EXCRETION OF ALKALOIDS BY MALPIGHIAN TUBULES OF INSECTS

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SUMMARY

Nicotine is transported at high rates by Malpighian tubules of larvae of Manduca sexta, Pieris brassicae and Rhodnius prolixus and the transport persists in the absence of alkaloid from the diet. In the fluid-secreting portion of Rhodnius tubules this transport is not coupled to ion transport, nor is it dependent on the physiological state of the animal. The transport, which can occur against a steep electrochemical gradient, shows saturation kinetics with a maximal rate of 700 pmol min⁻¹ per tubule and is half saturated at 2–3 mM. Nicotine transport independent of ion movements also occurs in the lower resorptive parts of Rhodnius tubules. Both portions of Rhodnius tubules can transport morphine and atropine. These alkaloids and nicotine compete with one another and are presumed to be carried by the same transport system. Nicotine transport in Rhodnius was unaffected by organic anions, such as amaranth and benzyl penicillin, or by the organic anion transport inhibitor, probenecid. Fluid secretion in 5-HT-stimulated tubules was reduced by atropine and nicotine, probably by blocking the 5-HT receptors. The Malpighian tubules of adult Calliphora erythrocephala and Musca domestica remove nicotine from bathing solutions, an unknown metabolite accumulating in the tubules. Adult P. brassicae and M. sexta do not exhibit transport of nicotine by their Malpighian tubules.

INTRODUCTION

Alkaloids are nitrogenous bases that occur in plants, particularly of the families Papaveraceae, Papilionaceae, Ranunculaceae and Solanaceae. They are believed to have been evolved to protect the plants possessing them against attack by insects (Gordon, 1961), and presumably, by other herbivores. Some herbivores, nevertheless, can thrive on a diet of alkaloid-containing plants. The present paper shows that many insect Malpighian tubules can remove from the haemolymph such alkaloids as nicotine, morphine and atropine at high rates and against large concentration differences. This ability must be an important element in allowing insects to exploit a wide range of food plants.

MATERIALS AND METHODS

Insects of the following species were taken from laboratory stocks: Manduca sexta (4th and 5th stage larvae and adults), Rhodnius prolixus (5th stage larvae), Calliphora erythrocephala (adults), Musca domestica (adults) and Pieris brassicae (larvae and adults). The techniques for isolating Malpighian tubules were the same as used by
Maddrell & Gardiner (1974). The physiological salines used had the following compositions: for *Manduca sexta* and *Pieris brassicae*, NaCl 15 mM, KCl 30 mM, CaCl$_2$ 2 mM, MgCl$_2$ 30 mM, K,HCO$_3$ 10 mM, KH$_2$PO$_4$ 5 mM, glucose 10 mM, maltose 10 mM, sodium citrate 5 mM, glycine 10 mM, alanine 10 mM, proline 10 mM, glutamine 10 mM, valine 10 mM, serine 5 mM, histidine 5 mM and the pH brought to 7.2 with KOH; for *Rhodnius prolixus* as in Maddrell (1969) and for *Musca* and *Calliphora* as for *Rhodnius* but with KCl and NaCl both at 75 mM.

$^{14}$C-labelled nicotine, $^3$H-labelled atropine and $^{14}$C-labelled morphine at specific activities of 56 mCi mmol$^{-1}$, 439 mCi mmol$^{-1}$ and 54 mCi mmol$^{-1}$ respectively were supplied by the Radiochemical Centre, Amersham. Radioactive samples were counted using conventional scintillation techniques with an Intertechnique ABAC SL40 scintillation counter.

Chromatograms of samples of radioactive fluid were run on silica gel thin layer plates and scanned radiometrically using a Berthold LB 2722 scanner.

All values are quoted as mean ± S.E. (number of observations).

**RESULTS**

A. Transport of nicotine by Malpighian tubules of *Manduca* sexta

Nicotine introduced into the haemolymph of larvae of *Manduca* either by feeding or by injection soon appears unchanged in the faeces (Self, Guthrie & Hodgson, 1964). We tested the ability of Malpighian tubules isolated from early 5th stage larvae of *Manduca* to transport nicotine. Approximately 3 cm lengths of the proximal parts of the tubules were placed in saline containing 0.004 mM $^{14}$C-labelled nicotine. They secreted fluid at a very slow rate so that only after 90 min was it possible to collect small drops of secreted fluid. These were found to contain nicotine at a concentration of 0.056 ± 0.0045 mM (n = 8), fourteen times more concentrated than the bathing fluid. The difficulty of collecting secreted fluid from these Malpighian tubules arises from their distensible nature and the fact that after dissection they are deflated; fluid secretion, which is slow in any case, has first to refill the tubules before fluid emerges from the cut ends. To avoid these difficulties, further experiments were done on cannulated tubules perfused with K-rich saline (similar in composition to that secreted by Malpighian tubules).

For these experiments we used the lengths of Malpighian tubule that lie on the surface of the midgut, the so-called proximal and medial tubules (Nijhout, 1975). Because it is easier to get a satisfactory fluid-tight seal between the cannula and a tubule into which it is inserted if the tubule is not too large, we used tubules from early 4th stage larvae rather than from the 5th stage larvae used earlier.

The rate of nicotine transport was measured in tubules bathed in saline containing 5 mM $^{14}$C-labelled nicotine. Nicotine appeared in the fluid perfused through the lumen of these tubules at an average rate of 3290 ± 520 pmol min$^{-1}$ (n = 6). The rate of perfusion of fluid was 150 nl min$^{-1}$ and the average concentration of nicotine in it was 21.9 mM, more than four times higher than in the bathing fluid, despite the high rate of perfusion. Larvae of *Manduca* can be raised on an artificial diet (Yamamoto, 1969). We reared *Manduca* on a nicotine-free diet and on a diet containing 28 g nicotine kg$^{-1}$, a concentration approximating to that found in tobacco plants (Self et al. 1964). We
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Fig. 1. Plots of the output of a radioactivity scanner to show the position of spots containing radioactivity on TLC plates. The samples run were of the bathing fluid containing 14C-nicotine and of the fluid secreted by upper Malpighian tubules of Manduca placed in it. The solvent systems used to develop the plates were (a) chloroform: methanol: acetic acid (75:20:5), (b) chloroform: methanol (80:15) and (c) cyclohexane: chloroform: diethylamine (5:4:1). The arrows mark the position of the origins.

also reared some larvae on Atropa belladonna, another Solanaceous plant which contains atropine, hyoscyamine and other alkaloids. Malpighian tubules from 4th stage larvae reared on all these diets all proved to be capable of transporting nicotine at rates higher than 1500 pmol min\(^{-1}\). Clearly, the ability to transport nicotine does not depend on its presence in the larval diet.

Adult Manduca, which feed on nectar, presumably have no need to excrete nicotine. Proximal lengths of adult Malpighian tubules isolated into saline containing 1 mM cyclic AMP readily secrete fluid. In such a saline containing 0.5 mM 14C-labelled nicotine, nicotine appeared in the secreted fluid at a rate of only 4.07 ± 0.87 pmol min\(^{-1}\) (n = 15), and at a concentration of 0.28 ± 0.03 mM (i.e. less than in the bathing fluid). Assuming that these Malpighian tubules are as permeable to nicotine (molecular weight 162) as to xylose (molecular weight 150), it can be calculated from Maddrell & Gardiner’s (1974) figures that the concentration of nicotine in the secreted fluid could reach 0.39 mM purely passively. So, in marked contrast to tubules from the larva, the adult tubules show no evidence of being able to excrete nicotine other than by passive means.

B. Transport of alkaloids by Malpighian tubules of Rhodnius

Transport of nicotine

Larvae of Manduca sexta feed exclusively on plants of the family Solanaceae which characteristically contain large amounts of alkaloids, so it is not surprising to find that Malpighian tubules of Manduca can transport nicotine. Unexpectedly it was found that the Malpighian tubules of the blood sucking insect, Rhodnius, can also transport nicotine at a high rate. In the first experiments the upper fluid-secreting...
parts of the Malpighian tubules from unfed 5th stage insects were isolated into saline containing 0.1 mM nicotine (labelled with ^14C nicotine) and were stimulated to secrete by the addition of 10^-6 M 5-HT. The fluid secreted by these tubules contained labelled material at a concentration about five times higher than the bathing solution. Tests with three different chromatographic separation techniques showed that all this material behaved exactly as authentic nicotine (Fig. 1) and so is presumably unchanged.

The question arises as to whether this transport of nicotine is an active process or not. Nicotine at physiological pH exists almost completely as the charged, cationic form of the base so that as the lumen of 5-HT-stimulated tubules is about 30-40 mV negative to the bathing solution (Maddrell, 1971), some concentration might occur passively. However, in an experiment with tubules not stimulated with 5-HT, nicotine was found to be concentrated in the secreted fluid from a bathing fluid containing 0.01 mM nicotine, by a factor of 39-71 ± 2.45 (n = 19). Under these conditions the tubule lumen is at a potential only about 15 mV negative to the bathing solution (Maddrell, 1971), and this could not passively support such a large concentration gradient. The transport of nicotine into the lumen is clearly an active process.

**Effects of the rate of fluid transport on nicotine transport**

Tubules bathed in a solution containing 0.01 mM nicotine transported nicotine at a rate of 6.2 ± 0.7 pmol.min^-1 (n = 8) when stimulated with 5-HT and at only 0.38 ± 0.11 pmol.min^-1 (n = 24) when unstimulated. Does 5-HT stimulate nicotine transport as well as fluid transport? Nicotine is a relatively small molecule (its molecular weight is 162) and, as we have emphasized (Maddrell & Gardiner, 1974), Malpighian tubules are very permeable to such compounds. Nicotine transported into the lumen is likely to diffuse back out again and this would go on faster in unstimulated tubules where the luminal concentration is higher. This loss would reduce the rate of nicotine transport. That this is the explanation of the large difference in net nicotine transport between stimulated and unstimulated tubules is shown by the following two lines of evidence.

If the net transport of nicotine is reduced by back diffusion this should be more marked at low rates of fluid secretion because the concentration of nicotine in the lumen would be greater under such conditions. In Fig. 2 the rates of nicotine transport are plotted against the rates of fluid secretion, showing a strong positive correlation between the two. Although the faster rates of secretion are by tubules from newly moulted insects, the correlation shown in Fig. 2 is not a reflexion of changing rates of nicotine transport with age because, as we shall see (p. 272), nicotine transport rates do not change with age in the same way that transport of organic anions does (Maddrell & Gardiner, 1975). A very similar correlation, however, in this case between net dye transport and fluid secretion, is shown by the permeable Malpighian tubules of Calliphora (Fig. 6 in Maddrell, Gardiner, Pilcher & Reynolds, 1974) where fast back diffusion of transported material is known to occur.

By perfusing nicotine-free fluid from a cannula through the lumen of a tubule it should be possible to keep down the concentration of nicotine in the lumen and so substantially reduce back diffusion. Fig. 3 shows that the net rate of nicotine transport in an unstimulated tubule bathed in 0.01 mM nicotine and perfused with fluid at a rate of 50 nl.min^-1 is of the order of 4 pmol.min^-1 (i.e. ten times higher than in
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Fig. 2. The relationship between rate of nicotine transport and rate of fluid secretion in unstimulated upper Malpighian tubules of Rhodnius. The saline bathing the tubules contains 0.01 mM nicotine.

Fig. 3. The rates of nicotine transport (open circles) in a cannulated and perfused upper Malpighian tubule of Rhodnius before and after treatment with 10^{-6} M 5-HT. The rates at which fluid emerged from the cut end of the tubule is also shown (closed circles). The bar at the top of the graph indicates the time during which 5-HT was present.
Possible effects of physiological age on nicotine transport

In *Rhodnius*, transport of organic anions by Malpighian tubules depends very much on the physiological state of the animal; in unfed insects the rate is low but it rises during the first 2-3 days after a protein-containing meal before declining again (Maddrell & Gardiner, 1975). While making measurements of the rates at which the tubules would transport p-aminohippuric acid (PAH), we also measured the nicotine transporting ability of the same tubules. The results showed clearly that, apart from increases associated with increasing size of the insect as it grows, there are no changes in ability to transport nicotine. As an example, the results of one part of this series of experiments are shown in Fig. 4; in contrast to PAH transport, nicotine transport by the Malpighian tubules of adult *Rhodnius* is scarcely affected when the insect feeds.
Concentration dependence of nicotine transport

The dependence of the rate of nicotine transport by isolated Malpighian tubules of *Rhodnius* on the concentration of nicotine in the bathing solution is shown in Fig. 5. The transport shows saturation kinetics with a maximal rate of about 700 pmol min⁻¹ and is half-saturated at a concentration of nicotine in the bathing solution of about 2–3 mM. A complicating feature of these experiments was that at concentrations of nicotine higher than about 2 mM, the rate of fluid secretion is reduced. Why this should be so is considered on p. 277. In the present context it meant that determinations of rates of nicotine transport at the higher concentrations had to be made using tubules that were cannulated and perfused with fluid (at about 70–80 nl min⁻¹) to avoid the extensive back diffusion of transported nicotine from the high luminal concentrations that occur in tubules secreting fluid only slowly.

Nicotine transport by the lower Malpighian tubules

*Rhodnius*’ Malpighian tubules each consist of an upper, fluid secreting, region and a lower one, now known to carry out absorption of potassium and chloride ions from the lumen so that its contents become hypo-osmotic to the bathing solution (Maddrell & Phillips, 1975a, b).

We tested the ability of this part of the tubule to transport nicotine by isolating whole tubules so that the upper ends were bathed in a drop of nicotine-free 5-HT-containing solution and the lower ends were bathed in a drop of saline containing ¹⁴C-nicotine (Fig. 6). Any nicotine secreted by the lower tubule thus appears in the fluid passed through it from the upper tubule. We found that the lower tubule is able to transport nicotine into the lumen at rates which, considering the lower tubule is
Saline containing 5-HT

Upper tubule

Saline containing [\(^6^C\)] nicotine

Lower tubule

Liquid paraffin

Wax

Fig. 6. Side view of the experimental arrangement involved in measuring nicotine transport by lower Malpighian tubules of *Rhodnius*. The upper tubules pass fluid through the lumen of the lower tubule so that transported nicotine is swept out and appears in the fluid emerging from the cut end of the tubule.

Fig. 7. The dependence of the rate of transport of nicotine by the lower Malpighian tubules of *Rhodnius* on the concentration of nicotine in the bathing solution. Each point is the mean of not less than eight determinations and the vertical lines attached to the points represent ± S.E. of the mean.

only half the length of the upper one, are not much less than those shown by the upper tubule. The dependence of the rate of transport on the concentration in the bathing saline is shown in Fig. 7. Just as in the upper tubule, nicotine transport shows saturation kinetics; the maximal rate is of the order of 250 pmol min\(^{-1}\) and transport is half-saturated at about 2 mM.

The ability of the lower tubule to absorb potassium chloride from the lumen depends on the presence of a stimulant such as 5-HT and on the potassium concentration of the bathing fluid (Maddrell & Phillips, 1975b). We have tested the dependence of nicotine transport on these factors. Lower tubules were bathed in saline containing
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0.5 mM 14C-labelled nicotine with or without 10⁻⁶ M 5-HT. Six such tubules showed no higher rate of nicotine transport in the presence of 5-HT than in its absence. To test the effects of an elevated potassium concentration (which depresses KCl absorption, Maddrell & Phillips, 1975b) lower tubules were bathed in saline containing 0.5 mM nicotine (labelled with 14C-nicotine), 10⁻⁵ M 5-HT and a potassium concentration either of 5 mM or of 80 mM. These tubules transported no less nicotine when bathed in 80 mM K than in 5 mM K. As with the upper tubule then, nicotine transport is not linked to the ion transport system of the tubule.

Transport of atropine and morphine

We have tested the ability of both parts of Rhodnius Malpighian tubules to transport two other alkaloids, atropine and morphine.

3H-labelled atropine is transported, unchanged, at high rates by both upper and lower Malpighian tubules. Chromatography using three different solvent systems showed that the labelled material in the fluid from the lumen of the tubules was all in the form of unchanged atropine. The transport showed saturation kinetics with the following characteristics; for the upper tubule a maximal rate of about 350 pmol.min⁻¹ and half saturation at about 5—6 mM and for the lower tubule a maximal rate of 100 pmol.min⁻¹ and half saturation at about 6 mM. Atropine is thus transported only about half as fast as nicotine.

Concentrations of atropine higher than about 0.02 mM greatly slow fluid secretion by 5-HT-stimulated upper tubules (this point is discussed on p. 277); at 0.34 mM, ten tubules secreted fluid at a rate of only 3.20 ± 0.49 nl.min⁻¹ and the secreted fluid contained 14.43 ± 1.22 mM atropine (i.e. a concentration 42 times higher than in the bathing fluid).

14C-labelled morphine is also transported by both upper and lower Malpighian tubules, and chromatography in two different solvent systems showed that it was unchanged.

With only labelled morphine available it was impracticable to use concentrations higher than about 0.5 mM. However, at 0.014 mM in the bathing solution, morphine was transported by the upper tubules at 3.49 ± 0.40 pmol.min⁻¹ (n = 8), at 0.04 mM at 7.51 ± 1.16 pmol.min⁻¹ (n = 6) and at 0.46 mM at 123.22 ± 3.79 pmol.min⁻¹ (n = 14). The lower tubules transported morphine from a solution containing 0.44 mM at an average rate of 33.22 ± 1.85 pmol.min⁻¹ (n = 10). These rates are closely similar to the rates at which nicotine is transported.

Competition studies

If nicotine, atropine and morphine are transported by a common mechanism, it should be possible to demonstrate competition between them. Table 1 summarizes the results of experiments designed to test this; it shows that there is competition for transport between the different alkaloids. Atropine is the most successful competitor in that it can suppress transport of both nicotine and morphine. Nicotine interferes with morphine transport but not with atropine transport. Morphine affects neither nicotine nor atropine transport. As an example of the competition, Fig. 8 shows the effects of atropine on nicotine transport by lower tubules.
Table 1. 

<table>
<thead>
<tr>
<th>Part of tubule</th>
<th>Alkaloid and concentration used</th>
<th>Presumptive competitor and concentration used</th>
<th>Effect</th>
<th>Number of tubules tested</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lower tubule</td>
<td>0.04 mM morphine</td>
<td>0.4 mM nicotine</td>
<td>&gt; 85% inhibition of morphine transport</td>
<td>5</td>
</tr>
<tr>
<td>Lower tubule</td>
<td>0.4 mM morphine</td>
<td>6 mM atropine</td>
<td>&gt; 80% inhibition of morphine transport</td>
<td>8</td>
</tr>
<tr>
<td>Upper tubule</td>
<td>0.01 mM morphine</td>
<td>0.1 mM atropine</td>
<td>&gt; 65% inhibition of morphine transport</td>
<td>7</td>
</tr>
<tr>
<td>Upper tubule</td>
<td>0.4 mM morphine</td>
<td>0.04 mM atropine</td>
<td>No effect</td>
<td>8</td>
</tr>
<tr>
<td>Lower tubule</td>
<td>0.5 mM nicotine</td>
<td>3 mM atropine</td>
<td>&gt; 85% inhibition of nicotine transport</td>
<td>8</td>
</tr>
<tr>
<td>Upper tubule</td>
<td>0.04 mM atropine</td>
<td>0.4 mM morphine</td>
<td>No effect</td>
<td>6</td>
</tr>
<tr>
<td>Upper tubule</td>
<td>0.01 mM atropine</td>
<td>0.5 mM nicotine</td>
<td>No effect</td>
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<tr>
<td>Lower tubule</td>
<td>6 mM atropine</td>
<td>6 mM nicotine</td>
<td>No effect</td>
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<tr>
<td>Lower tubule</td>
<td>0.5 mM atropine</td>
<td>5 mM nicotine</td>
<td>No effect</td>
<td>6</td>
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Fig. 8. The effect of treatment with 3 mM atropine on the rate of transport of nicotine by six lower Malpighian tubules of Rhodnius. The solution bathing the tubules contained 0.5 mM nicotine. The points are the mean values and the attached vertical lines represent ± S.E. of the mean. The bars at the top of the graph indicate the times during which atropine was present in the bathing solution; in the first such period, all six tubules were treated with atropine, while in the second, three tubules (upper line) were left untreated as controls for the three tubules to which atropine was added (lower line).

Effects on nicotine transport of organic anions and inhibitors of organic anion transport

As nicotine transport by Rhodnius tubules is much less affected by the physiological state of the animal than is PAH transport (Fig. 4), it seems likely that different systems are responsible for the transport of organic anions and of alkaloids. That this is so was shown by the following experiments. When 3.32 mM amaranth was added to the fluid bathing 6 upper tubules their secretion of nicotine from a fluid containing 0.5 mM
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nicotine was unaffected. Similarly 25 mM benzyl penicillin did not reduce nicotine transport by eight upper tubules. So neither of these substances, representatives of the two classes of transported organic anions, acylamides and sulphonates (Maddrell et al. 1974), interferes with nicotine transport.

Probenecid is a powerful inhibitor of organic anion transport systems (Despopoulos, 1965). We tested the effects of this drug on PAH and nicotine transport by the upper Malpighian tubules. The results are shown in Fig. 9 and they show that probenecid indeed inhibits PAH transport, but it has no effect on nicotine transport.

Another clear indication of the existence of two systems is that the lower tubule while able to transport nicotine into the lumen cannot so transport PAH (Maddrell & Phillips, 1975a).

Action of alkaloids on 5-HT induced secretion by upper tubules

As indicated on pp. 273 and 275 both nicotine (> 2 mM) and atropine (> 2 x 10⁻⁶ M) reduce the rate of fluid secretion by upper tubules bathed in saline containing approximately 10⁻⁸ M 5-HT. Fig. 10 gives the dose-inactivation curves for these two compounds. These effects may be due to an action, antagonistic to 5-HT, at receptors on the tubule cells. Morphine and atropine are known to act in this way in other systems (Offermeier & Ariens, 1966).

One of the consequences of this action is that the secreted fluid contains extraordinarily high concentrations of alkaloid. For example six tubules bathed in saline containing 20 mM nicotine secreted fluid containing 134.2 ± 10.1 mM nicotine! In contrast, the sodium concentration in fluid secreted by tubules in K-free saline containing 20 mM nicotine was only 104.6 ± 7.6 mM (n = 8). Such tubules can therefore secrete nicotine faster than they secrete monovalent cations.

Fig. 9. The effects of treatment with 0.2 mM probenecid on rates of fluid secretion, nicotine transport and PAH transport by upper Malpighian tubules of Rhodnius. The bathing solution contained 0.5 mM nicotine and 0.24 mM PAH. The stippled columns represent the mean of data from eight tubules treated with probenecid and the open columns the mean of data from eight untreated control tubules. The vertical lines represent ± S.E. of the mean.
C. Excretion of alkaloids by other insects

In these experiments we compared results from Manduca and Rhodnius with two dipteran species, Calliphora erythrocephala and Musca domestica and with a lepidopteran species Pieris brassicae, which does not feed on alkaloid-containing plants. Malpighian tubules from adult Calliphora and Musca all appeared to transport nicotine and atropine at high rates; labelled material appeared in the secreted fluid at 5-10 times the concentration in the bathing fluid. However, chromatography of the secreted fluid showed that in the case of nicotine, at least, the substance excreted was no longer nicotine (Fig. 11).

Tubules from adult Pieris brassicae appeared to transport nicotine at a low rate. But as the concentration rose to no higher than about 20% of that in the bathing solution, this apparent transport is probably no more than passive diffusion into the lumen, as in tubules of adult Manduca (p. 269). On the other hand, six tubules from larvae of Pieris, taken half-way through the 5th instar, secreted fluid containing labelled material at a concentration 12-58 ± 1-73 times higher than in the bathing fluid which contained 5 mM 14C-labelled nicotine.

To collect enough luminal fluid for chromatographic analysis, tubules from larvae of Pieris were cannulated and perfused with fluid. In one experiment, fluid perfused through a tubule at the high rate of 145 nl min⁻¹ was found to contain labelled material 2.95 times more concentrated than in the bathing fluid (0.1 mM). Chromatography using two different solvent systems showed that this was all in the form of unchanged nicotine. These larval tubules are plainly capable of transporting nicotine at very high rates.
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Fig. 11. Plots of the output of a radioactivity scanner to show the position of labelled spots on TLC plates. The samples run were of the bathing fluid containing \(^{14}\text{C}-\text{nicotine}\) and the fluid secreted by Malpighian tubules of \textit{Musca domestica}\ or of \textit{Calliphora erythrocephala}. The solvent systems used to develop the plates were (a) and (c) cyclohexane: chloroform: diethylamine (5:4:1), (b) and (d) chloroform: methanol (80:15). The arrows mark the positions of the origins.

**DISCUSSION**

All the five species of insects examined (\textit{Manduca sexta}, \textit{Rhodnius prolixus}, \textit{Pieris brassicae}, \textit{Calliphora erythrocephala} and \textit{Musca domestica}) have Malpighian tubules which can rapidly remove nicotine from the fluid bathing them. In three cases (\textit{Manduca}, \textit{Rhodnius} and \textit{Pieris}) nicotine is transported unchanged while in \textit{Musca} and \textit{Calliphora}, nicotine is transformed to an unidentified substance. In \textit{Rhodnius}, atropine and morphine are transported by the same system. Vertebrates have long been known to possess a renal transport system for organic bases such as \(N\)-methyl-nicotinamide, choline and histamine. Recently it has been shown that atropine is one of the compounds handled by the system (Acara & Rennick, 1972). It seems likely, therefore, that the system in vertebrates is rather similar to that we have now described for insects. Possibly these systems have evolved in both groups of animals as a counter-measure to alkaloid production by the plants on which they feed.

In \textit{Manduca} the ability of the tubules to transport nicotine correlates well with the nature of the food consumed; tubules from the nectar-feeding adults do not transport nicotine while those from the herbivorous larvae have a pronounced ability to transport nicotine. However, it is not clear why \textit{Rhodnius}, which feeds on blood, should need an ability to transport organic bases. It is conceivable that some product of digestion of the blood meal is excreted by this transport system.

While an ability to excrete alkaloids would seem to be a necessary prerequisite for feeding on alkaloid containing plants, an animal which does so may also need to prevent the access of alkaloid to sensitive sites. 5th stage larvae of \textit{Manduca sexta} injected with 500 \(\mu\)g of nicotine were able to continue feeding normally in spite of
initially having something like $3 \times 10^{-4}$ M nicotine in the haemolymph (Self et al. 1964) but larvae of *Pieris brassicae* were readily killed by eating young cabbage plants which had taken up nicotine through their roots (David & Gardiner, 1953) although they are able to excrete nicotine. *Pieris* larvae of course do not normally feed on an alkaloid-rich diet.

For an insect which may be exposed to alkaloids in its diet or to metabolically produced toxic bases, it would be reasonable to expect it to maintain a transport system for such compounds even if it is not continually used. If it had to rely on the system being induced by the appearance of such a compound in the body, the slowness of the response might be fatal. In the present work we have found that tubules from *Manduca* larvae retain the ability to transport nicotine when fed on an alkaloid-free diet and *Rhodnius* Malpighian tubules are also always capable of nicotine transport, regardless of the animal's physiological state.

Nijhout (1975) has recently shown that Malpighian tubules from larvae of *Manduca sexta* can secrete basic dyes but that this ability is lost as the last larval instar ceases to feed. It seems probable that these dyes are transported by the same system that excretes nicotine. Preliminary experiments have shown that *Rhodnius* tubules will concentrate the basic dye, methylene blue, into the lumen considerably faster than they will concentrate acidic dyes, such as indigo carmine or amaranth. This parallels the much higher rates at which they will transport alkaloids as compared with PAH or benzyl penicillin. It may be possible, therefore, to use the rate of excretion of a dye such as methylene blue as a convenient indicator of an insect's ability to transport organic bases. If so, then the finding that tubules of larvae of *Chironomus* rapidly concentrate neutral red (Salkind, 1930) but tubules of *Periplaneta orientalis*, *Forficula auricularia* and *Carausius morosus* cannot (Lison, 1938), would suggest that a transport system for organic bases is not universal in insects.

**REFERENCES**


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