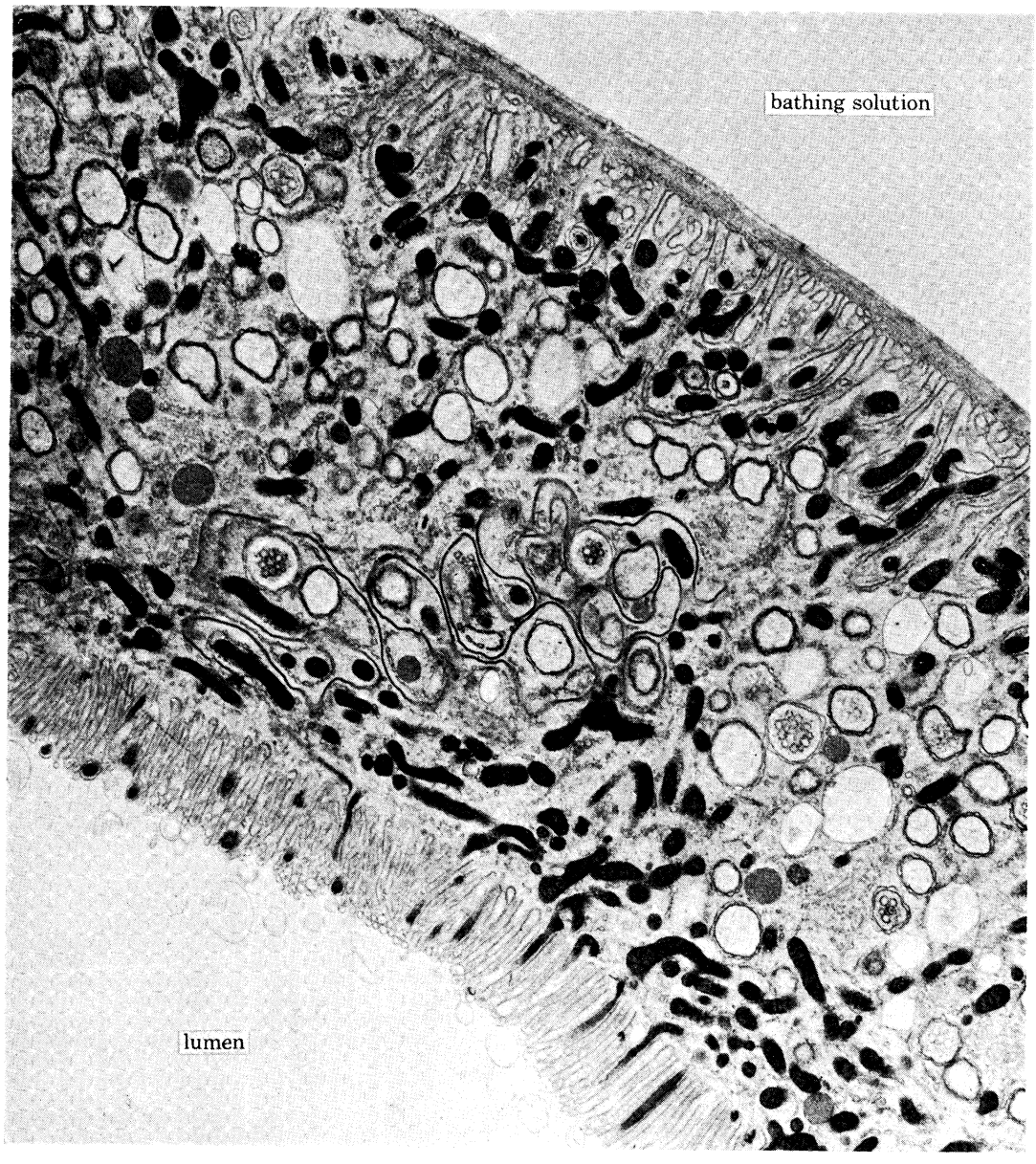

FIGURE 4. Electron micrograph of a section through the wall of the Malpighian tubule of *Periplaneta*. Photograph by courtesy of Dr J. L. Oschman. (Magn. × 6000.)

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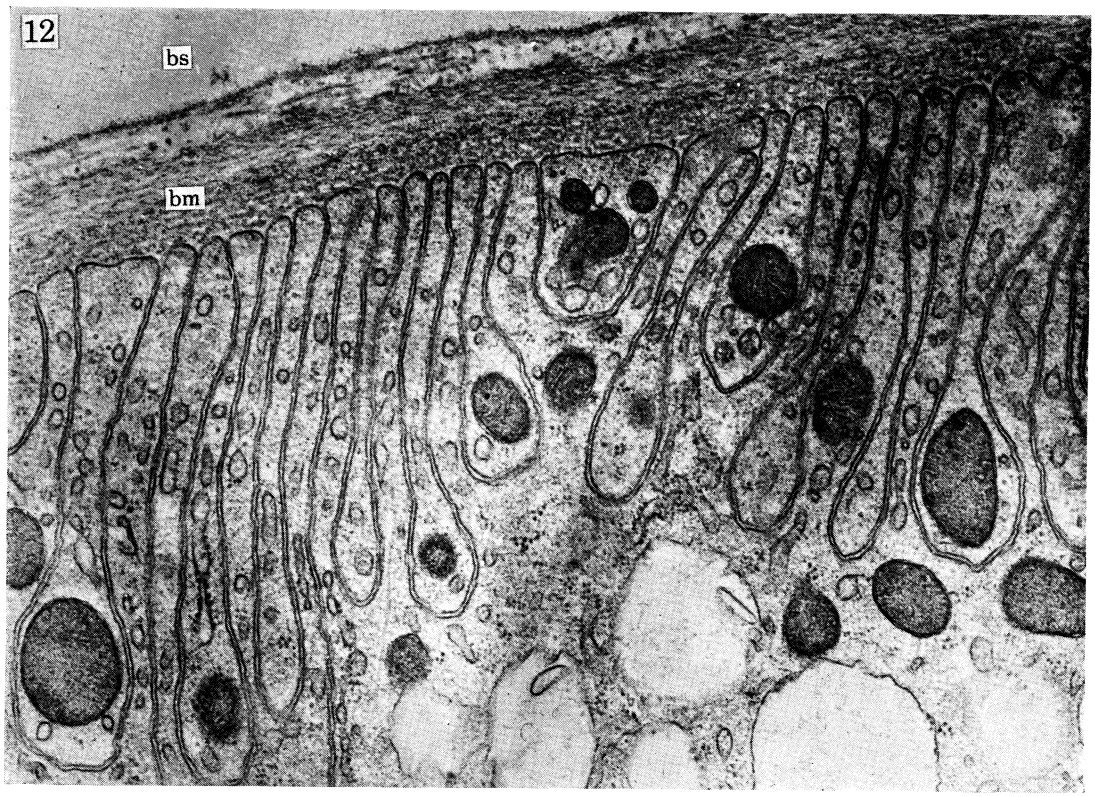

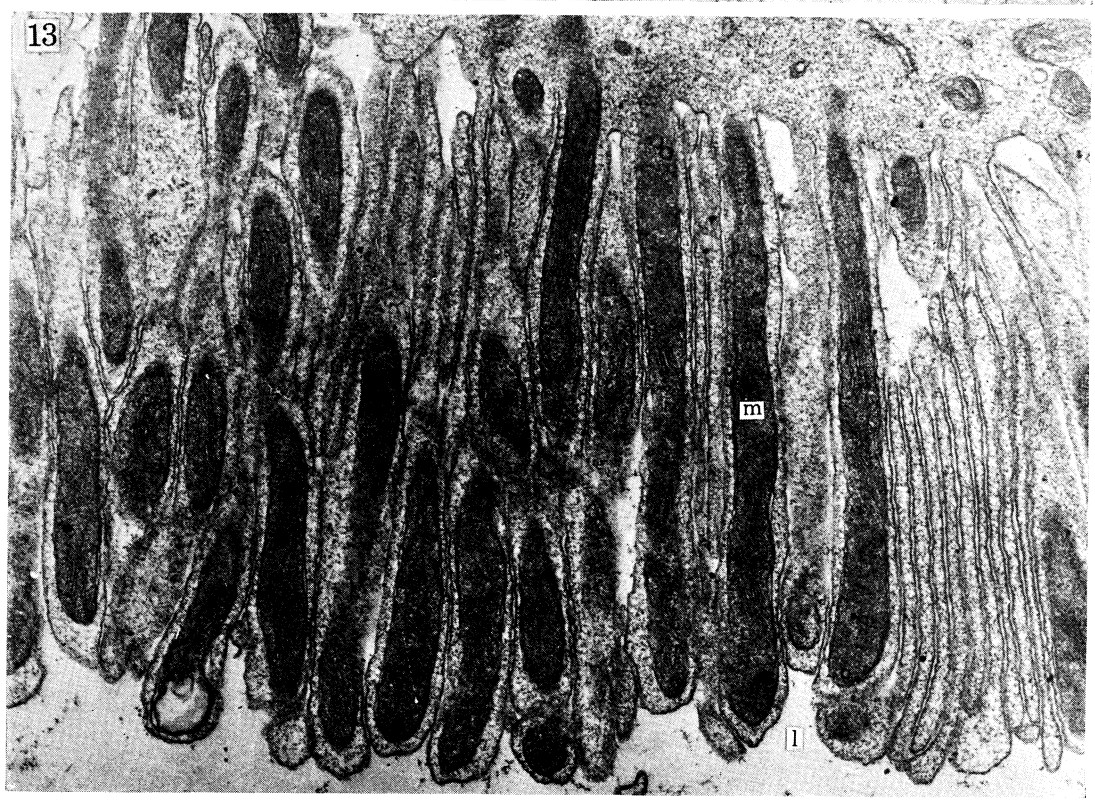

FIGURE 12. Electron micrograph of a section through the wall of the Malpighian tubule of *Calliphora* to show the infoldings of the basal cell membrane. The bathing solution (bs) is separated from the infoldings only by the basement membrane (bm) known to be very permeable. Photograph by courtesy of Dr J. L. Oschman. (Magn. × 28 000.)

FIGURE 13. Electron micrograph of a section through the wall of the Malpighian tubule of *Calliphora* to show the apical microvilli projecting into the lumen (l). Note that a proportion of the microvilli contain a mitochondrion (m). Photograph by courtesy of Dr J. L. Oschman. (Magn. × 28 000.)

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Malpighian tubules the cells of the wall are all of one type, but in others there are two cell types and although one is heavily outnumbered by the other, it none the less complicates one's efforts to understand how the tubules work. Just how these extra cells (called stellate cells because of their shape (figure 2)) might affect tubule function has not yet with any success been accommodated in any theory of tubule action.

A more complete idea of structure can of course be gained by using the electron microscope. Figure 4, plate 29 is of a cross-section of the wall of a Malpighian tubule to show the structure of the typical cells forming the wall. The points to note are:

(1) The basement membrane covering the haemolymph (basal) side of the cells. There is evidence that this is a very permeable structure. In some insects it is even permeable to substances as large as horseradish peroxidase which has a molecular weight as high as 40000 (Kessel 1970), though in others such large molecules cannot penetrate the membrane (Locke & Collins 1968).

(2) Deep and tortuous infoldings of the basal cell membrane.

(3) Long closely packed apical microvilli, a proportion of which have a long mitochondrion running along their length.

(4) The cells are joined laterally to one another by very extensive septate desmosomes.

The significance of some of these structural points will become clear from a consideration of the ways in which the Malpighian tubules handle ions and water.

The account which follows is based on the work of Ramsay (1953, 1954, 1955, 1956, 1958) and Pilcher (1970) on tubules of the stick insect, *Carausius morosus*, of Berridge (1968, 1969) on the tubules of the blowfly, *Calliphora erythrocephala*, of Coast (1969) on the tubules of the leather-jacket, *Tipula paludosa* and of Irvine (1969) on the tubules of larvae of the American skipper, *Calpodes ethlius*. Where it is relevant and comparable I shall also refer to my own work on *Rhodnius prolixus* (Maddrell 1969), but these tubules are unusual in that they ordinarily secrete considerably slower than do other Malpighian tubules and it is only under hormonal stimulation (Maddrell 1963) that they reach a high rate of secretion, which then is between ten and a hundred times faster than other Malpighian tubules. The ionic basic of this secretion (Maddrell 1969) is, perhaps not surprisingly, different from that to be described here for more typical tubules. None the less these tubules have a similar structure and face the same problems in coupling solute and water movements, so that they can usefully be considered under these headings.

One can somewhat arbitrarily divide the performance of Malpighian tubules into how they secrete ions and how they secrete water. I shall in fact argue that water movements are consequent upon ion movements so that I shall deal first with ion movements and then consider how water movements might be coupled with them.

Secretion of ions by Malpighian tubules

To study Malpighian tubules *in situ* is not easy and of course does not allow the composition of the bathing fluid to be changed easily. Ramsay was the first to address himself to this problem, and he devised a method for isolating Malpighian tubules from insects so that they could be studied under more controlled conditions (Ramsay 1954). Taken with the important discovery by Berridge (1966) that such tubules survive much better if they are supplied with an energy source (such as glucose) in the bathing medium, it is now possible to study Malpighian tubules

in isolation under controlled conditions in which they will survive and secrete for protracted periods. Figure 5 illustrates the experimental arrangements used in isolating a single tubule.

The fluid secreted by Malpighian tubules bathed in Ringer's solution has a composition which is summarized in table 1.

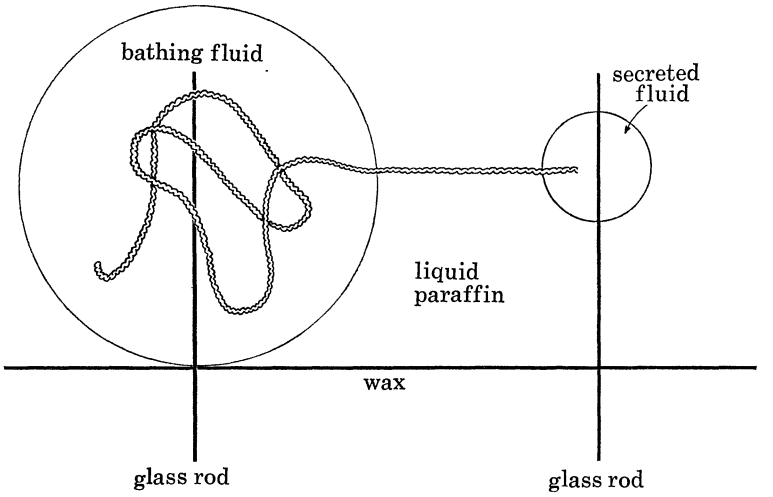

FIGURE 5. Side view of the experimental arrangements used to study the secretory behaviour of an isolated Malpighian tubule.

TABLE 1. THE COMPOSITION OF THE FLUID SECRETED BY MALPIGHIAN TUBULES BATHED IN A RINGER'S SOLUTION

constituent	concentration in the bathing fluid/mmol l^{-1}	concentration in the secreted fluid/mmol l^{-1}
K^+	10	125
Na^+	125	20
Ca^{2+}	2	< 1
Mg^{2+}	5	< 1
Cl^-	120	95
phosphate	20	50
glucose	20	8
osmotic concentration (as melting-point depression)	Δ = 0.520 °C	Δ = 0.525 °C

These figures have been compiled using results from more than one insect and so are to be taken as representative rather than exact. As indicated earlier the tubules of *Rhodnius* behave very differently from the other tubules in their handling of ions and so are not considered here. However, all tubules so far studied agree in that the osmotic concentration of the secreted fluid is very close to being iso-osmotic with the bathing fluid. Also all tubules secrete a fluid which is very rich in potassium ions. The tubules of *Calliphora* and *Carausius* secrete fluids containing elevated concentrations of phosphate ions; no figures are available for the concentration of this ion in the fluids secreted by *Calpodes* and *Tipula*. Chloride concentrations in the secreted fluid are usually somewhat depressed in comparison to the concentration in the bathing fluid. Calcium and magnesium ions cross the tubule wall with difficulty (Ramsay 1956).

How are these ion concentrations achieved?

Let us first consider the movements of potassium ions. There are several reasons for considering them first. Not only are these ions concentrated in the lumen at levels many times higher than is commonly found in the bathing fluid but measurements of the trans-wall potential show the lumen to be of the order of 30 mV positive with respect to the bathing solution. Movement of these ions into the lumen is therefore thermodynamically very much uphill. It therefore looks as if potassium is actively transported into the lumen. However, one must remember that in a Malpighian tubule with fluid flowing centripetally through the wall, there are created ideal conditions for frictional interactions between solvent and solute molecules—so-called solvent drag. This may account for some fraction of the potassium movement.

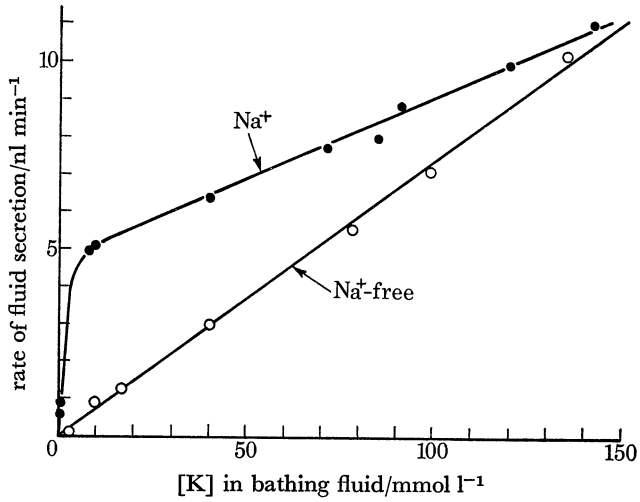

FIGURE 6. The rate of secretion of fluid by isolated Malpighian tubules of *Calliphora* as a function of the concentration of potassium ions in the bathing solution, either in the presence (●) or absence (○) of sodium ions. Redrawn from Berridge (1968).

The most important reason for considering potassium ions before others is that their secretion into the lumen is an essential feature of normal tubule function. The rate of fluid secretion depends very much on the concentration of potassium ions in the bathing fluid and in the absence of potassium ions, fluid secretion is slowed very considerably—usually to less than 10 % of its normal rate (figure 6). This relationship strongly suggests that water movements are secondary to potassium movements and not vice versa. It is very likely then that potassium transport is active.

From the few intracellular measurements of potential that have been made (by Ramsay 1953) it transpires that the cell interior is about 20 mV negative to the bathing solution and 50 mV negative to the lumen. The likeliest site for a potassium pump is therefore on the apical cell membrane.

The potential drop at the basal side of the cell would contribute to a passive entry of potassium into the cell. However, with respect to the trans-wall movement of potassium ions, there is the further point that the presence of small amounts of sodium ion very much accelerate potassium transport and so fluid movements when it (potassium) is present at only low concentrations, as is shown in figure 6. As a result it is clear that potassium ions can cross the cell at a high rate even when it is present in the bathing medium at very low concentrations, but that it requires the presence of sodium ions to be able to do so. This might have several explanations. It is unlikely that this is a direct effect on the apical membrane because there is some evidence that

the basal membrane is rather impermeable to sodium ions and yet the effect is produced by as little as 1 to 2 mmol l^{-1} of sodium ions (Pilcher 1970).

An effect of sodium ions on potassium movements across the basal side of the cell could be produced in several ways:

(1) By involvement in a sodium/potassium pump. However to do this, sodium ions would still have to cross the basal cell membrane and as we have seen this is unlikely to occur sufficiently fast to have a big effect. There is the further objection that secretion is unaffected by ouabain (Berridge 1968; Pilcher 1970) which one would expect to interfere with such a pump as it does in insect nerves (Treherne 1966).

(2) By an increase in the permeability to potassium.

(3) By the acceleration of a basal potassium pump not involving exchange with sodium ions.

There is as yet not enough evidence to allow a decision between these alternatives so that all that can be concluded is that potassium entry across the basal cell wall is sodium dependent.

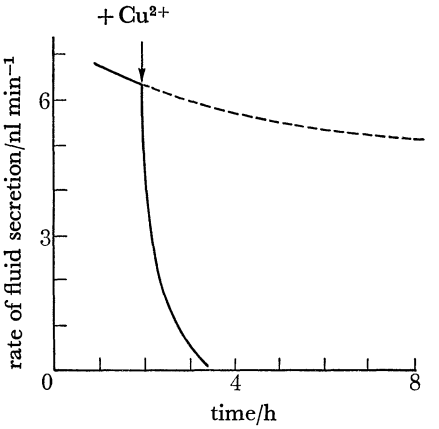

FIGURE 7. The effect of adding cupric ions (10^{-4} mol/l) to a phosphate-free chloride-based solution bathing isolated Malpighian tubules of *Calliphora*. Redrawn from Berridge (1969).

Sodium movements can be explained by supposing there to be a luminal sodium pump and rather restricted passive basal entry. Such an arrangement would account both for the ability of tubules slowly to secrete a sodium-rich fluid in the complete absence of potassium ions from the bathing fluid and for the low permeability of the basal wall to sodium ions.

Chloride movements may well be passive across both sides of the cell. On the luminal side the potential gradient is such as to encourage movement into the lumen. As far as the basal side is concerned it is known that adding copper ions, which are thought to prevent passive entry of anions, prevents the tubule from secreting chloride ions (figure 7).

Phosphate ions by contrast seem either to be transported actively into the lumen or to cross the tubule wall in some facilitated ways. First, phosphate ions support secretion very much faster than do anions of similar size (figure 8). As figure 8 shows, smaller anions in general allow faster secretion than do larger ones. This probably means that the cell membranes of the tubule cells are characterized by sites of low electric field strength (Diamond & Wright 1969). In such a case phosphate ions would be expected to allow only slow secretion. As is clear from figure 8, not only is this not true but phosphate ions allow secretion to go on faster than any other anion.

The second point is that although secretion in a chloride or nitrate-based Ringer's solution is stopped by copper ions, these ions, in *Calliphora* at least, have no effect on secretion in a

phosphate-based solution. Berridge (1969) whose work this is, suggests that phosphate may enter the cells by a process akin to phosphorylation on the outside of the basal membrane and dephosphorylation on the inside. This would explain the ability of arsenate ions to block phosphate transport in a manner similar to its action on phosphorylation in intermediary metabolism. Phosphate ions may well also cross the apical cell membrane in a facilitated way, particularly since it has been demonstrated that there exists there a non-specific alkaline

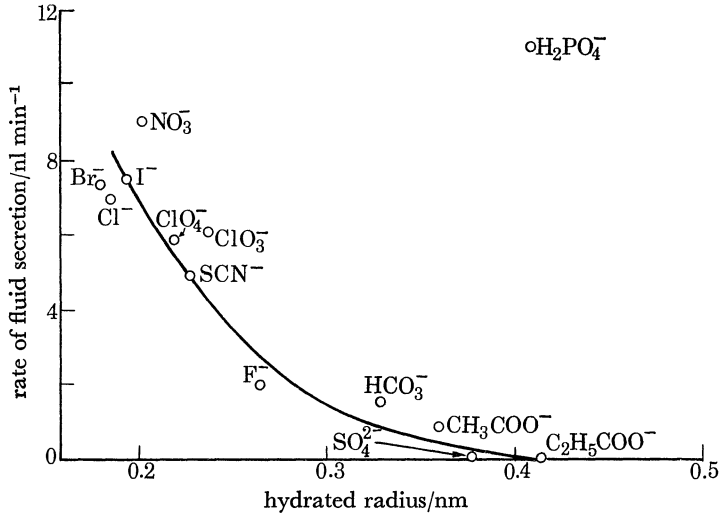

FIGURE 8. The ability of anions of different hydrated radii to support fluid secretion by isolated Malpighian tubules of *Calliphora*. Redrawn from Berridge (1969).

phosphatase (Berridge 1967). Whether or not phosphate movements are active, that is energy-dependent, is not clear. Phosphate ions are usually more concentrated in the lumen than in the bathing solution, but first, the lumen is positive with respect to the bathing solution, so that on this score some concentration might be achieved passively, and second, the luminal solution is usually slightly more alkaline than the bathing solution (Ramsay 1956) so that monovalent orthophosphate ions will be partly converted to the divalent form and make the concentration gradient for monovalent phosphate ions more favourable for entry into the lumen.

Finally, very little is known about tubular transport of calcium and magnesium other than that only very low concentrations of these ions appear in the secreted fluid (Ramsay 1956). As a pure speculation, one might suppose that their transport is passive.

These suggestions as to how trans-wall transport of the various ions might be achieved are assembled and illustrated in figure 9. *It must be strongly emphasized that while it is thought that the suggestions made in this figure are reasonable, they do not represent the only possible interpretations; it is clear that more evidence is required before more definite conclusions can be reached. The value of such tentative models lies in the experiments they suggest.*

THE COUPLING OF WATER MOVEMENTS TO SOLUTE MOVEMENTS

How might such ionic movements as are illustrated in figure 9 be related to water movements in the same direction? Several pieces of evidence suggest that water movements are consequent upon and determined by ion movements. In most Malpighian tubules the secreted fluid is

marginally hyper-osmotic† to the bathing fluid over a wide range of osmotic concentration (figure 10). Further than this, if one observes the rates of fluid secretion which result from change in the osmotic concentration of the bathing fluid, it is clear that there is a nearly exact inverse relationship between the rates of secretion and the osmotic concentration at which they were determined (figure 11). In other words, the rate of *solute* transport over a wide range of osmotic concentrations is nearly constant, and all that alters is the rate at which water crosses the tubule wall and this changes precisely to make the fluid secreted very slightly hyperosmotic. These facts plainly indicate that water movements are defined by and are secondary to movements of ions.

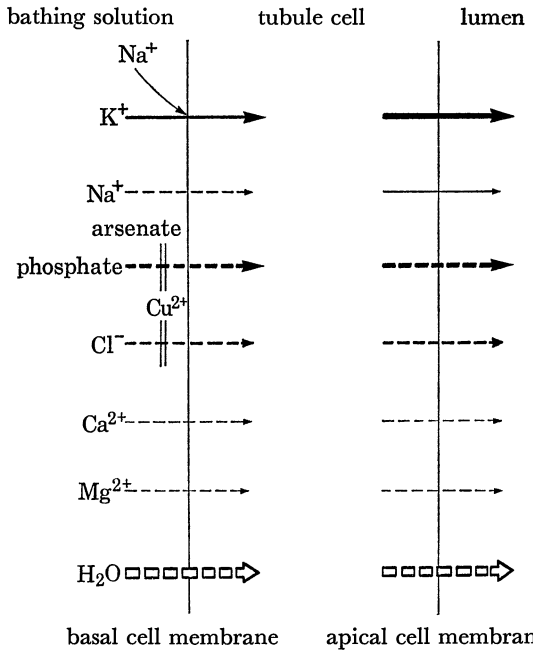

FIGURE 9. Hypothetical scheme for operation of Malpighian tubules. Active movements are indicated by unbroken lines and passive movements by broken lines.

How this result is achieved is at the moment not certain. However, when one looks at the ultrastructure of the tubule, very noticeable features are the elaborate infoldings of the basal and apical cell membranes—as long infoldings on the basal side and as apical microvilli on the luminal side (figures 12 and 13, plate 30). It is very tempting to suppose that these structures could constitute the long narrow channels which Diamond & Bossert suggest could well support the secretion of iso-osmotic fluids by the maintenance of standing osmotic gradients (Diamond & Bossert 1967). The trouble with this suggestion is that the infoldings and microvilli are not very long—of the order of 5 to 10 μm only. On theoretical grounds, the secretion of ions into such short channels would not be expected to produce iso-osmotic flow (see figure 5 in Diamond & Bossert 1967). However this expectation is based on calculations using average values of such parameters as transport rate and osmotic permeability. It is quite likely that,

† Tubules of *Carausius*, however, produce a fluid consistently hypo-osmotic to the bathing fluid (Ramsay 1954; Pilcher 1970). This may be due to the recently discovered fact that *Carausius* tubules have among a majority of typical tubule cells a small number of structurally different cells. There is as yet no evidence at all as to the role of these less common cells, but it is at least possible that they resorb ions without a concomitant amount of water. What is certain is that *Rhodnius* tubules, which consist only of one type of cell, secrete a very slightly hyperosmotic fluid.

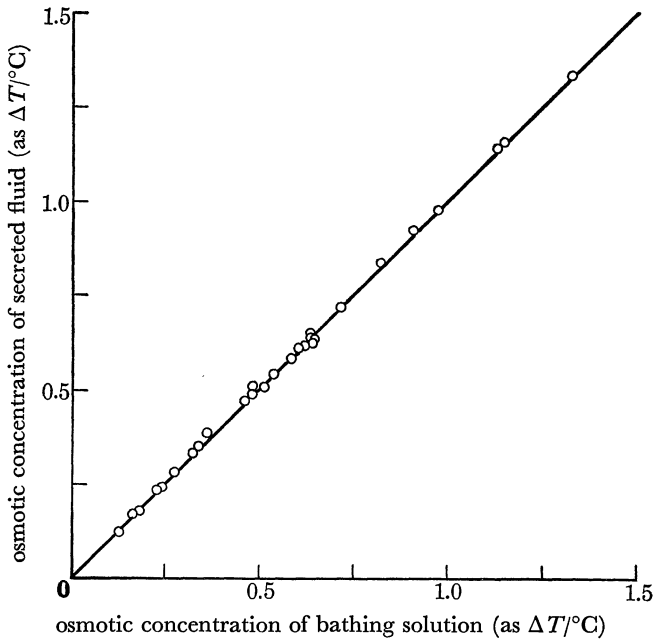

FIGURE 10. The osmotic concentration of the fluid secreted by isolated Malpighian tubules of *Rhodnius* as a function of the osmotic concentration of the bathing solution. Redrawn from Maddrell (1969).

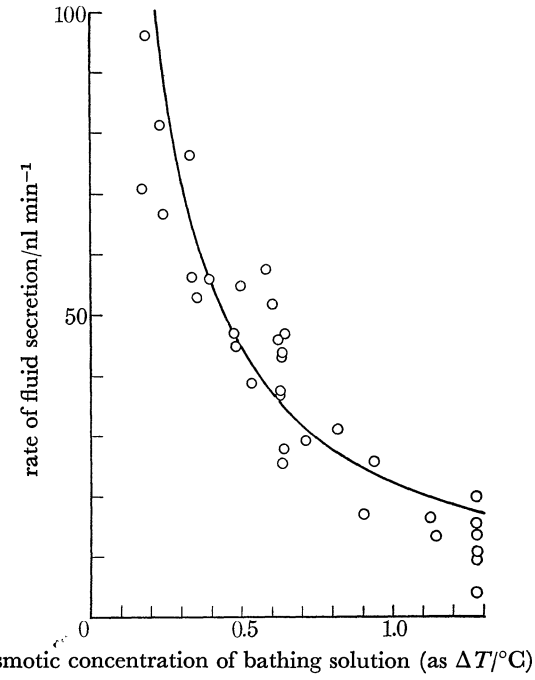

FIGURE 11. The rate of fluid secretion by isolated Malpighian tubules of *Rhodnius* as a function of the osmotic concentration of the bathing solution. The curve drawn is that of an exactly inverse relationship between the rate of secretion and the osmotic concentration of the bathing solution. Redrawn from Maddrell (1969).

as in the gall-bladder and proximal kidney tubule which also secrete fluid at high rates, the osmotic permeability of the walls of Malpighian tubules is in fact very high. One of the conclusions from Diamond & Bossert's paper was that osmotic permeability has a very great effect on the osmotic concentration of the fluid produced by a system of blindly ending channels. It

is a distinct possibility, therefore, that if, as may be the case, the osmotic permeability of Malpighian tubule cell walls is 20 to 40 times greater than average values of this parameter, this might well make large reductions in the osmotic concentration of fluid expected to be produced by such a system of short channels as in Malpighian tubules, perhaps so much as to lead to its being iso-osmotic. What is now needed is a recalculation using the different parameters for these shorter channels.

There is one further feature of the basal infoldings and apical microvilli which is worth mentioning and that is that they are arranged so as to produce a close-packed array of channels alternately opening to the cytoplasm and to the extracellular fluid (figure 14). 'Backward-facing' and 'forward-facing' channels (Diamond & Bossert 1967, 1968) are effectively arranged in parallel. As a result, ion movements from one side of the membrane to the other will tend not only to establish a hyperosmotic fluid on one side, but a hypo-osmotic fluid on the other because neither fluid can equilibrate as quickly with the cytoplasm or extracellular fluid as would otherwise be the case. This must make the osmotic gradient across the cell wall steeper, and thus the solvent/solute coupling more efficient. This factor will help to counteract the short length of the channels in Malpighian tubules.

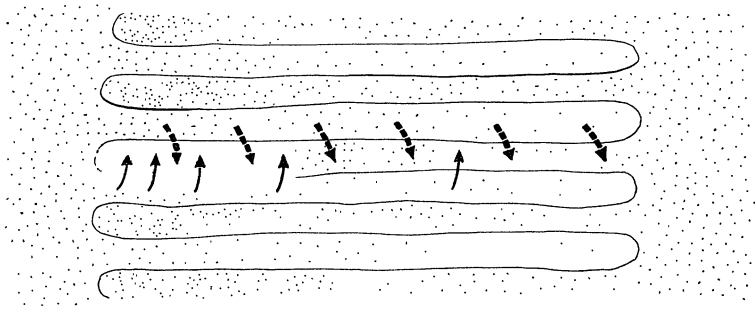

FIGURE 14. Hypothetical scheme for the coupling of ion and water movements at a folded cell membrane. Active ion movements are indicated by the smaller continuous arrows and passive water movements by the larger broken arrows. The density of stippling indicates the osmotic concentration of the fluids on either side of the cell membrane. This scheme might apply equally to the basal infoldings and apical microvilli; in the first case the left-hand side of the diagram would represent the extracellular fluid and in the second case the right-hand side would represent the extracellular fluid.

A further consequence of the alternative array of forward and backward channels is that, as indicated in figure 14, ion transport will effectively be focused at the side of the system towards which the backward channels face. This is because ion transport out of the backward channels will diminish the concentration of ions in these channels and the effect will increase with distance as the closed end is approached. Ion pumps on the cell membrane near the closed end of the channel will have fewer ions available to transport and so their effect will be relatively less. This effect will promote iso-osmotic flow from the forward-facing channels as more ions will enter their lumina near the closed ends than nearer the open ends; such an arrangement will ensure the production of a more nearly iso-osmotic secretion (Diamond & Bossert 1967).

It seems very reasonable to suppose that the basal infoldings and apical microvilli are the structural means whereby ion pumps sited on these cell membranes entrain water so as to produce an essentially iso-osmotic secretion.

Conclusion

The picture that emerges from this examination of Malpighian tubule function is as follows. The tubule walls are specialized to transport potassium ions across into the lumen. This may depend on an electrogenic potassium pump situated on the luminal membrane and a sodium-dependent entry across the basal membrane. As a result of the potential difference established, chloride ions move into the lumen passively. Phosphate ions may well also do so, but in this case their entry is by facilitated diffusion. In the absence of potassium ions, secretion is reduced to a very low level; that any secretion occurs at all is attributable to a slow active transport of sodium ions into the lumen.

These ion movements, because the cell membranes are much folded in such a way as to restrict diffusion away from the cell surfaces, result in the production of standing osmotic gradients. These gradients are small because the channels in which they occur are short. However, the cell membranes probably have a high osmotic permeability, so that water is able osmotically to equilibrate with the channel contents. As a result, the secretion produced by the Malpighian tubules is nearly iso-osmotic with the bathing medium.

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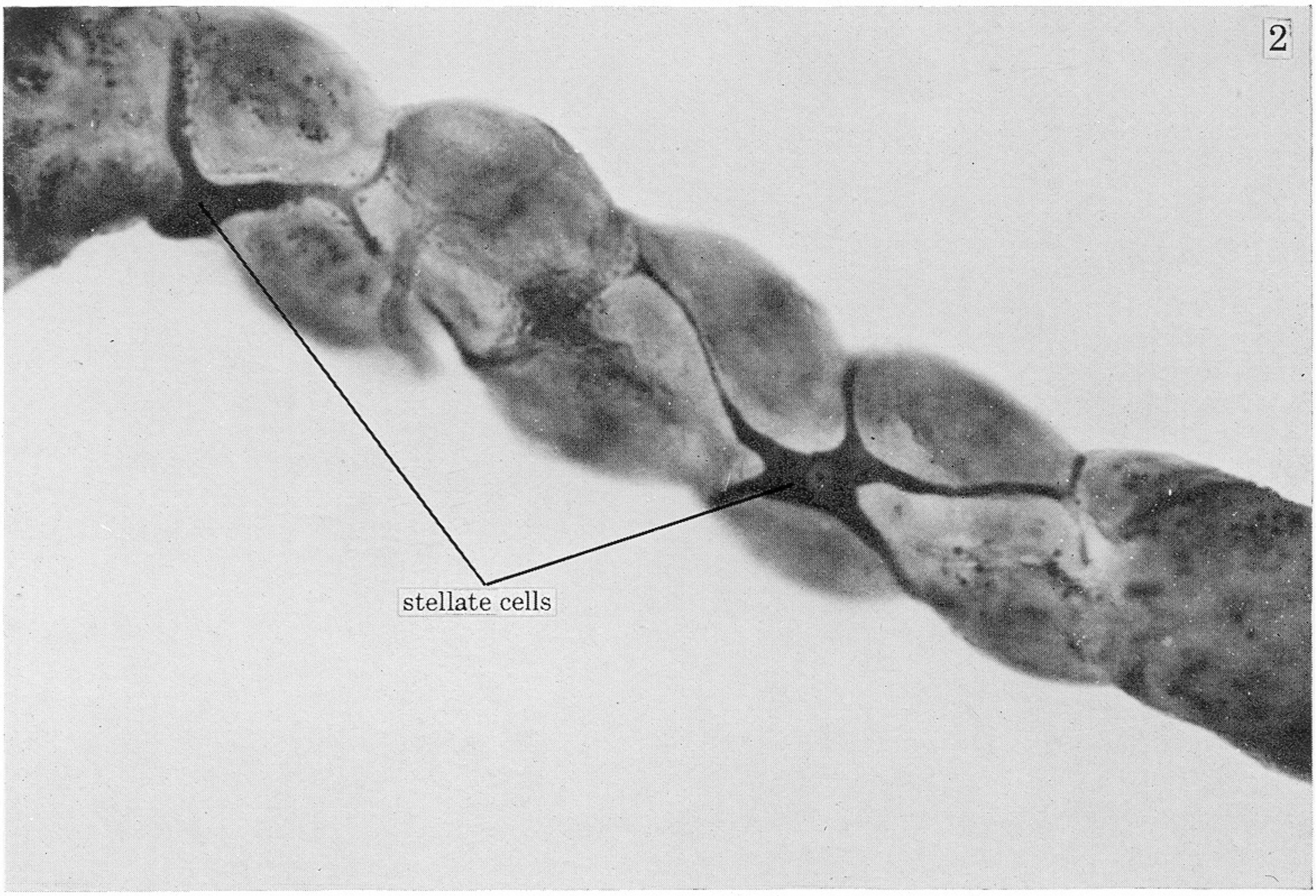
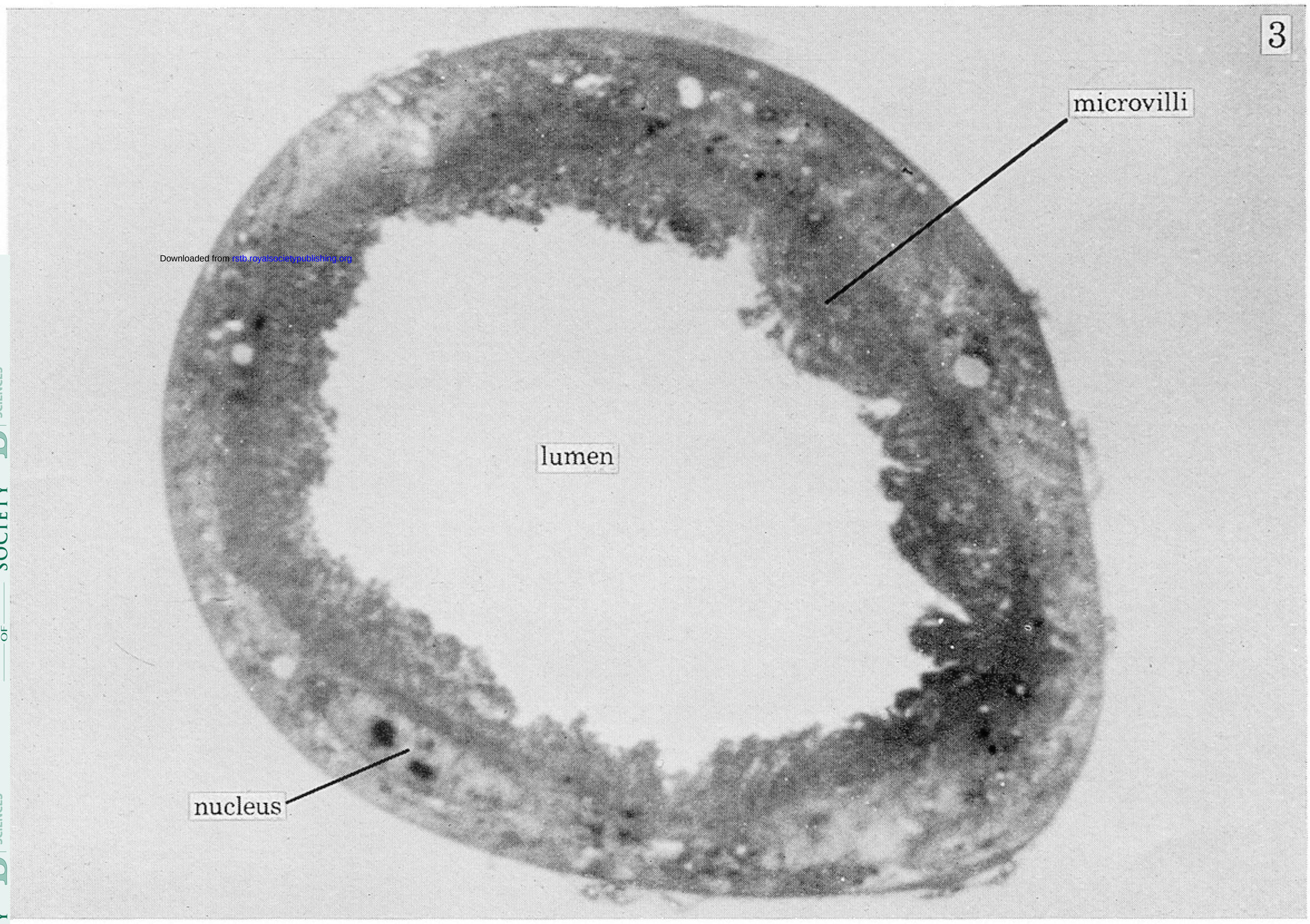




GURE 2. A length of Malpighian tubule from *Calliphora* fixed in glutaraldehyde and treated sequentially with lead nitrate and ammonium sulphide solutions. Note the intensely stained stellate cells. Photograph by courtesy of Dr R. C. Joyner. (Magn. × 300.)

GURE 3. A cross-section of a Malpighian tubule of *Rhodnius*. Note that the wall consists of a single layer of cells. (Magn. × 1000.)

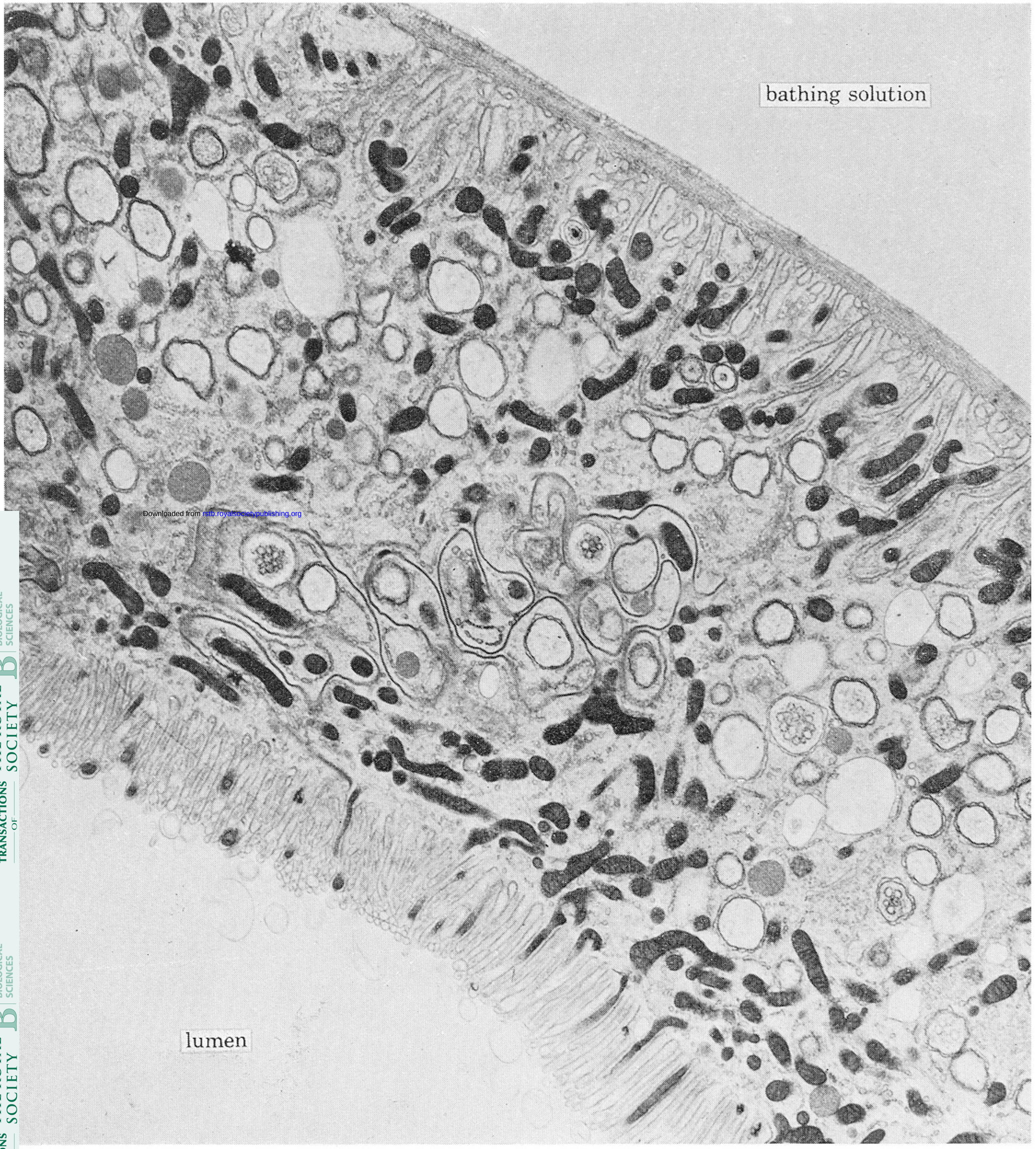
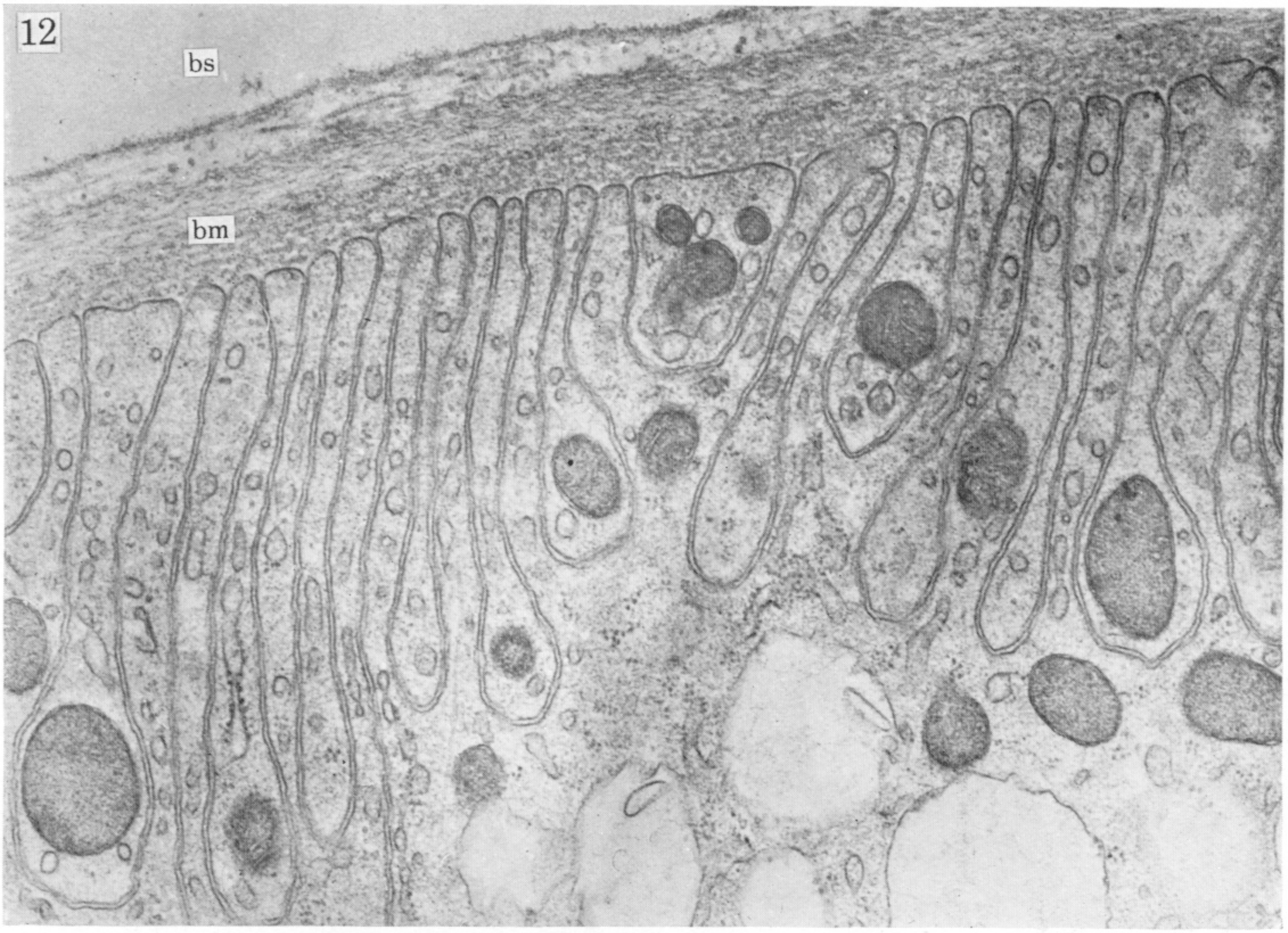


FIGURE 4. Electron micrograph of a section through the wall of the Malpighian tubule of *Periplaneta*. Photograph by courtesy of Dr J. L. Oschman. (Magn. × 6000.)



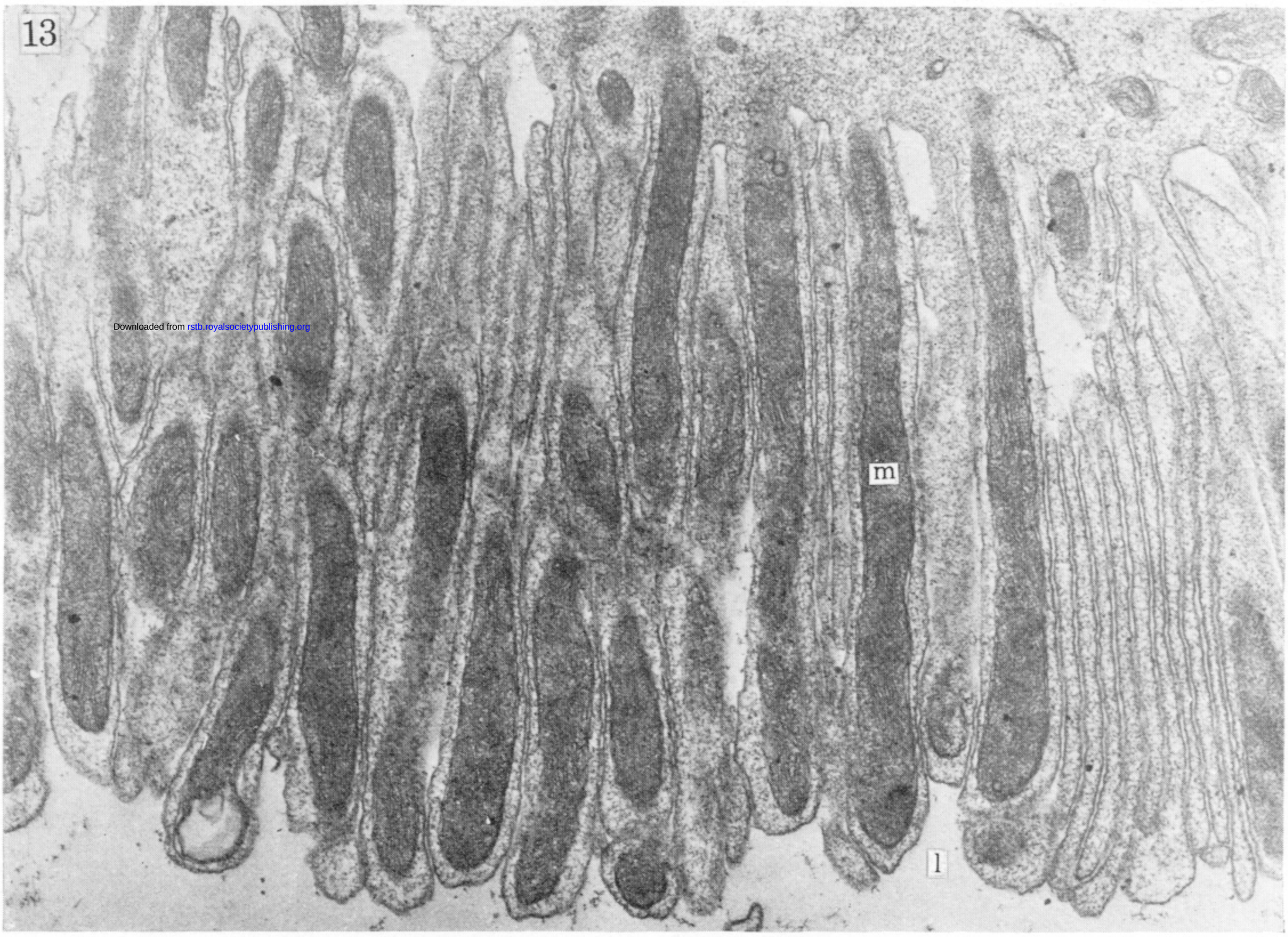


FIGURE 12. Electron micrograph of a section through the wall of the Malpighian tubule of *Calliphora* to show the infoldings of the basal cell membrane. The bathing solution (bs) is separated from the infoldings only by the basement membrane (bm) known to be very permeable. Photograph by courtesy of Dr J. L. Oschman. (Magn. × 28 000.)

FIGURE 13. Electron micrograph of a section through the wall of the Malpighian tubule of *Calliphora* to show the apical microvilli projecting into the lumen (l). Note that a proportion of the microvilli contain a mitochondrion (m). Photograph by courtesy of Dr J. L. Oschman. (Magn. × 28 000.)