THE RETENTION OF AMINO ACIDS IN THE HAEMOLYMPH DURING DIURESIS IN RHODNIUS

BY S. H. P. MADDELL AND B. O. C. GARDINER

A.R.C. Unit of Invertebrate Chemistry and Physiology, Department of Zoology, Downing Street, Cambridge CB2 3EJ, U.K.

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SUMMARY

The haemolymph of Rhodnius is rich in amino acids. During the rapid diuresis after a blood meal, no more than trace amounts of amino acids are lost in the urine. There is no significant reabsorption of amino acids in the excretory system. That they escape elimination can instead be attributed to a combination of the low permeability of the Malpighian tubules to amino acids, the very high rate of fluid secretion by the tubules, and the dilution of the haemolymph by an expansion in its volume after feeding. Amino acid losses are low in spite of the fact that the tubules actively accumulate high concentrations of amino acids in their cells and passive losses from these stores augment to some extent the flux of amino acids into the lumen.

At times other than during diuresis, fluid secretion by the Malpighian tubules is slow. Calculations show that haemolymph solutes can then passively reach the higher concentrations in the lumen that are required for the operation of the excretory system (which relies on unselective passive entry and active reabsorption of useful substances).

An advantage of the extraordinarily high rate of fluid secretion during diuresis is that fluid excretion can be rapidly completed. There is then little time for significant amounts of haemolymph solute to be lost passively.

INTRODUCTION

The haemolymph of the blood sucking bug, Rhodnius prolixus contains considerable concentrations of amino acids (Harington, 1961). During the very rapid excretion that follows a blood meal, however, the urine contains only very low concentrations of amino acids, 1% or less of those in the haemolymph. The amino acids that occur in the haemolymph are mostly rather small molecules which would be expected to penetrate the Malpighian tubules with ease (Ramsay, 1958, Maddrell & Gardiner, 1974).

That amino acids escape elimination in vivo suggests that they are rapidly reabsorbed from the primary fluid produced by the Malpighian tubules. This paper reports experiments made to see whether this is the case. Surprisingly we have found that amino acids are not reabsorbed at significant rates. Instead, amino acids are retained by the unexpectedly low permeability of the Malpighian tubules in Rhodnius and by the very high rate of fluid secretion. Amino acids cross the wall of the tubules at a rate which is so slow relative to the rate of fluid secretion that only small amounts are lost before diuresis ends.
MATTERIAls AND METHODS

The insects used were 5th instar *Rhodnius prolixus* taken from a laboratory culture 1–2 weeks after ecdysis from the 4th instar. The insects were kept at 28 °C and all experiments were done at room temperature (21–24 °C).

Preparations of isolated Malpighian tubules were made as previously described (Maddrell, 1969). A recent improvement has been the discovery that the tubules secrete faster and for longer if they are each kept in bathing drops larger than 100 µl. Most of the experiments used bathing drops of 125 or 150 µl. To stimulate fast fluid secretion, 5-hydroxytryptamine (5-HT) at about $10^{-5}$ M was included in the bathing solution (Maddrell, Pilcher & Gardiner, 1971).

As tracer substances, we used radioactive amino acids and urea from the Radiochemical Centre, Amersham. One difficulty we had is worth recording. In our experiments we consistently found that $^3$H-labelled compounds appeared to cross the tubule wall faster than did $^{14}$C-labelled substances. We believe that this is due to the small but non-negligible amounts of tritium label that exchange with hydrogen in the solvent water molecules. Such labelled water molecules are rapidly transported into the lumen in the rapid secretion of fluid carried out by the tubule. There they contribute to the radioactive content of the secreted fluid. In subsequent chromatography of the secreted fluid, the ‘extra’ counts are lost by evaporation and the drops of secreted fluid appear only to contain radioactive amino acid. To avoid this difficulty, we have used only $^{14}$C-labelled substances in the experiments we describe here. We used standard scintillation counting techniques, first with an Intertechnique ABAC SL40, but after its demise through old age, we subsequently used a Packard Tri-Carb 3225.

To determine whether $^{14}$C-labelled amino acids were metabolized in crossing the walls of the Malpighian tubules, samples of the secreted fluid and of the amino acids as supplied were subjected to thin layer chromatography using Polygram CEL 300. For each amino acid, at least two of the following solvent systems were used; n-butanol : acetic acid : water, 12 : 3 : 5; ethanol : ammonia : water, 20 : 1 : 4; n-butanol : pyridine : water, 1 : 1 : 1. The developed chromatograms were scanned with a Berthold Scanner LB 2722.

The technique for the cannulation and perfusion of Malpighian tubules is described in Maddrell *et al.* (1974).

The saline solutions used were as follows. Standard saline contained (mM); NaCl 129, KCl 8.6, CaCl$_2$ 2.0, MgCl$_2$ 8.5, NaHCO$_3$ 10.2, Na$_2$HPO$_4$ 4.3, glucose 34; this gives a solution of pH 6.7 and of osmotic concentration 342 m-osmol l$^{-1}$. A variant of this was made to give a medium containing several amino acids in amounts corresponding to those found in a preliminary amino acid analysis of the haemolymph of recently fed insects. It contained (mM): NaCl 120, KCl 5, CaCl$_2$ 2, MgCl$_2$ 2, NaHCO$_3$ 10.6, Na$_2$HPO$_4$ 4.4, glucose 15, sodium citrate 5, sodium succinate 5, L-proline 18, L-alanine 6.33, L-serine 1.94, L-valine 1.75, glycine 1.62, L-iso-leucine 0.90, taurine 0.64, L-leucine 0.42, L-histidine 0.41, L-lysine 0.30, and L-arginine 0.26. Fluid perfused through Malpighian tubules was either the standard saline or one modified so as to be similar in composition to that naturally occurring in the lumen during fast fluid secretion (Maddrell, 1969). The latter solution had the following composition...
Retention of amino acids in the haemolymph

Table 1. Concentrations of amino acids in the haemolymph and urine of Rhodnius 1–2 h after feeding

<table>
<thead>
<tr>
<th>Amino Acid</th>
<th>Haemolymph (mm)</th>
<th>Urine (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Proline</td>
<td>18 ± 2.9</td>
<td>0.87</td>
</tr>
<tr>
<td>Serine</td>
<td>1.9 ± 0.4</td>
<td>0.002</td>
</tr>
<tr>
<td>Glutamate</td>
<td>0.2 ± 0.1</td>
<td></td>
</tr>
<tr>
<td>Glycine</td>
<td>1.6 ± 0.3</td>
<td>0.012</td>
</tr>
<tr>
<td>Alanine</td>
<td>6.2 ± 0.4</td>
<td>0.031</td>
</tr>
<tr>
<td>Valine</td>
<td>1.8 ± 0.3</td>
<td>0.011</td>
</tr>
<tr>
<td>iso-Leucine</td>
<td>0.9 ± 0.1</td>
<td></td>
</tr>
<tr>
<td>Leucine</td>
<td>0.8 ± 0.1</td>
<td></td>
</tr>
<tr>
<td>Phenylalanine</td>
<td>0.2 ± 0.1</td>
<td></td>
</tr>
<tr>
<td>Lysine</td>
<td>0.4 ± 0.1</td>
<td>0.011</td>
</tr>
<tr>
<td>Histidine</td>
<td>0.6 ± 0.2</td>
<td>0.009</td>
</tr>
<tr>
<td>Arginine</td>
<td>0.4 ± 0.1</td>
<td>0.014</td>
</tr>
<tr>
<td>Taurine</td>
<td>0.6</td>
<td>0.160</td>
</tr>
<tr>
<td>Ornithine</td>
<td>Present not estimated</td>
<td></td>
</tr>
<tr>
<td>Tyrosine</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Three samples of haemolymph were analyzed and, except for taurine (one determination only), the values shown are the mean ± S.E.M. The urine concentrations were so low that one analysis only was performed on a large pooled sample.

Amino acids in the haemolymph and urine

Samples of haemolymph and urine were taken from fed 5th stage Rhodnius shortly after feeding, in the period when rapid excretion is occurring. The amino acid contents of these fluids is shown in Table 1. It is clear that although there are considerable quantities in the haemolymph, losses of most amino acids in the urine are minimal. An exception to this is taurine which is lost much more quickly. Taking the volume of the haemolymph after feeding to be about 35 μl (p. 324), its content of amino acids is about 1225 nmol (35 μl at 35 mm). Assuming that a fed insect eliminates about 140 μl of urine (Maddrell, 1964) of the composition shown in Table 1, then about 24 nmol of amino acids other than taurine would be lost. This represents less than 2% of the haemolymph content.

The question arises as to how the excretory losses of amino acid are kept so low. An obvious possibility was that amino acids might be reabsorbed after they had penetrated into the lumina of the Malpighian tubules.
Table 2. Appearance of amino acids and urea in the fluid secreted by upper Malpighian tubules of Rhodnius

<table>
<thead>
<tr>
<th>Amino acid</th>
<th>Concentration in secreted fluid as % of that in bathing fluid</th>
<th>Rate of fluid secretion (nl min⁻¹)</th>
<th>Number of tubules</th>
</tr>
</thead>
<tbody>
<tr>
<td>Proline</td>
<td>56.9 ± 2.7</td>
<td>57.4 ± 2.3</td>
<td>65</td>
</tr>
<tr>
<td>Alanine</td>
<td>31.0 ± 3.2</td>
<td>55.4 ± 1.8</td>
<td>49</td>
</tr>
<tr>
<td>Serine</td>
<td>11.7 ± 1.6</td>
<td>44.9 ± 2.0</td>
<td>13</td>
</tr>
<tr>
<td>Valine</td>
<td>18.4 ± 1.9</td>
<td>58.7 ± 2.1</td>
<td>30</td>
</tr>
<tr>
<td>Glycine</td>
<td>56.1 ± 3.2</td>
<td>58.5 ± 1.6</td>
<td>96</td>
</tr>
<tr>
<td>Lysine</td>
<td>29.9 ± 1.6</td>
<td>59.7 ± 2.2</td>
<td>14</td>
</tr>
<tr>
<td>Urea</td>
<td>82.1 ± 2.6</td>
<td>58.0 ± 2.5</td>
<td>20</td>
</tr>
</tbody>
</table>

In each case, the concentration of the test substance in the bathing medium was close to 0.01 mM.

Penetration of amino acids through the walls of the Malpighian tubules

For these experiments, we isolated the upper fluid secreting parts of the Malpighian tubules into standard saline containing 10⁻⁵ M 5-HT and about 0.01 mM of one of the following radioactive amino acids: proline, alanine, serine, valine, glycine, and lysine giving a medium containing around 20000 cpm μl⁻¹. During the next hour we collected samples of the secreted fluid and measured their radioactive content and compared it with that of the bathing medium. The results are shown in Table 2 as are those of similar experiments using urea. It appeared that all these amino acids, among which are the five most concentrated in the haemolymph (Table 1), crossed the Malpighian tubule walls without difficulty. This was not unexpected as urea, of molecular weight 62, crossed even more readily (Table 2) and is similar in structure to, and a little smaller than, glycine.

At first sight, then, it seems that extensive reabsorption of these amino acids must occur in vivo, if the final urine eliminated is to have the composition shown in Table 1. To test this, the following experiment was devised.

Possible reabsorption of amino acids in the lower Malpighian tubules

Potassium chloride secreted into the lumina of the upper Malpighian tubules is reabsorbed, during diuresis, by the downstream, lower lengths of the tubules (Maddrell & Phillips, 1975). It seemed reasonable therefore to suppose that amino acid reabsorption might also occur in the lower tubules, especially as they secrete no fluid. That only the lower-most one third of the lower tubule absorbs KCl (Maddrell, 1978), made it attractive to suggest that the remainder of the lower tubule might be involved in the rapid recovery of amino acids. To test these ideas two types of experiments were carried out. In the first, pairs of tubules from the same insect were isolated, one of them entire, i.e. both the upper and lower regions, and the other consisting of the upper part only. Both tubules were placed in a single bathing drop of standard saline with 10⁻⁵ M 5-HT and 0.01 mM radioactive glycine. We compared the radioactive content of the drops appearing at the cut ends of the tubules which were held a short distance away from the bathing drop. The results showed no evidence of any reabsorption by the lower tubule; the fluid emerging from the cut end of the lower tubule contained radioactive glycine at a concentration of 50.1 ± 3.2 μl⁻¹.
Retention of amino acids in the haemolymph

Table 3. Appearance of amino acids in fluid secreted by Malpighian tubules of Rhodnius bathed in amino acid-containing saline

<table>
<thead>
<tr>
<th>Amino acid</th>
<th>Concentration of amino acid in secreted fluid as % of that in bathing medium</th>
<th>Number of tubules</th>
</tr>
</thead>
<tbody>
<tr>
<td>Proline</td>
<td>1.8 ± 0.2</td>
<td>12</td>
</tr>
<tr>
<td>Alanine</td>
<td>2.2 ± 0.2</td>
<td>28</td>
</tr>
<tr>
<td>Valine</td>
<td>2.3 ± 0.4</td>
<td>8</td>
</tr>
<tr>
<td>Glycine</td>
<td>2.9 ± 0.3</td>
<td>17</td>
</tr>
<tr>
<td>Serine</td>
<td>1.0 ± 0.2</td>
<td>10</td>
</tr>
</tbody>
</table>

In each case, the rate of fluid secretion by the tubules was about 60 nl min⁻¹.

(n = 12) of that in the bathing medium while the fluid from the upper tubule alone contained glycine at 50.7 ± 3.2% (n = 11) of the concentration of the medium.

A second type of experiment was done in which fluid similar in composition to that secreted by the upper tubules and containing 0.01 mM radioactive glycine was perfused through a lower tubule from a cannula. The fluid emerging from the cut end was assayed for its radioactive content. In none of four trials, in two of which 5-HT at 10⁻⁵ M was included in the bathing medium, did the emergent fluid prove to have significantly less radioactive content than the perfused fluid (the emergent fluid content of glycine was 99.3 ± 0.7% (n = 8) of that in the perfused fluid).

The possibility of reabsorption of amino acids by the upper Malpighian tubules

The results of both the above series of experiments give no evidence of amino acid reabsorption by the lower tubule. One possible explanation for this might be that the standard saline was lacking some important constituent. It is known, for example, that Malpighian tubules of Pieris brassicae require a saline containing several amino acids for maintained secretion (Nicolson, 1976) and we have found that the lower Malpighian tubules of Rhodnius similarly require a medium enriched with amino acids if they are to reabsorb KCl for extensive periods (Maddrell & Gardiner, unpublished results). Accordingly, we repeated the experiments examining the handling of radioactive amino acids by entire tubules and upper tubules, with the tubules bathed in the more complete medium containing amino acids in amounts similar to those found in the haemolymph (for details of the composition of this medium see p. 317). Under these conditions the fluid produced by the tubules contained amino acids at concentrations which were much smaller fractions of those in the bathing fluid than before (Table 3). It seemed, therefore, that reabsorption of amino acids had occurred and, surprisingly, it appeared that this had already happened by the time the fluid had left the upper tubule. It seemed odd that reabsorption should occur in the permeable upper part of the tubule, but a precedent is that glucose reabsorption occurs in the permeable Malpighian tubules of Calliphora vomitoria (Knowles, 1975). In an attempt to investigate the phenomena further, we cannulated upper Malpighian tubules of Rhodnius and perfused them with simulated secreted fluid containing 0.01 mM radioactive glycine and bathed the tubules in an amino acid-containing medium with the same concentration of radioactive glycine. In one of four trials did the fluid that was passed through the tubule lumen lose signifi-
A Reabsorption of amino acids from intercellular clefts

B Lumen-directed active transport of amino acids

Slow passive penetration of amino acids

Fig. 1. Alternative ways in which amino acids might be treated by the walls of Malpighian tubules. In (A), amino acids diffusing passively (broken line) into the lumen through the intercellular clefts are reabsorbed actively (open arrow) into the bathing medium. In (B), slow passive movements of amino acids into the lumen (broken line) are supplemented by an easily saturated, lumen-directed active transport process (open arrow).

cant amounts of radioactive glycine. In case this was due to suppression of reabsorption by the glucose content of the perfused fluid, the experiments were repeated with sugar-free fluid run through the tubule lumen. No amino acid reabsorption occurred (four trials). At this stage it appeared that amino acid reabsorption must occur not from the lumen but as the amino acids diffused through the tubule wall – presumably from the intercellular clefts. Alternatively, the rapid penetration of radioactive ami
Retention of amino acids in the haemolymph

Table 4. Concentrations of glycine in fluid secreted by Malpighian tubules at rates reduced with atropine or by omitting either sodium or 5-HT from the bathing solution. In each case, the bathing solution contained 0.01 mM glycine

<table>
<thead>
<tr>
<th>Bathing solution</th>
<th>Average rate of fluid secretion (nl min⁻¹)</th>
<th>Average concentration of glycine in secreted fluid as % of that in bathing medium</th>
<th>No of tubules</th>
</tr>
</thead>
<tbody>
<tr>
<td>Standard saline + 10⁻⁶ M 5-HT</td>
<td>12.2</td>
<td>230</td>
<td>4</td>
</tr>
<tr>
<td>Standard saline + 2 x 10⁻⁶ M atropine</td>
<td>24.4</td>
<td>130</td>
<td>4</td>
</tr>
<tr>
<td>Standard saline + 1 x 10⁻⁶ M atropine</td>
<td>11.2</td>
<td>235</td>
<td>6</td>
</tr>
<tr>
<td>Na-free saline + 10⁻⁶ M 5-HT</td>
<td>0.42</td>
<td>535</td>
<td>7</td>
</tr>
</tbody>
</table>

Concentrations of glycine in fluid secreted by Malpighian tubules at rates reduced with atropine or by omitting either sodium or 5-HT from the bathing solution. In each case, the bathing solution contained 0.01 mM glycine.

Acid into the lumina of tubules bathed in fluid containing only trace amounts of that amino acid and the suppression of this in a medium containing several mM of amino acid, might result from an active transport of amino acid towards the lumen which would be easily saturated by more than a relatively low concentration of amino acid in the bathing medium. This latter possibility seemed unlikely as such a process would act to drain the haemolymph of its content of amino acids. In addition, the tubule would have to have a low passive permeability to amino acids, even those of small size such as glycine, in order to explain the low concentrations of amino acids which appear in fluid secreted by tubules bathed in amino acid-enriched media. The two alternative possibilities are illustrated in Fig. 1.

Surprisingly, it turns out that there is active transport of amino acids towards the lumen and that the tubule wall is relatively impermeable to amino acids. The experimental evidence for this is that when Malpighian tubules were bathed in saline containing 5-HT to stimulate fluid secretion, but with atropine added to reduce the rate of secretion (Maddrell & Gardiner, 1976a), the tubules slowly produced fluid containing radioactive glycine at concentrations higher than was in the bathing medium (Table 4). Similar results came from experiments with tubules bathed in stimulant-free saline (Table 4). Interestingly, when the secretory rate of the tubules was slowed by the omission of sodium from the 5-HT containing saline (Maddrell, 1969), the tubules still produced fluid containing high concentrations of glycine (Table 4). Many other amino acid transport systems, in insects as well as in vertebrates are sodium-dependent (Smith & Ellory, 1971; Balshin & Phillips, 1971).

Active accumulation of amino acids by Malpighian tubule cells

What might be the function of this apparently wasteful process? The most likely explanation is that Malpighian tubule cells normally contain significant amounts of amino acids and that they actively accumulate these from the extracellular fluid as do many tissues in both invertebrates (Evans, 1973) and vertebrates (Heinz & Geck, 1974). After dissection in amino acid-free saline they might well, therefore, rapidly
take up amino acid when subsequently placed in bathing fluid containing amino acid. If the tracer content of the cell rose to high levels, even a slow passive loss to the lumen might significantly raise the concentration there. That this is most likely what actually occurs was shown by experiments in which the cellular content of tracer amino acid was monitored after a 25 min period of secretion in tracer-containing bathing medium. To do this we compared the amount of radioactive glycine to be found in whole tubules with that found in equal volumes of luminal fluid. The results showed that the tubule walls contained levels of glycine up to 300 times more concentrated than the bathing medium (average $224 \pm 18$, $n = 12$). It is easy to believe that slow loss of tracer to the lumen through the very large interface that the cells have with the lumen by virtue of their close packed apical microvilli could result in tracer concentrations in the lumen of the same order as that in the bathing medium.

Further support for this explanation comes from measuring the rate of accumulation of tracer glycine in the cells and at the same time following the appearance of glycine in the secreted fluid. Both follow similar time courses (Fig. 2), as they must of course if they are causally related. We propose then that significant quantities of amino acids reach the lumen of the Malpighian tubule by being first actively taken up into the cells of the wall and then leaking passively into the lumen.

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**Fig. 2.** The rates of appearance of radiolabelled glycine in the cells (broken line) and in the lumina (continuous line) of Malpighian tubules. The values plotted are the means and the vertical lines attached represent ± S.E.M.
Retention of amino acids in the haemolymph

Permeability of the upper Malpighian tubule to amino acids

As amino acids are subject to transport towards the lumen, one cannot measure directly the passive permeability of the tubule wall to amino acids. However, as Table 3 shows, by making measurements on tubules which are bathed in a saline containing amino acid levels corresponding to those in the haemolymph, the rate of clearance from the bathing solution is much reduced. Presumably the mechanism for active uptake into the cells is then saturated and becomes of less importance in determining the overall entry of amino acid into the tubule lumen.

To investigate this, we measured the rates at which glycine appeared in the secreted fluid from various concentrations in the bathing fluid. The results are shown in Fig. 3 and can be interpreted as showing both a saturable active process and a passive component in which glycine slowly diffuses through the tubule wall into the lumen. The rapidly saturating component has the properties of an active transport of glycine towards the lumen with a $V_{\text{max}}$ of about 9 pmol min$^{-1}$ and a $K_t$ of close to 0.5 mM. That a fraction of the glycine reaching the lumen arrives there passively is indicated by the finding that glycine passage into the lumen continues to increase, though more slowly, as the concentration in the bathing medium is raised above about 1.5 mM. This passive entry is at a rate consistent with a wall permeability to glycine of about 0.10 nl min$^{-1}$ mm$^{-2}$.

From the point of view of the overall loss of amino acids through the Malpighian tubules in vivo, it is, of course, the net effect of both active and passive transport which is important. We have measured the transport of various amino acids into the
fluid secreted by Malpighian tubules bathed in saline containing amino acids at concentrations similar to those of the haemolymph. If one ignores for the moment that entry into the lumen is partly actively assisted, one can calculate ‘effective permeabilities’ from the results as if entry was purely passive. The effective permeabilities were as follows: for proline, $0.09 \pm 0.01 \text{ nl min}^{-1} \text{mm}^{-2}$ (12); alanine $0.18 \pm 0.02 \text{ nl min}^{-1} \text{mm}^{-2}$ (28); serine $0.07 \pm 0.02 \text{ nl min}^{-1} \text{mm}^{-2}$ (10); valine $0.19 \pm 0.02 \text{ nl min}^{-1} \text{mm}^{-2}$ (8); and for glycine $0.16 \pm 0.02 \text{ nl min}^{-1} \text{mm}^{-2}$ (30). In fact, of course, since active uptake into the cells is responsible for adding to the overall movement into the lumen, the actual passive permeability of the wall of the upper Malpighian tubule of *Rhodnius* to these amino acids must be even lower.

In these experiments to determine the permeability of the tubules to amino acids, it was important of course to be sure that the radioactive counts appearing in the secreted fluid could be attributed to amino acid in unchanged form. To confirm this, samples of the secreted fluid were subjected to chromatography. This showed that none of the five important amino acids, glycine, alanine, proline, serine, and valine had been significantly metabolized in these experiments.

*The role of increased haemolymph volume in restricting amino acid loss.*

Samples of haemolymph are considerably easier to obtain from freshly fed *Rhodnius* than they are from unfed insects. It was found that the haemolymph volume rapidly increases from $16.9 \pm 1.1 \mu l$ ($n = 12$) before feeding to $36.2 \pm 1.3 \mu l$ ($n = 45$) within about half an hour of the termination of feeding. It seemed likely that this would give rise to a corresponding decrease in the concentrations of amino acids in the haemolymph. Measurements showed that there is indeed such an effect. Using a ninhydrin technique, we found that the concentrations of proline and other ninhydrin-positive substances in the haemolymph were reduced by $43.7 \pm 2.9\%$ (proline; $n = 43$) and $33.7 \pm 2.5\%$ (other ninhydrin-positive substances; $n = 40$) within an hour of feeding. The importance of this in the present context is that the reduction decreases the driving force for diffusive loss of amino acids into the fluid secreted by the Malpighian tubules. The effect that this is likely to have is shown in Fig. 4.

**DISCUSSION**

The most important finding of the present work is that *Rhodnius* Malpighian tubules have only a low permeability to amino acids. Two questions arise. First, is the low permeability of *Rhodnius*’ tubules sufficient to explain the very low amino acid content of the urine during diuresis? Secondly, how is such a low permeability to be reconciled with the widely accepted idea that Malpighian tubules are freely permeable to a range of haemolymph solutes, and that the excretory system functions by producing a primary excretory fluid, rich in most substances from the haemolymph, which is altered by selective reabsorption so that a fluid of appropriate composition is excreted?

To answer the first question, a simple calculation will allow one to estimate the concentrations of amino acids expected in the primary excretory fluid produced *in vivo* by the upper Malpighian tubules. At $24^\circ C$ a 5th stage *Rhodnius* eliminates fluid
Table 5. Concentrations of amino acids in the haemolymph and urine of fed Rhodnius and the concentrations of amino acids expected to be in the fluid secreted by the upper Malpighian tubules

<table>
<thead>
<tr>
<th>Amino acid</th>
<th>Concentration of amino acid in the haemolymph (mM)</th>
<th>Concentration of amino acid expected in tubule fluid (μM)</th>
<th>Concentration of amino acid found in urine (μM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Proline</td>
<td>18</td>
<td>63</td>
<td>87</td>
</tr>
<tr>
<td>Alanine</td>
<td>6</td>
<td>37</td>
<td>31</td>
</tr>
<tr>
<td>Serine</td>
<td>1.9</td>
<td>4.6</td>
<td>2.2</td>
</tr>
<tr>
<td>Valine</td>
<td>1.8</td>
<td>11.9</td>
<td>11.2</td>
</tr>
<tr>
<td>Glycine</td>
<td>1.6</td>
<td>8</td>
<td>12.4</td>
</tr>
</tbody>
</table>

at about 800 nl min⁻¹ (Maddrell, 1964). Assuming this accurately reflects the rate of fluid secretion by the four upper tubules, it follows that each secretes fluid at 200 nl min⁻¹. Taking the effective permeabilities shown on p. 324, one can now calculate the concentrations of amino acids expected to be reached passively in the fluid secreted by the upper Malpighian tubules. This is done by substituting the permeability values in the formula derived by Ramsay (1958):

\[ S = \frac{Hb}{a + b} \]  

where \( S \) is the concentration of the amino acid in the secreted fluid, \( H \) is the concentration of the amino acid in the haemolymph, \( b \) is the effective permeability of the wall (nl min⁻¹ mm⁻²), and \( a \) is the rate of fluid secretion per unit area of tubule (nl min⁻¹ mm⁻²). This formula effectively makes allowance for the fact that the net rate of entry of solute into the tubule lumen is significantly reduced by passive loss from the lumen when the solute concentration there is an appreciable fraction of that in the bathing medium. The results of the calculations are shown in Table 5, as are the amino acid levels found to occur in the urine. Although the fit is not perfect, the two sets of figures agree closely enough to make it unnecessary to look further than the low permeability of the upper Malpighian tubules for an explanation of the low rate of loss of amino acid in the urine of fed Rhodnius.

To answer the second question, it is helpful to recall that for more than 99% of the time, Rhodnius Malpighian tubules secrete fluid in vivo at very slow rates, of the order of 1000× more slowly than during the diuretic phase, with which we have been concerned in this paper. Reworking the figures given in Table 5 for tubules secreting 1000× more slowly gives radically different results. The calculations show that glycine would appear in the fluid formed by the upper Malpighian tubules at 89% of the concentration of that occurring in the haemolymph. The figure for alanine is 86%, for proline 77%, for serine 72%, and for valine 86%. In short, at their usual slow rates of fluid secretion, the upper Malpighian tubules function as organs producing a primary excretory fluid not dissimilar in amino acid composition from that of the haemolymph. So, although their permeability is low, it is adequate for them to function appropriately at slow rates of fluid secretion. At high rates of fluid secretion, on the other hand, their low permeability automatically confers the advantage of retaining haemolymph solutes without the need for reabsorption.
Presumably, however, at low rates of fluid secretion, reabsorption of useful solutes does occur. Since this would only need to be slow, it is not then surprising that no evidence of reabsorption was seen in the experiments described on pp. 319 and 320 in which fluid was run through the tubules at high rates. It will be interesting to repeat the experiments using very much lower rates of perfusion.

An explanation is needed for the very high rates of penetration of urea shown in Table 2. How can such rapid permeation occur through the relatively impermeable walls of the tubules? It is not the case, as with amino acids, that lumen-directed active transport occurs; penetration is just as rapid in the presence of 40 mM-urea (unpublished results). The effective permeability of the tubule wall to urea is more than 500 times higher than for glycine. The most likely explanation for this is that urea is able not only to cross the walls through the intercellular clefts but also to move through the cell membranes, as in other tissues (Wright & Pietras, 1974). The basal infoldings and apical microvilli of the tubule cells offer an extremely large area of membrane through which such movement might occur.

To return again to the main theme of this discussion, it is now possible to see the very rapid rate of fluid excretion by fed *Rhodnius* in a new light. If the prevention of loss of haemolymph solutes during diuresis depends very largely on the low permeability of the tubules and not to any extent on a reabsorptive process, it becomes advantageous for fluid secretion to be as rapid as possible. In this way not only is the loss of haemolymph solutes kept at a minimum, but the insect rapidly recovers its shape, reduces its weight, and concentrates the meal prior to digestion. To emphasize the importance of rapid fluid secretion in avoiding the loss of haemolymph amino acids, Fig. 4 shows how the loss to be expected by diffusion through the walls of the Malpighian tubules during diuresis depends on the rate at which they secrete fluid. The calculations are based on equation 1, which gives the concentration of amino acid in the fluid secreted by the upper tubules. If it is assumed that no further amino acid is added to or removed from the excretory fluid, the instantaneous rate of loss of amino acid through one tubule is

\[
\frac{RHb}{a+b},
\]

where \( R \) is the rate of fluid secretion by the Malpighian tubule, and \( H, a \) and \( b \) have the same meanings as before. The total loss to be expected during diuresis has to take into account the declining concentration in the haemolymph and the fact that the faster the fluid elimination, the less time there is during which loss of amino acid can occur. These factors are taken into account in the following manner.

If the instantaneous rate of loss of amino acid through a single tubule is given by equation 2 above, then the rate of change of concentration brought about by all four tubules is

\[
\frac{dH}{dt} = \frac{-4RHb}{V_h(a+b)}
\]

where \( V_h \) is the haemolymph volume. This has the form \( dH/dt = -kH \) and yields, on integration,
Retention of amino acids in the haemolymph

Retaining of amino acids in the haemolymph

70 r

100 150 200

Rate of fluid secretion by each Malpighian tubule (nl min⁻¹)

Fig. 4. To show the losses of haemolymph amino acids expected during diuresis in fed Rhodnius when fluid excretion occurs at different rates. For the calculation it was assumed that the tubule wall has an effective permeability to amino acids of 0.15 nl min⁻¹ mm⁻¹, and that diuresis involves the elimination of 140 µl of fluid. The lower curve shows the reduced losses to be expected from the doubling of the haemolymph volume after feeding, assuming that this halves the concentrations of amino acids in the haemolymph. The upper curve shows the losses that would occur if there were no change in haemolymph volume.

\[ \ln H = -kt + \text{constant} \]  

\[ H_t = H_0 e^{-kt} \]  

The accrued loss of amino acid from the haemolymph at time \( t \) is \( V_h(H_0 - H_t) \) and the loss as a fraction of that initially present is

\[ \frac{V_h(H_0 - H_t)}{V_h.H_0} \quad \text{or} \quad \text{fractional loss} = 1 - \frac{H_t}{H_0} \]

and substituting from 5 and 3,

\[ \text{fractional loss} = 1 - e^{-\left(\frac{4Rb}{V_h(a+b)}\right)t} \]  

The total loss incurred during diuresis depends on the time taken to eliminate all excess fluid. If \( V_e \) is the volume eliminated, then the time taken to eliminate it is
Substituting this value in equation (7) we have that the fractional loss incurred in diuresis is

\[
1 - e^{-\left[\frac{V_ub}{V_a(a+b)}\right]}. \tag{8}
\]

The way that this depends on the rate of fluid secretion by the tubules is shown in Fig. 4. It is clear from Fig. 4 that amino acid losses are dramatically cut when the rates of fluid secretion are as high as those found in vivo. The tubules of many other insects can reach rates of fluid secretion as high as 30–40 nI min\(^{-1}\) during hormonal stimulation (Maddrell, 1979). Fig. 4 shows that such relatively low rates would be of little use to \textit{Rhodnius} as it could then lose more than a third of its haemolymph content of amino acids. It seems that this consideration must be an important factor in explaining the apparently needlessly high rates of fluid secretion by the Malpighian tubules of blood sucking insects immediately after feeding; a tubule which secretes fluid at 300 \mu l min\(^{-1}\) (which can be achieved in vivo, see Maddrell, 1964) is transporting fluid at a rate of close to 70 \mu l s\(^{-1}\) gm of tubule\(^{-1}\). Fig. 4 also shows how the doubling of the haemolymph volume that occurs after feeding further reduces the loss of amino acid from the haemolymph, effectively by reducing the concentration of amino acid in the haemolymph.

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\textbf{REFERENCES}


Retention of amino acids in the haemolymph


