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Review

Insect homeostasis: past and future

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Summary

Most of my work has been on the hormonal control of fluid secretion by insect Malpighian tubules. My present purpose is mostly to describe some previously unpublished results in this area and put them in context of what was already known. In this, I hope to draw attention to some areas where future research might be productive.

Key words: Malpighian tubule, diuretic hormones, ions and water, epithelial transport, fluid secretion, transport of organic compounds.

Control of haemolymph volume in fed *Rhodnius*

I want to start by considering the problem of how insects might match the incoming and outgoings of fluid and ions, in particular in *Rhodnius prolixus* (Stål). This blood-sucking insect takes, in its young stages, blood meals of a spectacular size, up to 12 times its unfed weight (Buxton, 1930). It then greatly accelerates the action of several systems that together allow it to rid itself of the surplus fluid and ions contained in the meal by transporting them out of the midgut into the haemolymph and then out of the haemolymph in the urine produced by the Malpighian tubules. This is done at a huge rate. The Malpighian tubules secrete approximately 1000 times faster when they are hormonally stimulated on the occasion of the meal (Maddrell, 1963).

A fed *Rhodnius* is capable of excreting its own unfed body mass every forty minutes or so. That means that a volume of fluid equal to that of the entire haemolymph volume passes through the haemolymph every 10–15 min as it is transferred from midgut to haemolymph and then from the haemolymph out of the insect *via* the Malpighian tubules. One might think that the relatively small amount of haemolymph would be overwhelmed by this and would be in danger of big changes in volume and composition. But in fact the haemolymph is relatively little affected.

Perhaps the most interesting problem in *Rhodnius* has to do with how the rate of fluid flow into the haemolymph from the midgut is matched by the rate of fluid flow out of the haemolymph *via* the Malpighian tubules. In other words, how is the haemolymph volume protected against the potentially embarrassingly large changes in volume that would occur were these two fluid flows not matched?

I previously suggested that this matching might be achieved if the midgut was less sensitive to a circulating diuretic hormone than the Malpighian tubules (Maddrell, 1977). In addition, I suggested that the maximal rate of stimulated fluid transport by the midgut must be higher than the maximal rates of fluid secretion by the four Malpighian tubules. Since that time, it has been found that there are at least two hormones released into circulation to control these fluid transport processes (Lange et al., 1989; Maddrell et al., 1991a). Nonetheless, a composite figure for the response of the midgut and Malpighian tubules to the hormones might be as shown in Fig. 1. The curves have to be drawn rather more steeply than if the epithelia were being stimulated by a single hormone because this is one of the effects of synergism between the, at least, two diuretic hormones released after feeding (Maddrell et al., 1993).

The dose/response curves cross at two points. The higher of the two is a stable point. For there, if the concentration of hormones were to increase, the midgut would secrete fluid faster into the haemolymph, thereby bringing the hormone concentration down again. And, conversely, if the hormone concentration decreases, the midgut activity also decreases so reducing the haemolymph volume and increasing the hormone concentration.

What is the evidence to support this proposal?

The dose/response curves of the two fluid-transporting epithelia differ – at least in their responses to serotonin (5-HT), known to be one of the diuretic hormones that *Rhodnius* uses (Maddrell et al., 1991a). The midgut is less sensitive to 5-HT than the Malpighian tubules are (Farmer et al., 1981). If we then suppose that the midgut can transport fluid faster than can all four Malpighian tubules, we have the basis for an automatic matching of their rates when both are stimulated by this hormone. There is no direct evidence for this but it is fairly easy to believe, as one can calculate that the area of the midgut epithelium is approximately 8 times larger than the total area of the fluid-secreting parts of the four Malpighian tubules (admittedly this ignores the fact that both tissues have extensive elaborations of their basolateral and apical membranes and they may differ in the extent of this). It follows that the rate of fluid transport across a unit area of midgut epithelium is considerably lower than that of the Malpighian tubules when both are transporting at the same overall rate.

One method of obtaining hormones from their release sites is to treat the sites with K^+-rich saline (Maddrell and Gee, 1974). This was done with the mesothoracic ganglionic mass and its abdominal nerves – the release sites for the diuretic hormones. The hormone-rich saline was then tested on the midgut and the Malpighian tubules from the same insect as used to prepare the test saline. The results showed that the Malpighian tubules are 2–3 times as sensitive to the hormone-laden saline than is the midgut wall. So this aspect of the proposed relation shown in Fig. 1 has support.

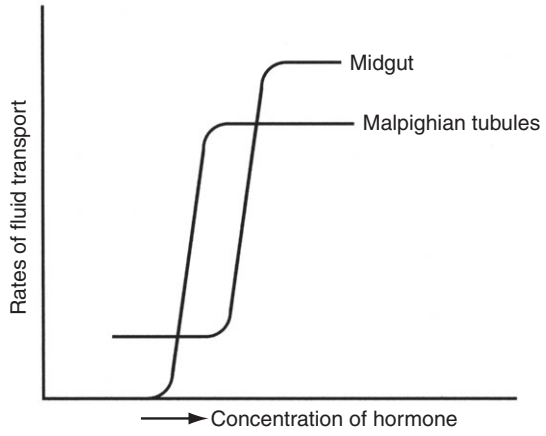

Fig. 1. Proposed relationship between concentration of hormones and responses of the midgut and Malpighian tubules in fed *Rhodnius*.

Rather similarly, it can be shown that the haemolymph of recently fed insects always contains hormone concentrations more than enough, by approximately 2–3 times, that are required to elicit the maximal rate of fluid secretion by the Malpighian tubules. So if the Malpighian tubules are maximally stimulated in the intact fed insect, the rate of diuresis should be more or less constant – and it is (Maddrell, 1964).

By contrast, testing haemolymph samples from fed insects on isolated midguts usually produced little effect. However, we know that target tissues can inactivate hormones. Early on, I showed that samples of haemolymph from fed *Rhodnius* lose their diuretic activity much more rapidly if they bathe a Malpighian tubule than if they are merely left by themselves (Maddrell, 1964). In addition, we would expect from the model that the haemolymph hormone concentration should only be sufficient partially to stimulate the midgut. So it is credible to suppose to that hormone-rich haemolymph taken from a fed *Rhodnius* would be sufficiently rapidly inactivated by the midgut epithelium to prevent its having much effect on that epithelium, especially given the large surface area of the midgut.

And of course we already know that 5-HT affects the Malpighian tubules at concentrations too low to affect the midgut.

Further support for the model comes from some experiments not previously published. In these experiments, I removed Malpighian tubules from a series of 5th stage *Rhodnius* leaving some with three Malpighian tubules, others with two Malpighian tubules and some with only one. Then when these insects were fed blood meals, not surprisingly the rates of diuresis were, respectively, cut by 25%, 50% or 75% (Fig. 2). And diuresis lasted 50% longer, twice as long or 4 times as long, respectively. But what was as interesting was that the volume of haemolymph did not appear to change to any marked degree. Because the plasticised and extended abdominal endocuticle is more or less transparent, one can illuminate the insect from below and clearly see how much haemolymph there is outside the midgut.

So what was going on? If the Malpighian tubules' output is reduced, the haemolymph volume is bound to start to increase from the faster export of fluid into it from the midgut. This will immediately lower the hormone concentration and fluid transport by the midgut will slow. Not so for the Malpighian tubules as they will still be maximally stimulated. The midgut transport rate then slows until it again matches the Malpighian tubule output at which

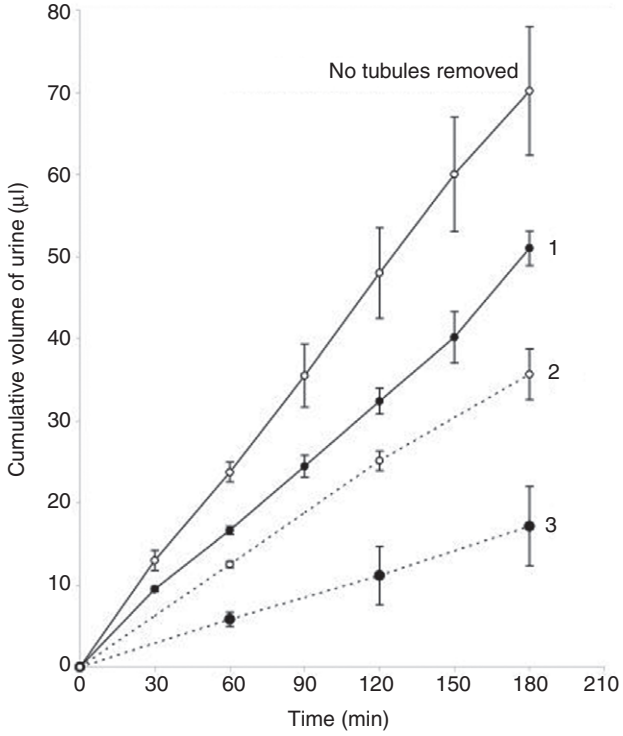

Fig. 2. Diuresis in fed 5th stage *Rhodnius* with one, two or three Malpighian tubules removed.

point the situation is again stable. And this adjustment should occur whether the insect lacks, one, two or even three of its complement of Malpighian tubules (the full complement is four). Interestingly enough, a few fed insects from whom three of their Malpighian tubules had been removed failed to excrete fluid or only minimal amounts. I think you can see why this might be from a redrawing of Fig. 1 to take into account the reduced rates of fluid secretion in insects with fewer Malpighian tubules (Fig. 3). Unstimulated midguts transport fluid at significant rates (Farmer et al., 1981) – unlike Malpighian tubules, where unstimulated tubules secrete fluid 100–1000 times as slowly as they do when maximally stimulated. So if the midgut transport rate in a fed insect with only one Malpighian tubule is higher than the maximal rate of fluid secretion by that single Malpighian tubule, it will overwhelm transport by the Malpighian tubule, the haemolymph volume will rapidly increase and the Malpighian tubule will shut down as the hormone concentration becomes insufficient to stimulate it. And, indeed, observation of such insects shows that they have greatly increased volumes of haemolymph.

So in Fig. 3 we have a working model to explain how the rates of fluid flow into the haemolymph can be matched to the outflow almost regardless of how fast the Malpighian tubules secrete fluid. In this way, the insect is protected against large changes in volume of haemolymph from any shortfall in fluid secretion by its Malpighian tubules.

Other general problems with insect hormones

One set of problems here concerns how the correct concentration of a hormone in circulation is maintained. Several hormones have different effects at different concentrations. For example a small release of the moulting hormone, 20-HE, cause wandering behaviour in a lepidopteran 5th stage larva whereas a later, much

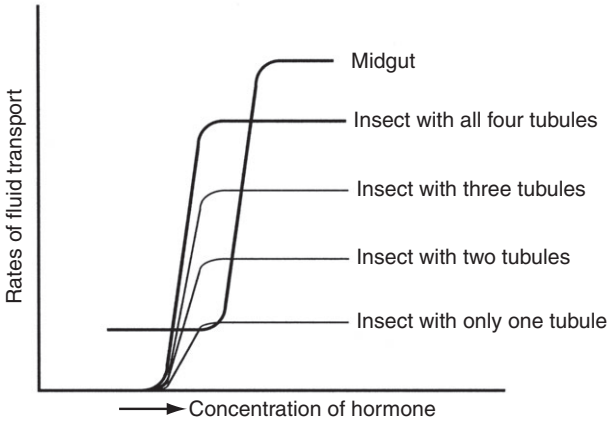

Fig. 3. Proposed relationship between the concentration of hormones and the responses of the midgut and Malpighian tubules in fed *Rhodnius* with one, two or three Malpighian tubules removed.

larger release causes pupation (Truman and Riddiford, 1999). So there is a general problem of how insects can assess the level of hormones in circulation. Perhaps the easiest solution would be if the releasing sites (the neurohaemal areas for neurohormones) were themselves directly sensitive to the concentrations in circulation of the hormones they release. As far as I know, this has yet to be looked at.

Another problem concerns the apparently over-large number of hormones that can act on Malpighian tubules – and, it can be imagined, on other organs, such as the heart.

The fact that many different hormones affect fluid secretion by Malpighian tubules had been known for some time [see paper by Coast in this issue (Coast, 2009)] (Donini et al., 2008). There is evidence for the Malpighian tubules of *Manduca sexta* that at least eight different sorts of compounds can significantly affect the rates of their fluid secretion (Skaer et al., 2002). All the substances tested on the Malpighian tubules also accelerated the rate of heartbeat in *Manduca* and at similar concentrations.

What is one to make of this? What we said at the time was ‘we suggest that there may exist in the extracellular fluid a continuous broadcast of information in the form of a chemical language, to which many or all parts of the body continuously respond on a moment-to-moment basis and which, because of the greater information in it, ensures a more effective and efficient coordination of function than could be achieved by a series of single, tissue-specific hormones that force stereotypical responses by their target tissue(s)’.

And indeed this idea may explain the apparently overly complex array of hormonal signals affecting such processes as moulting in insects where many hormones contribute to varying degrees. If it is the case that events in an animal are at least partly controlled by an internal language with a rich array of ‘words’ (each a circulating chemical signal), then this complexity should not be surprising but should be expected.

It is consistent with these ideas that a hormonal signal is not a command but merely a signal to which a tissue or cell may or may not respond. So, for example epidermal cells in an insect may respond to the appearance of 20-HE in circulation by moulting the cuticle above them. Other cells respond quite differently, with many of them content to ignore the signal completely. Similarly, the appearance of bursicon in circulation is read by most cells in the developing wings of, say, a dipteran fly as notice that they should undergo programmed cell death and disappear completely so as to lighten the resulting wing. Other cells, even some of those in direct contact with the dying cells, remain unaffected. These undisturbed cells line the haemolymph spaces in the wing veins and they need to be able to continue to carry out their normal functions. So, apparently very similar cells respond completely differently to the same hormone regime – as one would expect if hormones were only elements in a supply of information to the tissues of the body.

Possible differential sensitivity of Malpighian tubule cells to hormones

Individual cells in Malpighian tubules may respond differently to hormonal signals. For example the different stellate cells of the Malpighian tubules of *Drosophila melanogaster* gave indications that they might respond to a greater or lesser extent to hormonal stimulation (O’Donnell et al., 1997).

A useful peculiarity of *Rhodnius*’ Malpighian tubules might allow single Malpighian tubule cells to be assessed directly. A few percent of *Rhodnius*’ Malpighian tubules have an abnormality in which a single upper Malpighian tubule cell appears among the first few cells of the lower Malpighian tubule (Fig. 4) (Maddrell and Overton, 1985). The whole Malpighian tubule can then be set up in a way that allows the physiological properties of the single cell to be examined (Fig. 5). For example one can measure the transport of sodium ions into the lumen (Fig. 6). As this figure shows, the sodium transport by one upper Malpighian tubule cell is very large and very easy to measure. It should be quite simple to construct a dose/response curve to a particular stimulant for that single cell. And if one first did a dose/response curve for the entire upper Malpighian tubule upstream, the two dose/response curves could be compared. In a few cases, more than one upper Malpighian tubule cell appears distant from its fellows, so the sensitivity of each could be studied. If they are in contact with each other, one could then investigate whether being in contact affects their hormone sensitivity.

Oscillations in trans-epithelial potential difference in isolated Malpighian tubules

Measurements of the trans-epithelial potential difference (TEP) in *Rhodnius* show that after stimulation with any of the known

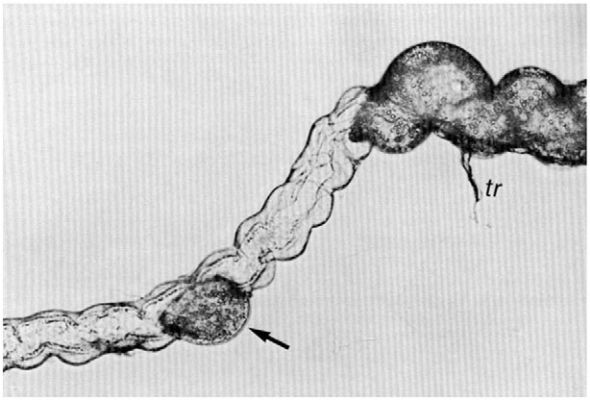

Fig. 4. A part of a Malpighian tubule from a 5th stage *Rhodnius* in which a developmental error has led to a cell (arrow) typical of the upper Malpighian tubule coming to lie in a background of lower Malpighian tubule cells. *tr*, a short length of trachea (air supply system) attached to one of the upper cells. Upper tubule ~70–80 μm in diameter. [From Maddrell and Overton (Maddrell and Overton, 1985).]

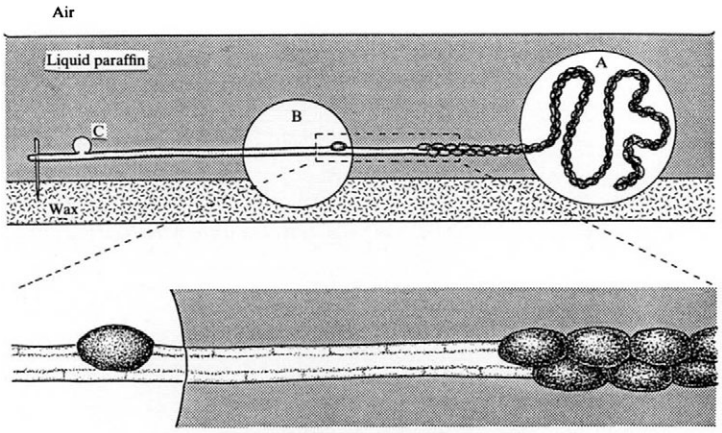

Fig. 5. Diagram of the experimental arrangement used to follow the transporting activity of single upper Malpighian tubule cells. Most of the upper, fluid-secreting region of the Malpighian tubule is placed in a 100 µl drop (A), held in position in a depression in the wax base. This drop contains 10^{-7} mol l^{-1} serotonin (5-HT) to stimulate fluid secretion by the upper Malpighian tubule. The lower Malpighian tubule is arranged so that the boundary between it and the upper Malpighian tubule is a short distance upstream from a 20 µl test drop (B). This allows the single upper cell, whose activity is being investigated, to be situated just within the test drop. The test drop contains the radioactive marker being used. Fluid passing down the lumen is collected from a cut in the wall of the Malpighian tubule at (C). The lower drawing shows, in close-up, the upper/lower Malpighian tubule boundary and the position of the single upper cell [from Maddrell and Overton (Maddrell and Overton, 1985)].

stimulant hormones there is a characteristic three-phase spike (O'Donnell and Maddrell, 1984) after which the lumen holds a near constant negative potential difference with respect to the bathing medium. Here, I report an unusual occurrence in which *Rhodnius*' upper Malpighian tubules stimulated with 5-HT showed a TEP that started to oscillate (Fig. 7). The size of the oscillations would grow until they were maybe 20 mV or more in size – then they faded away. What was striking was how regular the oscillations were. What appeared to cause this sort of behaviour was if the Malpighian tubule was rather bunched up and crowded at one side of the bathing drop. Indeed, the oscillations could be stopped by moving the Malpighian tubule until it was more central in the bathing drop and less bunched up. Or if more, fresh saline was added, that too would often stop the oscillations.

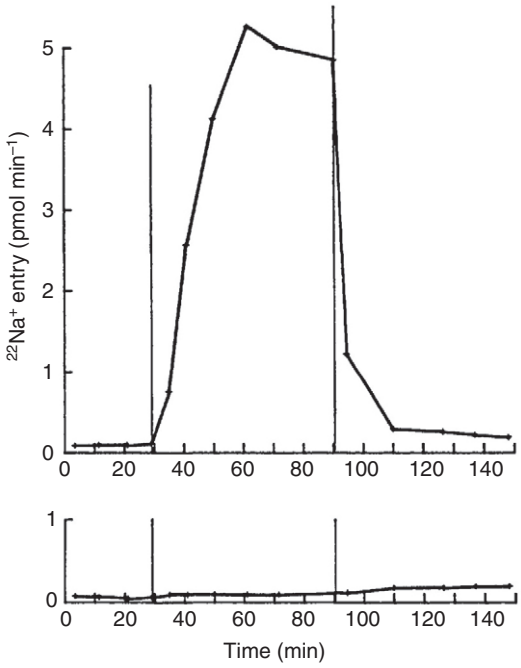

Fig. 6. The transport of radioactive $^{22}Na^+$ from a test droplet containing a single upper Malpighian tubule cell (upper panel) or where the test droplet does not contain an upper Malpighian tubule cell (lower panel). In each case, 10^{-7} mol l^{-1} serotonin (5-HT) was added at 30 min (the first vertical line) and the test droplet was replaced with a droplet of 5-HT-free saline at 90 min (second vertical line) [from Maddrell and Overton (Maddrell and Overton, 1985)].

The oscillations were very temperature sensitive. If the dish in which the experiment was going on was warmed, the oscillations would get faster and indeed bigger. And the frequency of oscillation was so predictable that one could assess the temperature of the bath surrounding the Malpighian tubule in its drop of bathing fluid from the periodicity or wavelength of the oscillations. The log of the interpeak time was more or less linearly related to the temperature over the range from 20–32°C (Fig. 8).

What is one to make of these oscillations? *Rhodnius*' Malpighian tubules do not make the writhing movements that many Malpighian tubules do, so they are likely instead to result from the Malpighian tubule being starved of something in some way. One obvious possibility might be oxygen, especially as the oscillations could usually be produced by crowding a Malpighian tubule into one part of the bathing drop of saline. This would tie in with the fact that the oscillations get bigger when the temperature is raised (they nearly double in size when the temperature is raised from 22°C to 28°C, for example), when presumably the Malpighian tubule is using up energy faster as its metabolic rate is raised. But why this should cause such oscillations is not too clear. Such regular oscillations look as if they may result from some negative feedback from one part of the system onto another. Negative feedback with a delay classically results in oscillations – as in insect asynchronous flight muscles, for example.

If we are to see what underlies the oscillations, we need to know what the various ion movements are and what are the electrical potential differences that they might cause. And, also, how variations in cellular second messengers affect potential differences. Unfortunately, we also need to know which of these is the most sensitive to oxygen change, if indeed that is the physiological factor that precipitates these nice oscillations. Given the predictability of the response and its size, it might be possible to find out what is the proximate cause of the oscillations. More usefully, however, these oscillations might be useful in a deeper understanding of the cellular responses to stimulation.

The future

Most of the work that has been done on Malpighian tubules since Ramsay's pioneering work in the 1950s has been on their fluid secretion and on their control by hormones. Some research has been done on their ability to transport inorganic solutes other than those directly involved in fluid secretion; some mosquito Malpighian

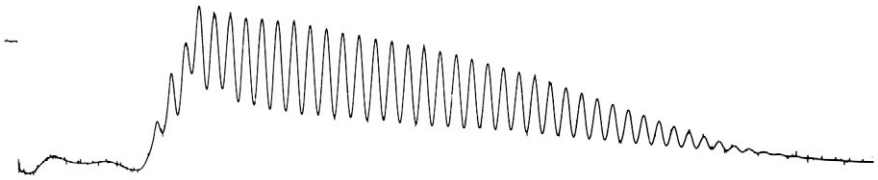

Fig. 7. Oscillations in transepithelial potential difference (TEP) measured in an upper Malpighian tubule from a 5th stage *Rhodnius* in a 100 μl droplet containing serotonin (5-HT) at 10^{-7} mol l^{-1}. The TEP was steady at approximately –30 mV at the start but started to rise and changed to oscillations of approximately 20 mV after 20 min. The wavelength of the oscillations here was approximately 3 min at the bath temperature of 24°C.

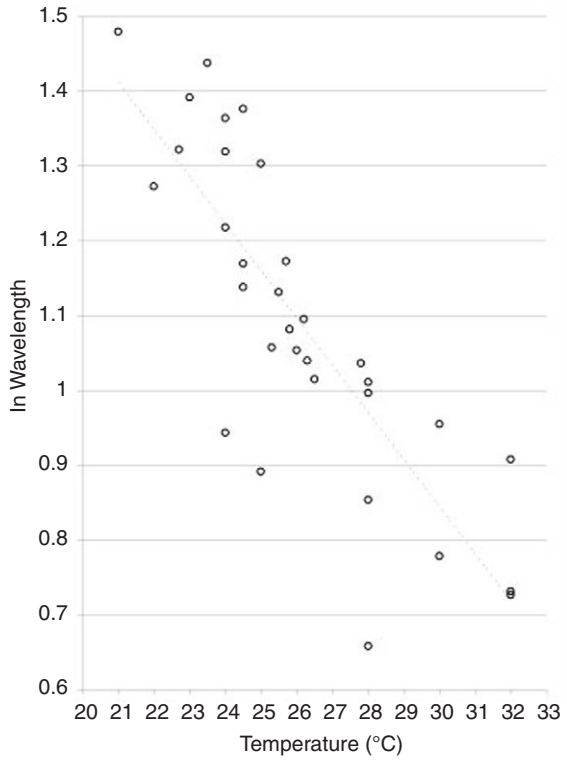

Fig. 8. The relationship between bath temperature and wavelength of oscillations in transepithelial potential difference (TEP) in Malpighian tubules from 5th stage *Rhodnius* stimulated with 10^{-7} mol l^{-1} serotonin (5-HT). A wavelength of 2 min has a ln wavelength of 0.69 and a wavelength of 4 min has a ln wavelength of 1.38.

tubules can, for example carry out active transport of magnesium and sulphate ions (Phillips and Maddrell, 1974; Maddrell and Phillips, 1975). And some Malpighian tubules can transport calcium ions (Herbst and Bradley, 1989; Maddrell et al., 1991b). A wide range of organic materials is also known to be actively transported by Malpighian tubules. There are early references to their ability to concentrate different sorts of dyes (Lison, 1937) but they are also able to transport cardiac glycosides (Rafaeli-Bernstein and Mordue, 1978), alkaloids (Maddrell and Gardiner, 1976), uric acid (Wigglesworth, 1931; O'Donnell et al., 1983), salicylate (Ruiz-Sanchez et al., 2007) and compounds related to para-amino hippuric acid (Maddrell et al., 1974). And there is something known of how such transport of organic materials is altered by the insect in response to the presence of absence or abundance in the food material or the physiological state of the insect (Maddrell and Gardiner, 1975; O'Donnell et al., 1983). But by comparison with what is known of ion and water transport, these very important aspects of Malpighian tubule function have been relatively little explored. With the new developments in genomics, proteomics and what has been termed metabolomics, things are certain to change (Kamleh et al., 2008) [and see paper by Dow in this issue (Dow, 2009)]. And it is likely that there will be surprises. One major one has already appeared. Who could have guessed that Malpighian tubules would be centrally involved in the immune response of insects to xenobiotics (McGettigan et al., 2005) [and see paper by Dow in this issue (Dow, 2009)]!

I think we can look forward in the next few years to a greatly increased knowledge of all aspects of insect homeostasis. Exciting times lie ahead!

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