

THE FUNCTIONAL DESIGN OF THE INSECT EXCRETORY SYSTEM

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(Received 22 April 1980)

SUMMARY

Insects have a slowly operating excretory system in which the passive rate of movement of haemolymph solutes into a slowly secreted primary excretory fluid is restricted by a reduction in the area available for passive transfer. They may have come to possess such an energy-saving system as a result of their evolution as small animals in osmotically and ionically stressful environments. Although the possession of a waxy cuticle is a major element in their ability to live in such environments, insects have a very high surface-area/volume ratio and this is likely to have conferred a selective advantage on individuals able to withstand unusually variable extracellular conditions. Among their major adaptations evolved to allow them to tolerate such conditions are the lack of a blood-borne respiratory pigment to be affected and the development of a system whereby their most sensitive tissues are protected by the regulatory activities of special covering epithelia. Because of these features it follows that there has been less evolutionary pressure for rapid excretory control of the haemolymph composition. With an excretory system that only slowly filters the haemolymph, less energy expenditure is involved in the production of the primary excretory fluid and in reabsorption of useful substances from it. In addition, insects are able to maintain in circulation high concentrations of substances such as amino acids, trehalose, and lipids. They can also eliminate excess fluid at very high rates with the loss of only trace amounts of haemolymph solutes. It is argued that terrestrial insects owe much of their success to their ability to recover virtually all the water from the slow flow of primary excretory fluid. The hindgut cells that are responsible for this recovery are aided by their cuticular lining which protects them from contact with the very high concentrations of potentially interfering compounds in the excretory material.

INTRODUCTION

The function of an animal's excretory system is to remove harmful or useless substances from the extracellular fluid. This is nearly always achieved by exposing the extracellular fluid to some form of filter through which it is driven by pressure or by secretion. The function of the filter is to hold back cells and large molecules such as proteins. The filtrate is then passed along a tube where useful substances are reabsorbed, and where harmful substances may be actively transported into it. The

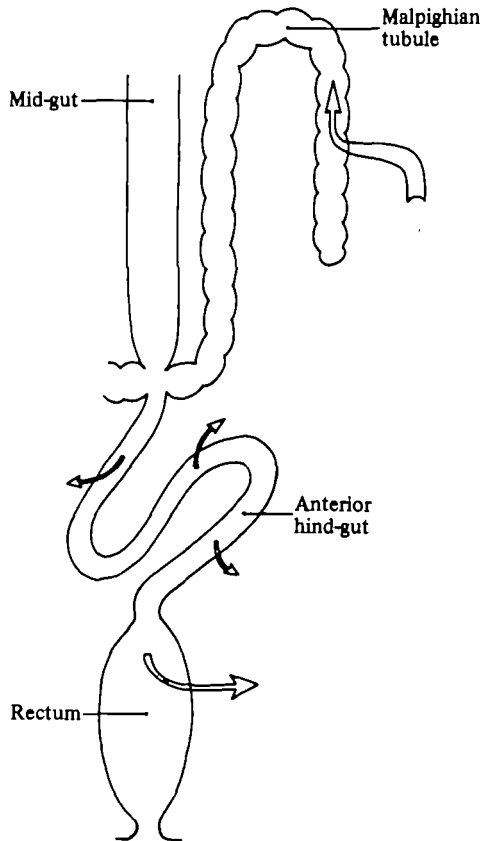


Fig. 1. Fluid movements in the insect excretory system. Fluid is transported into the Malpighian tubules where many haemolymph solutes can diffuse across the walls into the lumen. Much of the water is then recovered in the anterior hindgut and rectum together with other useful substances.

resulting fluid is then passed out of the body. Such a system is more energy consuming than might seem necessary. Because of the relatively unselective nature of the filtration process, large quantities of useful substances (water, ions, sugars, amino acids, etc.) have continually to be reabsorbed. It would seem that if the filter were more selective, savings of energy might be made and evolution would act to favour animals with such a selective filter. However, this would expose them to the danger of toxic material not able to pass through the filter. Although evolution has led to the development of active transport systems able to eliminate toxic materials that often appear in circulation, the animal would still be vulnerable to toxins not encountered before (Ramsay, 1958). Harmful proteins and/or cells held back by the filter are dealt with by separate mechanisms, e.g. antibodies and phagocytosis.

In accord with these ideas, the accepted view of the insect excretory system is that the haemolymph is 'filtered' through permeable Malpighian tubules, with reabsorption of useful substances from the filtrate, in the anterior hindgut and rectum (Fig. 1).

One of the main objects of the present paper is to draw attention to the fact, surprising at first sight, that the effective permeability of insect Malpighian tubules is

very considerably less than the analogous filtration sites in most other animals, both invertebrates and vertebrates, and to consider the implications of this for the operation of the insect's excretory system.

In the fluid filtered by pressure through the glomeruli of vertebrates, even substances as large as inulin occur at concentrations negligibly different from that in the blood on the other side of the filter (Smith, 1951). Similarly, Kirschner & Wagner (1965) showed that the filtration site of the crayfish antennal gland, the coelomosac, allows free penetration of compounds up to 50000 in molecular weight. They concluded that 'the evidence indicates that the permeability of the antennal gland exceeds that of the vertebrate glomerulus'. Braun, Kümmel & Mangos (1966), working on the protonephridium of the rotifer, *Asplanchna priodonta*, provided evidence that, as in the vertebrate nephron, [¹⁴C] inulin passes freely through the walls of the filtering terminal organs (the flame bulbs). Reabsorption of water and electrolytes in the succeeding protonephridial tubules produced a urine in which the inulin concentration always very significantly exceeded that of the body fluid. In sharp contrast, the fluid secreted by Malpighian tubules of insects contains even such small molecules as sugars and amino acids at concentrations markedly less than in the haemolymph (Ramsay, 1958; Maddrell & Gardiner, 1974). Rapidly secreting Malpighian tubules of *Rhodnius*, for instance, produce fluid containing glycine and inulin at concentrations which are only about 1% of those in the bathing fluid (Maddrell & Gardiner, 1974; Maddrell & Gardiner, 1980). It might be argued that these results are merely consequences of the fact that the force driving such solutes into the primary excretory fluid of insects is not pressure as in most other animals but only diffusion acting under the influence of the concentration gradient between the secreted fluid and the bathing extracellular fluid. This can be discounted, however, as the Malpighian tubules of the pill millipede, *Glomeris marginata*, which is not an insect, can rapidly secrete fluid extremely similar in composition to the bathing fluid (Farquharson, 1974*b*). Here, as with insect Malpighian tubules, the secreted fluid is not filtered by pressure into the lumina of the tubules and presumably most haemolymph solutes find their way into the lumen by diffusion. The secreted fluid nonetheless has virtually the same ionic composition as the bathing fluid even when this is varied over a wide range and even such large solutes as inulin and dextrans of molecular weight up to 16000 appear in the secreted fluid at concentrations only a little depressed from those of the bathing fluid (Farquharson, 1974*b*).

Another possible explanation of the under-representation of haemolymph solutes in the fluid secreted by insect Malpighian tubules could be that such solutes are actively reabsorbed from the lumen. Indeed, such reabsorption is known to occur – the Malpighian tubules of *Calliphora vomitoria* limit the loss of D-glucose from the haemolymph by a reabsorptive process which can be blocked by treatment with phlorizin (Knowles, 1975). However, neither L-glucose which is not actively transported, nor D-glucose in phlorizin-treated tubules appear in the luminal fluid at concentrations any higher than 50% of those occurring in the bathing fluid (Knowles, 1975).

To emphasize the low permeability of insect Malpighian tubules and, incidentally, display one of the advantages of such low permeability, it is worth recounting the results of some recent work on the blood sucking insect, *Rhodnius*. Amino acids

passively cross the walls of this insects's Malpighian tubules so slowly that when, after a blood meal, the tubules rapidly eliminate a volume of fluid equivalent to 5–10 times that of the haemolymph, there is a loss of only about 2% of the haemolymph content of amino acids (Maddrell & Gardiner, 1980). No reabsorption of amino acids is required during this rapid excretion of fluid.

Insect Malpighian tubules thus have only limited permeability to haemolymph solutes. Three questions now arise. (i) How can such low permeability be reconciled with the overriding need, emphasized above, for an excretory system to contain a relatively non-selective and permeable filtration site? (ii) How are the walls of Malpighian tubules structurally organized to provide a low effective permeability? (iii) What are the advantages to be derived from having an excretory system of only limited permeability to haemolymph solutes?

It will be the main object of the remainder of this paper to answer these questions.

OPERATION OF AN EXCRETORY SYSTEM OF LOW PERMEABILITY

To answer the first question, it has to be pointed out that although such small solutes as amino acids can only penetrate the walls of insect Malpighian tubules slowly, the tubules yet have measurable permeabilities to substances as large as inulin (Ramsay & Riegel, 1961; Maddrell & Gardiner, 1974). This is a clear indication that the apparently low overall permeability of the tubule wall might be due more to a limitation in area of the permeable sites than to any restriction at the sites of permeation. This is very important, as it means that toxic molecules, even those of moderate size, will still be removed passively, albeit slowly, from the insect's haemolymph. This in turn suggests that insects might in fact use the same basis for the operation of their excretory systems as do other animals, but that the rate of 'filtration' of the extracellular fluid is very much reduced. But how are insects able to survive with such a slowly operating system and gain the advantages described below, when other animals seem not to be able to?

A possible explanation is along the following lines. Insects as a group are characterized by their small bodily size, which gives them a high surface-area/volume ratio and makes their body fluids more liable to be affected by changes in the environment. In spite of this, insects seem only to be found in habitats which impose some form of osmotic and/or ionic stress (thus they appear on land, and in fresh, brackish, and hypersaline bodies of water, but not in such a stable environment as the sea). Of course, a crucial element in their ability to survive in such environments is the possession of a wax-covered integument which greatly restricts ion and water fluxes between their internal and external environments (Beament, 1961). It is often thought that the haemolymph of insects, because of its supposedly large volume, might also help in that it could act as a water store and as a buffer against changes in composition. While this could be true for some insects, such as caterpillars, which have particularly large volumes of haemolymph, recent measurements of haemolymph volume in other insects make this less likely. They show that many insects, particularly flying insects, have haemolymph volumes which are, if anything, *smaller* in relation to total body weight than, for example, the extracellular fluid of vertebrates. Table 1 shows the results of determinations of haemolymph volume in a series of insects. These results are to be compared with determinations of extracellular fluid volume in vertebrates.

Table 1. *Volumes of haemolymph in insects*

Order	Species	Stage	Haemolymph volume (as % of body weight)	References
Diptera	<i>Musca domestica</i>	Adult ♀	18.6	Bondaryk & Morrison (1966)
	<i>Musca domestica</i>	Adult ♂	20.6	Bondaryk & Morrison (1966)
	<i>Sarcophaga barbata</i>	Adult (30 h after emergence)	9	Cottrell (1962)
Lepidoptera	<i>Pieris brassicae</i>	Larva	34.5	Nicolson (1975)
	<i>Pieris brassicae</i>	Adult	7.2	Nicolson (1975)
Coleoptera	<i>Tenebrio molitor</i>	Larva	10	Jones (1957)
Hemiptera	<i>Rhodnius prolixus</i>	Young 5th instar	30.9	Maddrell & Gardiner (1976)
Orthoptera	<i>Carausius morosus</i>	Adult ♀	23	Nicolson <i>et al</i> (1974)
	<i>Carausius morosus</i>	Adult ♀	15	Ramsay (1955)
	<i>Periplaneta americana</i>	Larva	16.4	Edney (1968)
	<i>Periplaneta americana</i>	Adult ♂	17.8	Wall (1970)
	<i>Arenivaga</i> sp.	Larva	17.5	Edney (1968)
	<i>Schistocerca gregaria</i>	Adult ♂	13.7	Samaranayaka (1975)
	<i>Locusta migratoria</i>	5th instar	17.1	Loughton & Tobj (1969)
	<i>Locusta migratoria</i>	Adult	18.2	Loughton & Tobe (1969)

As far as blood volume goes there seems to be little variation between vertebrate species; determinations on eight different vertebrates gave closely similar results with an average value of 7% of the body weight (Sjöstrand, 1962). To this figure must be added the volume of the lymph, about 2% of the body weight, and the interstitial fluid, about 16% of the body weight (Pitts, 1968), giving a total extracellular fluid volume of about 25% of the body weight for vertebrates. (Direct measurements using a range of markers (Swan, Madisso & Pitts, 1954) gave extracellular fluid volumes of between 22 and 23% for dogs.) These figures suggest that, in general, insects are in no better a position than vertebrates to withstand water loss. Indeed, Edney (1977) has found that, even in desert-living insects, it is not so much tolerance of water loss but rather its prevention that is the important factor in their survival. They can withstand losses of 30% or so of their body weight, but this is not greatly different from the abilities of desert-living cattle, camels, and donkeys. These desert vertebrates are all well able to survive water losses of 20% of their body weight (Siebert & MacFarlane, 1975), and in extreme cases can tolerate losses up to 27% of body weight in the camel (Schmidt-Nielsen, 1964) and up to 30% in the donkey (Maloij, 1970).

In general, insects are very much smaller than vertebrates living in the same environment. For example, the smallest terrestrial adult insects, species of *Thrips*, weigh only a few micrograms (C. P. Ellington, personal communication) as against the smallest terrestrial vertebrates such as the Etruscan shrew which, as an adult, weighs about 2 g (Miller, 1964). At the other end of the scale, the largest terrestrial insects weigh less than 100 g, while a bull elephant can weigh more than 10 000 000 g. These differences imply a surface-area/volume ratio for terrestrial insects which is close to 100 times larger than for terrestrial vertebrates. So although insects are well protected by their wax-covered integument, they are so very much smaller than vertebrates and have a so much higher surface-area/volume ratio that it is hard to avoid the conclusion that insect tissues must have evolved a greater tolerance to varied conditions in the haemolymph. Perhaps this may explain the relative ease with which *in vitro* preparations of many insect tissues can be made. However, some insect tissues are less tolerant. In this respect, it is noteworthy that insects, alone among the

invertebrates, have developed a series of epithelia, characterized by the presence of simple tight junctions, to protect such potentially sensitive tissues as the central nervous system, the eyes, and the testis (Lane & Skaer, 1980). The regulatory properties of one of these epithelia, the perineurium, which envelops much of the nervous system, has been particularly well worked out (Treherne, 1974; Treherne & Schofield, 1978). Oxygen supplies for these tissues reaches them directly by the tracheal system so that their protective epithelia are no liability in this respect. In addition, as the haemolymph is not responsible for circulating oxygen, it contains no respiratory pigment which could be adversely affected by changes in the haemolymph. (The single exception to this is the occurrence of haemoglobin in the haemolymph of the aquatic larvae of certain Chironomidae. However, this pigment only releases oxygen under conditions of extreme oxygen paucity (Wigglesworth, 1974).) It may well, therefore, be possible for insects to survive haemolymph conditions which vary in composition within wider limits than would be tolerable in other animals.

If, then, insects can tolerate changes in their internal milieu, one can argue that the excretory system need not operate so rapidly as it otherwise would have to, to control the composition of the haemolymph. Perhaps insect tissues can tolerate or are protected from potentially deleterious changes in their environment for long enough to allow the excretory system to correct the situation at a relatively leisurely rate. It may not matter so much how quickly novel toxic materials are removed, so long as they are eventually removed. A system which provides for slow passive removal of substances, even those as large as inulin, would, therefore, suit insects well, and as we have seen, that is exactly what they have. To back up this passive system, evolution has led to the appearance in Malpighian tubules of active transport mechanisms that can rapidly eliminate a very wide range of substances that insects repeatedly need to excrete (Maddrell, 1977). Peculiarly, these mechanisms appear not to involve nitrogenous wastes, usually thought to be a very important class of substances for excretory systems to eliminate. Although insects excrete many different nitrogenous compounds, the most widely encountered is uric acid (Bursell, 1967). Surprisingly, the speed at which uric acid is eliminated from the body seems not to have been the subject of strong selection pressure in the evolution of insects. Indeed, deposits of uric acid occur in the fat bodies of parasitic insects (Corbet & Rotherham, 1965), and in the fat bodies of normally hydrated and fed cockroaches, where it is even suggested that they act as stores of nitrogen (Tucker, 1977). Uric acid also appears in great quantities in the accessory glands of male cockroaches (Roth & Dateo, 1965) and it is actually used as a pigment in the bodies and wings of other insects (Berridge, 1965; Harmsen, 1966). Even in *Rhodnius*, where the diet is very rich in protein, the indications are that uric acid crosses the wall of the upper tubule entirely passively (Maddrell & Gardiner, 1974). These findings all support the view that the slow elimination of nitrogenous waste in insects is readily tolerated and that selection pressure for an excretory system that operates more rapidly does not arise from this source.

THE STRUCTURE OF INSECT MALPIGHIAN TUBULES

The insect excretory system operates as if it has permeable areas limited in their extent. Does the structure of insect Malpighian tubules bear this out? Fig. 2 shows that it does. Compared with the situation in, say, the podocytes of the Bowman's

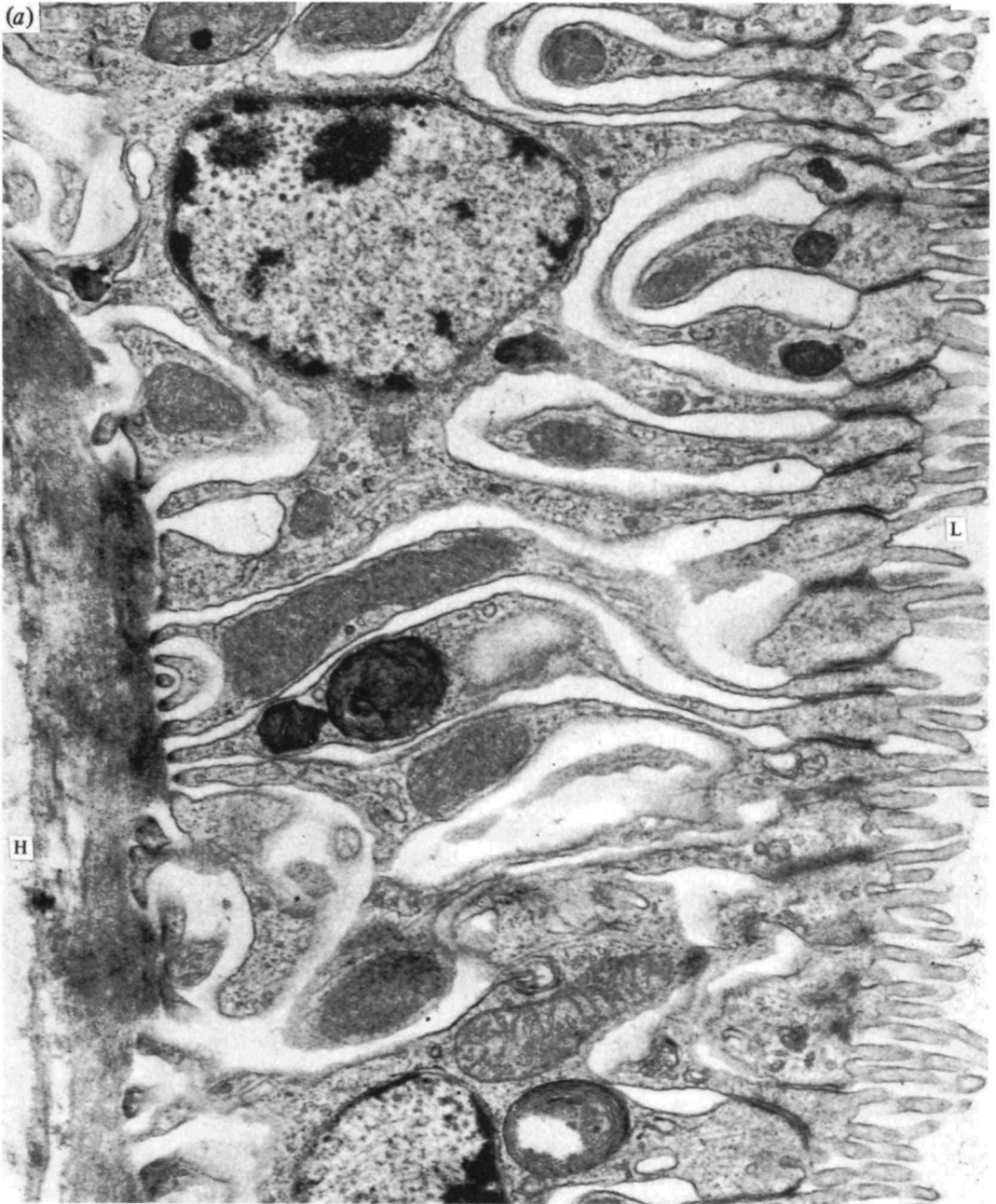
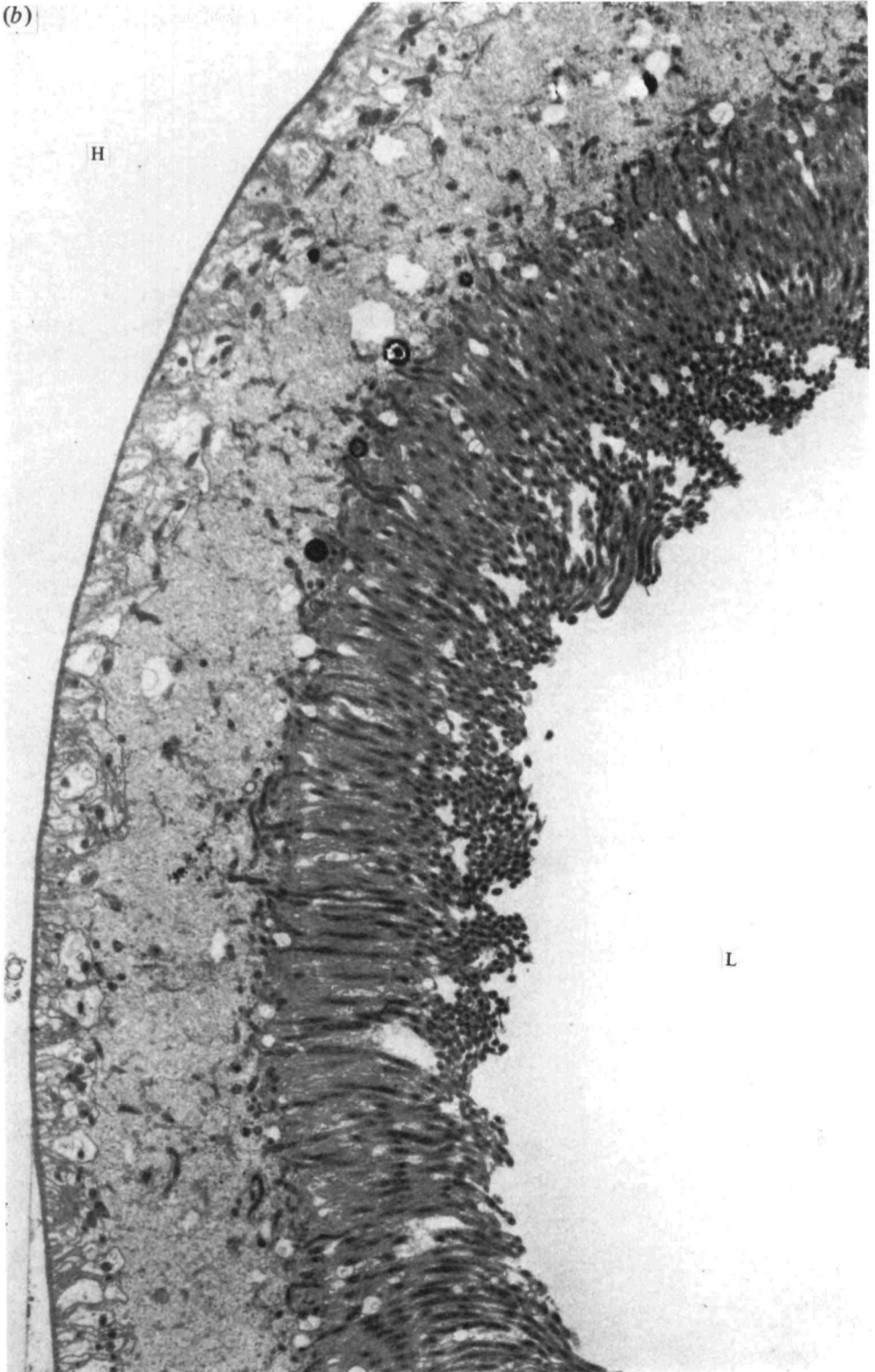


Fig. 2. Electron micrographs of sections through the walls of the Malpighian tubules of (a) a millipede, *Glomeris marginata* ($\times 20000$; micrograph courtesy of Dr J. A. Riegel), and (b) an insect, *Rhodnius prolixus* ($\times 3000$; micrograph courtesy of Dr B. S. Hill). In the wall of the tubule of *Rhodnius*, intercellular clefts that could act as pathways for materials to cross the wall passively are not often encountered even in low-power micrographs such as that shown. In contrast, the wall of the tubule of *Glomeris* shows many such clefts, even in a higher-power micrograph of a section taken from a part of the cell near the nucleus.



capsule of the vertebrate kidney or the podocytes of the antennal glands of Crustacea (or, indeed, the filtration sites of the excretory systems in virtually all other animals studied – see Kümmel (1973) for an excellent account of such filtration structures), the intercellular clefts of insect Malpighian tubules occupy very much less of the frontal area of the epithelium. In *Rhodnius*, for example, it has been calculated that the area of the clefts occupies only 0.03% of the epithelial surface (Maddrell, 1980a). The contrast with the multiply perforated wall of the very permeable Malpighian tubules of the millipede, *Glomeris marginata* (Fig. 2 and see Farquharson, 1974b) could scarcely be more marked. It is relevant to add that the overall dimensions of the tubules are rather similar. *Rhodnius* has four Malpighian tubules, each with a fluid secreting region 30 mm long and about 100 μm in diameter, while *Glomeris* has only two tubules but each has a fluid secreting portion about 60 mm long and around 80 μm in diameter (Farquharson, 1974a).

The restricted area of the intercellular clefts in insect Malpighian tubules raises another question. What is the function of the extensive area of the cells of the tubules' walls? Could not a low rate of passive filtration of the haemolymph simply be achieved by much less extensive tubules with intercellular clefts less widely separated? Part of the explanation could be that insects may need occasionally to be able to excrete large quantities of fluid or to filter their haemolymph much more rapidly, and this requires an accelerated rate of fluid secretion provided in turn by many tubule cells (Maddrell, 1980a).

Blood-sucking insects, sap-feeding insects, and insects that live in fresh waters are obvious cases where rapid fluid excretion is important. The need for the tubules of other insects to cater for varying rates of fluid production has a more subtle explanation. As will be argued below, an advantage of slow filtration of the haemolymph is that the energy-consuming process of reabsorption can then also be slow. The price of this, as we have seen, is that toxins are more slowly removed. Now, if passive filtration of haemolymph solutes is by diffusion, its rate will depend on the difference in concentration of these solutes between the haemolymph and lumen. If fluid secretion is slow, the luminal concentration will be high and solutes will enter the tubule only slowly; reabsorption need also only be slow. If, however, fluid secretion is accelerated, this will continuously carry away solutes diffusing across the walls and so lower the concentration of these solutes in the lumen. They will thus enter the tubule much faster. A similar argument applies to substances which are actively transported into the tubule lumen. These will tend to diffuse back out of the tubule through the permeable intercellular clefts at a rate dependent on their concentration in the lumen. So the rate at which they can be removed from the haemolymph will also depend on the rate of fluid secretion, as this strongly affects their concentration in the lumen. Fig. 3 shows, for one particularly well studied case, the Malpighian tubules of *Calliphora*, how the net rates of removal of substances from the haemolymph are affected by changes in the rate of fluid secretion. Clearly, the rate of fluid secretion can have very dramatic effects on the rates at which substances are removed from the haemolymph, regardless of whether they cross the tubule walls actively or passively. The shapes of the curves show that the rate of elimination of substances from the haemolymph is more affected by changes in rate of fluid secretion at low rates than at higher rates. So, if the insect is to enjoy the advantages of being able to control the

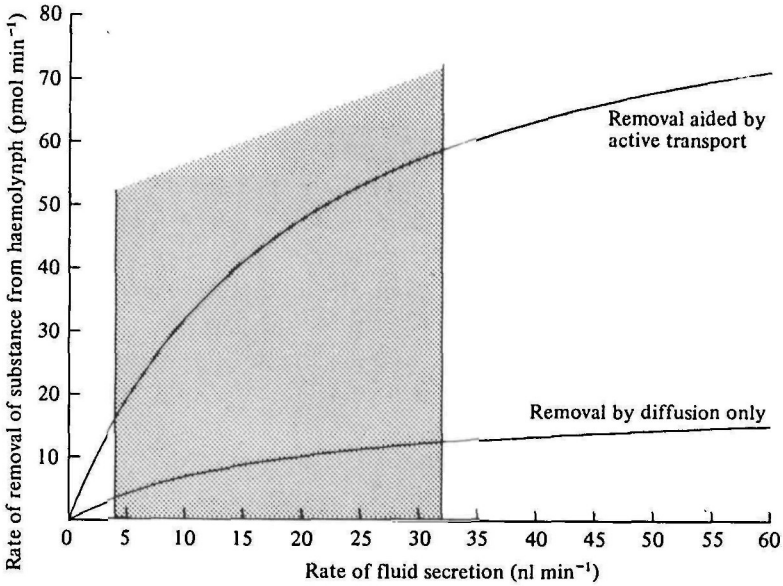


Fig. 3. Calculated rates at which a substance, present at 1 mM in the haemolymph, is removed from circulation by a Malpighian tubule secreting fluid at different rates. The calculations are based on the properties of Malpighian tubules from *Calliphora* (for details see Berridge, 1965; Maddrell & Gardiner, 1974; Schwartz & Reynolds, 1979). For the lower line it was assumed that loss was by passive diffusion across the tubule wall with the wall having a permeability to the substance of $10^{-5} \text{ cm s}^{-1}$. For the upper line it was further assumed that the substance was subjected to active transport towards the lumen at a rate of 75 pmol min^{-1} . For an explanation of the mathematical basis of the calculation see Maddrell & Gardiner (1980). The rates of fluid secretion normally shown by unstimulated and by maximally hormonally stimulated Malpighian tubules are shown by the vertical lines so that the stippled area between them encompasses the normal range of rates that the tubules can achieve.

rate of elimination of these substances, it would make more sense for it to control the rate of fluid secretion over a range where this has large effects on the rate of removal of substances from the haemolymph. Fig. 3 shows that for *Calliphora* this expectation is realized; the range of rates of secretion that can be achieved does not extend up to values where the rate of solute removal is becoming insensitive to changes in rate of fluid secretion.

If the above arguments have any force, then an ability to control the rate of fluid secretion by their Malpighian tubules should be widespread among insects. Table 2 lists those insects for which there is evidence that they can alter the rate of secretion by their tubules; the list covers virtually all those insects where fluid secretion by their Malpighian tubules has been studied. Control of fluid secretion by Malpighian tubules is known in most cases to be effected by hormones (these hormones are usually called diuretic hormones, but since tubule secretion can be accelerated greatly without there necessarily being a loss of water from the insect, the water often being reabsorbed in the hindgut, the term diuretic is not always appropriate). An excellent example of an insect deriving an advantage from accelerating fluid secretion by its Malpighian tubules is the locust. Locusts in flight are, of course, extremely active metabolically, and since metabolism is likely to produce useless, if not harmful by-products, there will, during flight, be a need to filter the haemolymph more

Table 2. *Insects for which there is evidence that the rate of fluid transport by their Malpighian tubules is regulated by hormones*

Order	Species	References
Hymenoptera	<i>Apis mellifera</i>	Altmann (1956)
Diptera	<i>Calliphora vomitoria</i>	Knowles (1976)
	<i>Calliphora erythrocephala</i>	Schwartz & Reynolds (1979)
	<i>Glossina morsitans</i>	Gee (1975)
	<i>Aedes taeniorhynchus</i>	Maddrell & Phillips (1978)
	<i>Anopheles freeborni</i>	Nijhout & Carrow (1978)
Lepidoptera	<i>Pieris brassicae</i>	Nicolson (1976)
	<i>Calpodex ethlius</i>	Ryerse (1978)
	<i>Danaus plexippus</i>	Dores, Dallman & Nerman (1979)
Coleoptera	<i>Anisotarsus cupripennis</i>	Nuñez (1956)
Hemiptera	<i>Rhodnius prolixus</i>	Maddrell (1962)
	<i>Triatoma infestans</i>	Maddrell (unpublished results)
	<i>Triatoma phyllosoma</i>	Maddrell (unpublished results)
	<i>Dipetalogaster maxima</i>	Maddrell (unpublished results)
	<i>Dysdercus fasciatus</i>	Berridge (1966)
Orthoptera	<i>Periplaneta americana</i>	Mills (1967)
	<i>Locusta migratoria</i>	Cazal & Girardie (1968)
	<i>Schistocerca gregaria</i>	Mordue (1969)
	<i>Carausius morosus</i>	Pilcher (1970)
	<i>Acheta domestica</i>	Geldiay & Edwards (1976)

rapidly. Appropriately, flying locusts release hormone(s) to accelerate both fluid secretion by their Malpighian tubules and fluid reabsorption in the hindgut (Mordue, 1969).

If we accept that insects need to be able to secrete fluid at a range of rates, then it now becomes clear why the Malpighian tubules have to have a considerable area of fluid secreting epithelium, and this the tubule cells provide.

ADVANTAGES OF AN EXCRETORY SYSTEM WITH RESTRICTED PERMEABLE SITES FOR FILTRATION

What advantages do insects derive from their somewhat unusual excretory system? As will be clear from what has already been said, an obvious advantage of a system which operates slowly is that reabsorption of useful substances from the filtered fluid need only be slow. Presumably the fluid flow in the system need also only be slow, at least when no hormonal stimulation of secretion goes on. It is possible to check this point by reference to the known rates of Malpighian tubule fluid production. Table 3 compares the rates at which the extracellular fluid is passed through the excretory system of insects with similar figures for vertebrates. Clearly, most vertebrates filter their extracellular fluid 10–20 times more rapidly than do most insects. Taking into account the fact that, in insects, many haemolymph solutes of even quite small molecular size only appear in the filtered fluid at concentrations in the range 10–50% of those in the haemolymph, it is clear that reabsorption of such solutes need only occur in insects at about 1–2% of the rate required in many vertebrates.

The figures in Table 3 for a snake and a frog suggest that filtration of extracellular fluid may be slower in ectothermic animals, perhaps reflecting their lower rates of metabolism. If so, then the slow rate at which insects pass fluid through their Malpighian tubules might be attributable more to the fact they are ectotherms than to

Table 3. *Times taken for volumes of fluid equivalent to the total extracellular fluid (a) to be filtered through the glomeruli of some higher vertebrates or (b) to be passed through the Malpighian tubules of insects*

(a) Vertebrates*	Times (min)	References
Frog	285	Osbaldiston (1974)
Snake	525	Osbaldiston (1974)
Chicken	138	} Renkin & Gilmore (1973)
Mouse	38	
Sand Rat	19	
Rat	42	
Rabbit	83	
Cat	127	
Dog	57	
Pig	178	
Goat	185	
Sheep	158	
Camel	218	Siebert & MacFarlane (1975)
Ox	82	Siebert & MacFarlane (1975)
Horse	322	Renkin & Gilmore (1973)
Man	135	Renkin & Gilmore (1973)
(b) Insects		
<i>Calliphora erythrocephala</i>	625	Berridge (1968)
<i>Dysdercus fasciatus</i>	1 500	Berridge (1966)
<i>Schistocerca gregaria</i>	1 700	Phillips (1964)
<i>Carausius morosus</i>	2 750	Nicolson, <i>et al.</i> (1974)
<i>Rhodnius prolixus</i>	10 000	Ramsay (1954)
	20 (during diuresis)	Maddrell (1964)
<i>Aedes taeniorhynchus</i>	2 000	Maddrell & Gardiner (1976)
		Maddrell & Phillips (1978)

*The extracellular fluid of these vertebrates is assumed to be 25% of the body weight (Pitts, 1968).

any difference special to insects. Very likely this is part of the explanation but there is another factor to be taken into account. The insects used as examples in Table 3 are all considerably smaller than frogs and snakes and so, of course, would be expected (Schmidt-Nielsen, 1979) to have higher specific metabolic rates (metabolic rate per unit of body weight). This would suggest that they should need to filter the haemolymph faster than do larger ectotherms. The few figures so far available (Table 3) indicate that, if anything, the opposite is the case and that insects filter the haemolymph considerably more slowly than would be predicted.

Direct evidence for the slow passive removal of substances from insect haemolymph comes from experiments on the rate of elimination of inulin injected into locusts. Although locust Malpighian tubules are permeable to inulin, its elimination is at a rate with a half-time of about 8 days (Maddrell & Gardiner, 1974). For comparison, it can be calculated from the known rate of glomerular filtration in man (Pitts, 1968) that inulin would be eliminated from the extracellular fluid with a half-time of about 100 min.

A major advantage logically follows from the fact that substances are only slowly removed passively from insect haemolymph. It becomes feasible for insects to maintain in the haemolymph high concentrations of useful substances of low molecular weight. Flying insects can, as fuels for flight, have 100 mM of the disaccharide trehalose and/or diglyceride lipid in the haemolymph (Weis-Fogh, 1967). Many

Insects in the orders Lepidoptera, Hymenoptera, and Coleoptera contain 100–200 mM of amino acids in the haemolymph (Maddrell, 1971). Finally, hormones that are released into circulation will only slowly be removed by the excretory system and so can more easily function for useful lengths of time. A potential disadvantage is that insecticidal substances once they reach the haemolymph have longer to produce damaging effects than if they were more rapidly eliminated (Maddrell, 1980b).

Insects have evolved a series of active transport mechanisms that move substances into or out of the tubule lumen. This would not be possible in a more permeable tubule as the resulting gradients would rapidly be cancelled by diffusion. Examples of these systems include active transport into the lumen of phosphate, magnesium, and sulphate ions, sulphonates, acylamides, alkaloids, and glycosides (Maddrell, 1977) and the active reabsorption of glucose (Knowles, 1975). Nearly all Malpighian tubules secrete fluid much richer in potassium than is the haemolymph. This has to be reabsorbed in some insects but in herbivorous insects that face the problem of excess potassium in the diet, the use of potassium transport as the driving force for fluid formation in the Malpighian tubules allows them both to rid themselves of potassium and to produce the essential primary excretory fluid with a single transport process. With a more permeable tubule, potassium excretion would require additional potassium transport in a less permeable part of the excretory system.

We have seen that acceleration of fluid secretion in the Malpighian tubules accelerates the 'filtering' of the haemolymph in that haemolymph solutes pass more rapidly into the tubule lumen (Fig. 3). This is closely similar to the effect of an increase in glomerular filtration rate in vertebrates. However, if the rate of fluid secretion is increased still more, the relation breaks down and the rate of passage of solutes into the lumen is scarcely any further affected (Fig. 3). Essentially this is because the acceleration of solute movement from the haemolymph follows from increasing dilution of solute in the tubule lumen at higher rates of fluid secretion; diffusion back into the haemolymph falls to low levels. Once the concentration of a solute in the lumen reaches a very low level, however, further increases in the rate of fluid secretion have virtually no more effect on solute entry. This allows the evolution in insects that need to eliminate fluid from the haemolymph of a system whereby the tubules secrete fluid at very high rates. Since the excess fluid is then quickly removed, and contains only traces of haemolymph solutes, no reabsorption of the solutes is needed. Such an adaptation is, of course, particularly suited to blood-sucking insects such as *Rhodnius*, which take very large blood meals that necessitate the elimination of much excess fluid. These ideas may well be important in explaining why many blood-sucking insects can excrete fluid at what seem unnecessarily high rates; *Rhodnius* after feeding can eliminate fluid equivalent in volume to its total haemolymph in as short a time as 30 min.

INSECTS IN THE TERRESTRIAL ENVIRONMENT

As pointed out earlier, insects seem only to be found in habitats characterized by osmotic and/or ionic stress. In particular, they are most numerous in the terrestrial environment. Here the problem is one of resisting desiccation and it is worth considering what role the insect excretory system plays in this. Obviously, its main

contribution must be that of limiting water loss. It seems that there are two distinctive features of insects which are important in this.

The first is that the flow of water through the excretory system is comparatively slow. Selection pressure for effective water reabsorption, strong in all terrestrial animals, is likely to be still more severe in insects because of their small size. It is scarcely surprising that many terrestrial insects have evolved with highly developed abilities for reabsorbing water from the primary excretory fluid. Given the low rate of production of this fluid, water reabsorption is very effective, recovering, in many cases, virtually all the water from the excretory material. So effective are these water recovery systems that in *Tenebrio* (Ramsay, 1971) and in *Thermobia* (Noble-Nesbitt, 1970), for example, water vapour can be taken up from moist air drawn into the hindgut.

A second characteristic of insects may have been of crucial importance in the evolution of such effective water reabsorption. This is that the hindgut, which is responsible for water recovery, is lined with cuticle. The significance of the restricted permeability of this cuticular lining was first recognized by Phillips & Dockrill (1968). They pointed out that the very effective water reabsorption carried out by the rectal cells might well only be possible because they are protected by the cuticle from mounting concentrations of potentially interfering compounds in the luminal fluid. As would be expected, then, evolution has favoured the hindgut as the site of mechanisms concerned with the final stages of water recovery in insects. The other very successful terrestrial animals, the vertebrates, do not have the advantage of such a cuticular intima and could not have developed such a system. Indeed, water recovery in vertebrates is achieved by epithelia that are in direct contact with the urine. It is possible that this difference was one of the most important factors in preventing the evolution of terrestrial vertebrates small enough to compete effectively with insects for the niches available for animals in this size range.

SUMMARIZING REMARKS

Insects have a slowly operating excretory system in which the rate of passive movement of haemolymph solutes into a slowly secreted primary excretory fluid is restricted by a reduction in the area available for passive transfer. They may have come to possess such an energy-saving system as a result of their evolution as small animals in osmotically and ionically stressful environments. Although the possession of a waxy cuticle is a major element in their ability to live in such environments, insects have a very high surface-area/volume ratio and this is likely to have conferred a selective advantage on individuals able to withstand unusually variable extracellular conditions. Among their major adaptations evolved to allow them to tolerate such conditions are the lack of a blood-borne respiratory pigment to be affected and the development of a system whereby their most sensitive tissues are protected by the regulatory activities of special covering epithelia. Because of these features it follows that there has been less evolutionary pressure for rapid excretory control of the haemolymph composition. With an excretory system that only slowly filters the haemolymph, less energy expenditure is involved in the production of the primary excretory fluid and in reabsorption of useful substances from it. In addition, insects

are able to maintain in circulation high concentrations of substances such as amino acids, trehalose, and lipids. They can also eliminate excess fluid at very high rates with the loss of only trace amounts of haemolymph solutes. In the terrestrial environment, where insects are particularly successful, the slow production of primary excretory fluid may have been important in allowing the evolution of very effective water recovery mechanisms. That these mechanisms have been developed in the hindgut is attributable to the advantage conferred by the cuticular lining of allowing water reabsorption by the hindgut cells to go on protected from mounting concentrations of potentially interfering compounds.

I am indebted to many of my colleagues and students who have debated with me many of the points that are discussed above. I particularly wish to thank Dr G. E. Pratt for some characteristically pertinent and helpful comments.

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