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ABSORPTION OF FLUID BY THE MIDGUT OF *RHODNIUS*

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SUMMARY

1. Isolated midguts of 5th-instar *Rhodnius prolixus* will transport fluid from the lumen that is close to iso-osmotic with the luminal contents.

2. The transported fluid contains sodium and chloride ions as its major constituents.

3. Fluid transport can be attributed to active transport of sodium ions from the lumen. The haemolymph side of the epithelium, towards which transport is directed, is at a potential positive with respect to the lumen; this potential difference is greatly increased if the lumen contains only impermeant anions, and the rate of fluid transport is strongly dependent on the concentration of sodium ions in the luminal fluid.

4. The rate of fluid transport is increased approximately six times by treatment with 5-hydroxytryptamine (2×10^{-7} M) or cyclic AMP (2×10^{-3} M). The transepithelial potential is increased by such treatment but the major effects are on the short-circuit current, which increases by about five times, and on the electrical resistance of the epithelium, which falls to about a quarter of its earlier value.

INTRODUCTION

Rhodnius prolixus is a blood-sucking bug which takes very large meals; in the young stages the meals are often twelve times the weight of the unfed insect (Buxton, 1930). To recover from some of the problems arising from the ingestion of such large amounts of fluid, the insect rapidly excretes a large quantity of urine (Wigglesworth, 1931*a*). The activity of the excretory system in this diuresis has been much studied (Wigglesworth, 1931*b*; Ramsay, 1952; Maddrell, 1963, 1976). This, however, is only part of the story as the excretory system removes fluid from the haemolymph while the ingested blood is taken into the expanded anterior part of the midgut. The present paper describes the other necessary part of the process – that in which excess fluid in the blood meal is first absorbed into the haemolymph by the activity of the anterior midgut so that it can then be eliminated by the excretory system. Rather little is yet known about fluid transport by the insect midgut (Treherne, 1967), so an investigation of the very rapid fluid absorption shown by *Rhodnius* midgut is especially timely. We have found that this absorptive mechanism appears to be driven by active transport of sodium ions, which is of interest as virtually all other fluid transport systems in insects depend primarily on transport of potassium ions.

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MATERIAL AND METHODS

Fifth-stage larvae of *Rhodnius prolixus* from a laboratory culture maintained at 28 °C were used in all the experiments. They were taken three to five weeks after their previous meal, at which time little residue of the meal was present in the anterior midgut. Midguts were isolated from these insects by dissection under standard *Rhodnius* saline solution (Maddrell, 1969).

Isolated preparations of midguts

For measurements of the rates at which midguts transport fluid they were mounted on cannulae made by pulling out 50 μl 'Microcap' pipettes (Drummond Scientific Co.) and held in a chamber through which fluid of any desired composition could be passed. The chamber, with an approximate volume of 200 μl, was moulded in 'Silastic' mould-making rubber (Dow Corporation). Solution entered at the bottom of the chamber via a pipe made from a 19-gauge syringe needle and was removed from the top through a similar tube connected to a vacuum pump. The cannula entered the chamber through a slit in the rubber; this gap was made watertight with a coating of silicone grease. The portion of the cannula outside the chamber was supported on a small platform; a linear scale, marked in millimetres, was fixed to this platform to lie parallel to the cannula, and the length of cannula projecting into the chamber was coated with a thin layer of rubber solution. The whole apparatus (Fig. 1) was viewed from above with a binocular microscope.

Technique for setting up the isolated preparation

Before starting an experiment, normal saline was run into the chamber from a tap-funnel. By applying suction through a vinyl tube connected to the wider end of the cannula the desired quantity of saline was drawn in. Further entry of fluid into the cannula caused by capillary action was prevented by closing the vinyl tube with a spring clip. At this stage the dissected tissue was placed in the chamber and the end of the cannula introduced into the lumen of the anterior midgut via the cut remnant of the narrow posterior part of the midgut. The preparation was then secured to the cannula by a ligature of fine silk tied round the intestine close to its junction with the anterior midgut. The coating of rubber applied to this region of the cannula ensured that the attachment did not slip and prevented fluid leaking past the ligature. A cut was made in the anterior midgut close to its junction with the foregut. By grasping this cut edge in forceps the sac of tissue could be carefully reflected along the cannula to expose the luminal surface of the epithelium to the fluid in the chamber. A flow of normal saline through the chamber was used to wash away the adherent gut contents. The use of starved insects kept such contaminants at a minimum. The tissue was then gently pulled back along the cannula and the open end of the preparation was tied off. By following this procedure, the tissue handled with forceps while setting up the preparation was separated by the ligatures from that region of the epithelium on which observations were to be made.

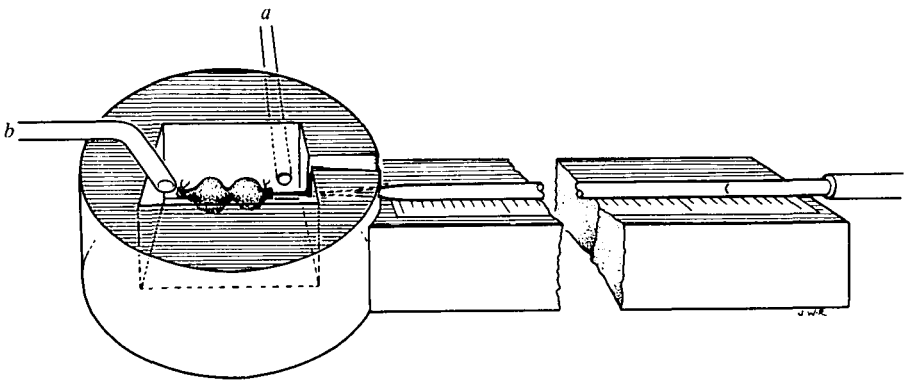

Fig. 1. Apparatus used to measure rates of fluid transport by isolated midguts of *Rhodnius*. The midgut is held in the chamber to the left. Saline is run into the chamber through pipe *a* and removed by suction through pipe *b*. For further explanation, see text.

Measurement of fluid movement across the anterior midgut

With the preparation tied in place, a volume of normal saline was contained in the closed system formed by the cannula and the sac of anterior midgut epithelium. Gentle suction applied via the vinyl tube and the capillary action of the glass were used to draw the fluid from the lumen of the preparation into the cannula. Observation from above with a binocular microscope fitted with a micrometer eyepiece enabled the position of the fluid meniscus in the cannula to be defined relative to the adjacent linear scale with an accuracy of one micrometer unit (0·08 mm). Approximately 10 μl of saline was transferred from the cannula to the lumen of the preparation by applying pressure via the vinyl tube, which was then held closed. Subsequent repetition of this sequence of actions revealed any movement of fluid across the anterior midgut epithelium as changes in the position of the meniscus in the cannula. As the unaltered portion of the 'Microcap' pipette is of constant bore these linear measurements were readily converted to measurements of volume. The linear measurements were reproducible within a range of 0·2 mm, giving an accuracy for any volume measurement of $\pm$ 0·1 μl.

Throughout any one experiment the luminal surface of the tissue was exposed only to that solution originally introduced into the cannula. However, the solution bathing the basal surface could readily be altered by connecting different tap-funnels to the inlet pipe of the chamber. Experiments with solutions coloured with dye showed that such an exchange of solutions was complete within 30 s. Oxygen, saturated with water vapour, was bubbled through the experimental solutions in the tap-funnels.

For determinations of rates of fluid movement, readings were made at 10 min intervals. The solution in the chamber was renewed or changed every 10 min. The experiments were performed at room temperature (20–23 °C) and the variation of temperature during any one experiment was never more than 0·5 °C.

Everted preparations

To measure the effects of fluids of different composition on fluid transport a different preparation was used in which the gut was in the form of an everted sac. To prepare this, the cannula was passed into the lumen of the midgut via a cut made in the anterior end and introduced into a short length of the posterior midgut which had been left attached. The preparation was tied in place at this point. Using forceps to grip the anterior edge the midgut was then turned inside out and the free end ligated to form a closed sac.

Preparations of the midgut as a flat sheet

For electrical measurements it was more convenient that a portion of the midgut epithelium was arranged as a flat sheet. This was achieved by slitting the gut longitudinally and opening it out flat over a vertically held, saline-filled tube of internal diameter in the range 1–3 mm. A portion of it could then be tied in place over the tube and the excess tissue trimmed off to give a flat sheet of epithelium in position over the mouth of the tube. For measurements of trans-epithelial potential difference, the tube was removed to a wax-lined dish of liquid paraffin and a drop of saline of suitable composition placed in contact with the exposed gut surface. Coarse-bore glass pipettes (of *ca.* 100 μm internal diameter) filled with KCl-Agar could then be introduced, using micromanipulators, into the fluids on either side of the epithelium. Through connexions of KCl-Agar-filled polythene tubing to calomel half-cells, potential differences were measured and recorded with a Keithley 600 B electrometer and pen recorder.

For measurements of short-circuit currents (I_{sc}), midguts were mounted as flat sheets in an Ussing-type chamber. The male half of the chamber (internal diameter 4 mm) was introduced through a hole in the bottom of a wax-lined Petri dish. An area of midgut epithelium was tied in place under saline, as previously described. The male chamber half was removed from the dish and clamped to the female half which was then filled with saline with the chamber upright in order to keep constant the hydrostatic pressure difference across the gut epithelium. Each chamber half held 1 cm^3. The delicacy of the midgut epithelium allowed only very gentle stirring with the gas lift pumps (95 % O_2–5 % CO_2) (Wood, 1976; Wood & Moreton, 1978). Due to the very low electrical resistance of this tissue relative to the saline, a three-electrode system was used (see Wood & Moreton, 1978). An amplifier used to voltage-clamp the tissue allowed both potential difference and I_{sc} to be recorded simultaneously on a twin-pen chart recorder. An automatic timing circuit interrupted the clamp for 60 s every 10 min so that the open-circuit epithelial potential difference and resistance could be measured.

Midgut preparations for analysis of absorbed fluid

To obtain samples of the fluid transported by the midgut, isolated closed sacs of epithelium were prepared and filled with solution of the desired composition. To do this, isolated guts were injected from a syringe with glass cannula temporarily tied into the anterior end of the midgut. After filling, the syringe and cannula were removed and the anterior and posterior ends of the gut closed with ligatures. The closed s[illegible]

was then lifted out of the dissecting dish on a 'spoon' of fine stainless steel mesh. Any saline carried over with the preparation was removed by applying filter paper to the underside of the mesh spoon, so avoiding damage to the tissue above. The preparation was then submerged in liquid paraffin contained in a polystyrene Petri dish and the mesh spoon was removed. No adherent fluid could be collected from the surface of the preparation at this stage, nor could any be seen under the binocular microscope.

Absorption of fluid from the midgut resulted in a film of aqueous fluid appearing between the surface of the epithelium and the surrounding liquid paraffin. Inclusion of small quantities of dye (either indigo-carmine or amaranth) in the injected saline enabled leakage of fluid from the lumen of the preparation to be detected. Once sufficient absorbate had accumulated it was aspirated from around the preparation with a fine pipette and stored, under liquid paraffin, in a refrigerator.

The concentration of chloride ions in such a sample was estimated by potentiometric titration against silver nitrate solution (Ramsay *et al.* 1955). Osmotic pressures were determined by a cryoscopic method (Ramsay & Brown, 1955). After dilution of the sample, the concentrations of sodium and potassium present were estimated by emission flame photometry using a Unicam SP 900 Spectrophotometer.

Composition of salines

The standard saline used contained (mM): NaCl 129, KCl 8·6, $CaCl_2$ 2, $MgCl_2$ 8·5, $NaHCO_3$ 10·2, NaH_2PO_4 4·3, and glucose 34, giving a solution of pH 6·7 and 342 mosmol l^{-1}. Saline solutions with different sodium and potassium levels were prepared by replacing sodium salts with their appropriate potassium equivalents. In experiments where sodium concentrations were changed under potassium-free conditions, the salines were prepared by mixing appropriate volumes of (i) a variant of the standard saline containing 137·6 mM of NaCl and no KCl and (ii) a saline containing 137·6 mM choline chloride but no KCl nor NaCl and buffered with 15 mM tris chloride instead of $NaHCO_3$ and NaH_2PO_4. Solutions of varied chloride concentrations were made up from mixtures of standard saline and a chloride-free saline which contained (mM): Na_2SO_4 81, K_2SO_4 7, $CaSO_4$ 2, $MgSO_4$ 5, $NaHCO_3$ 12, NaH_2PO_4 5, glucose 34, and sucrose 50.

RESULTS

Fluid absorption in standard saline

Observations were made with standard saline bathing both surfaces of isolated preparations of anterior midgut epithelium. In every case a net loss of fluid from the lumen of the preparation was recorded. In the intact animal a movement of fluid in this direction would result in the transfer of materials from the lumen of the midgut to the haemolymph and is therefore described here as 'absorption'. The rate of absorption of fluid by the isolated preparations was calculated from the volume of fluid lost in the last 70 min of experiments lasting 90 min. During this period the rate of absorption remained virtually constant; the observed rate of fluid absorption was $19 \pm 2{\cdot}2$ nl min^{-1} ($n = 5$). During the first 20 min the rate of fluid absorption varied somewhat, presumably reflecting the time taken for the tissue to equilibrate with the bathing medium.

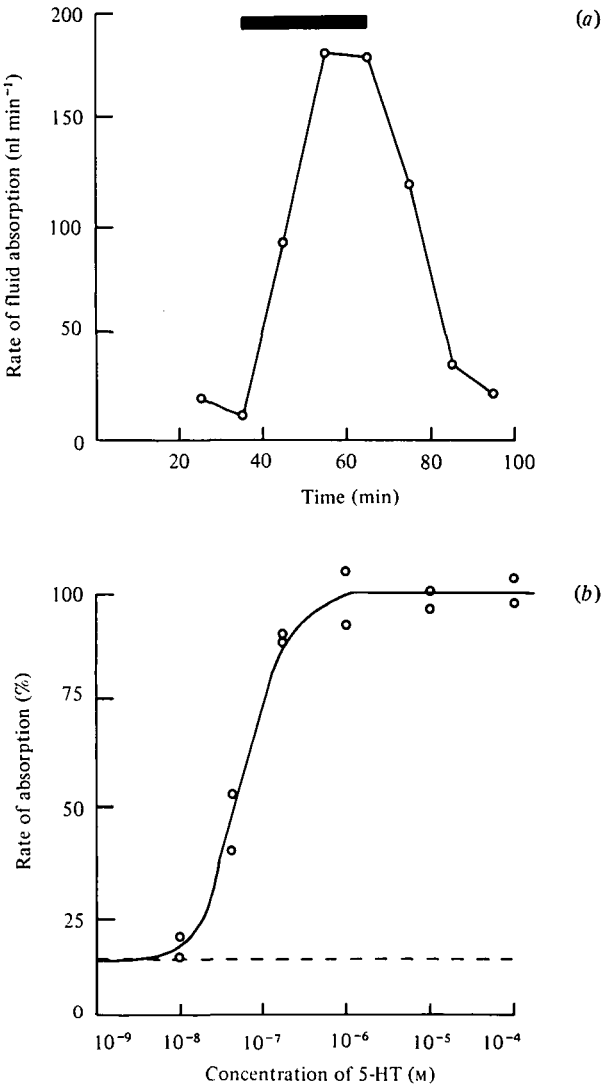

Fig. 2. (*a*) The rate of fluid absorption by an isolated midgut of *Rhodnius* when subjected to 10^{-4} 5-HT for 30 min (indicated by the bar). (*b*) Dose/response curve for the effect of 5-HT on the rate of absorption of fluid by isolated midguts. The rates are expressed as percentages of that observed in 10^{-4} M 5-HT. The broken line represents the rate of absorption characteristically shown by unstimulated preparations. In this and following figures, unless otherwise indicated, the points represent individual observations.

Stimulation of fluid transport by 5-hydroxytryptamine and cyclic AMP

As with many other fluid-transporting tissues in insects, fluid absorption by the isolated anterior midgut of *Rhodnius* is much accelerated by exposure to 5-hydroxytryptamine (5-HT) or to 3′-5′-cyclic adenosine monophosphate (cAMP). A typical result and the dose-response curves are shown in Figs. 2 and 3. The responses were elicited by applying these stimulants to the haemolymph-facing (basal) side of

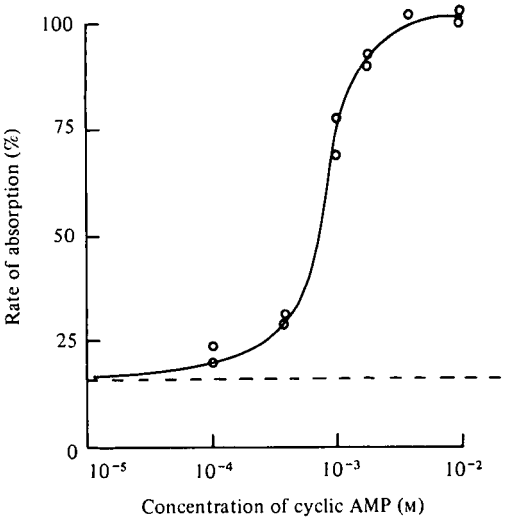

Fig. 3. Dose/response curve for the effect of cyclic AMP on the rate of absorption by isolated midguts. The rates are expressed as percentages of that observed in 10^{-2} M cAMP. The broken line represents the rate of absorption in unstimulated preparations.

epithelium. The mean maximal rates of absorption were, for 5-HT, 131 ± 6 nl min^{-1} ($n = 12$) and, for cAMP, 124 ± 5 nl min^{-1} ($n = 11$), that is more than six times faster in each case than in unstimulated preparations. Application of solutions containing 5-HT to the luminal surface of the epithelium had no stimulant effect. Dopamine, implicated as the transmitter regulating fluid secretion by the salivary glands of *Manduca sexta* (Robertson, 1974) and of *Nauphoeta cinerea* (House, Ginsborg & Silinsky, 1973) had no observable effects on fluid absorption by the midgut of *Rhodnius* when applied to the basal (haemolymph) surface of the preparation at concentrations of up to 10^{-3} M. All of the preparations treated with this compound were subsequently shown to respond to 5-HT.

The high concentration of cAMP, 2×10^{-3} M, needed to elicit maximal fluid transport is in line with that required to stimulate other insect tissues such as the salivary glands of *Calliphora* (6×10^{-3} M required for maximal secretion, Berridge & Patel, 1968) and presumably reflects a relatively low permeability of the cell membranes to this substance. Even insect epithelia such as Malpighian tubules where the cell membranes are greatly folded and relatively permeable (Maddrell, 1980), are only maximally stimulated when the bathing solution contains around 2×10^{-4} M cAMP (Maddrell, Pilcher & Gardiner, 1971).

Effects on fluid absorption of different sodium and potassium concentrations at the luminal surface

The luminal surfaces of everted anterior midgut preparations were exposed to solutions in which the concentration of sodium was altered by exchange with potassium. The effect of the sodium concentration on the rate of absorption under these conditions shown in Fig. 4. This experiment was repeated, substituting choline for sodium in

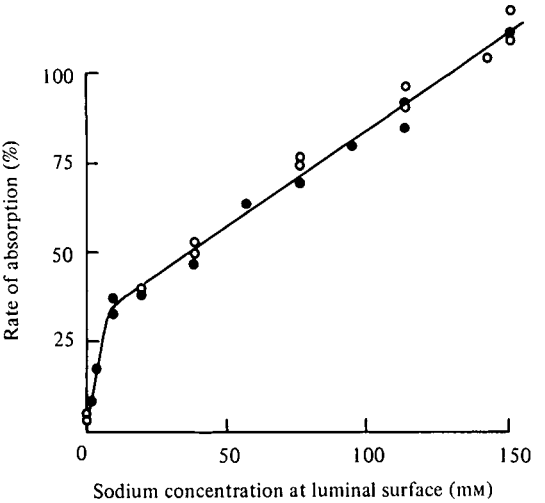

Fig. 4. The effects of changes in sodium concentration at the luminal surface on the rates of fluid absorption shown by isolated midgut preparations. The rates are expressed as percentages of that shown in standard saline solution. In some of the experiments the sodium concentrations were varied by replacement with potassium (○); in the remainder, sodium was replaced with choline (●).

saline containing no potassium. The results (see Fig. 4) are indistinguishable from those observed with potassium ions present at the luminal surface. By contrast, when the potassium concentration was varied by exchange with choline in sodium-free solutions, the rate of absorption was very low across the range of concentrations used.

Thus it is seen that the rate of absorption of fluid shown by the everted preparation of the anterior midgut depends on the concentration of sodium ions at the luminal surface, when this is varied between 0 and 152 mM. The presence of potassium at the luminal surface has no effect on the rate of absorption; it will not support absorption when present alone, and the dependence of the rate on the sodium concentration is unaffected by changes in the potassium concentration.

The effects on fluid absorption of different chloride concentrations at the luminal surface

The sulphate anion is known to permeate epithelia far less readily than does chloride. When sulphate was substituted for chloride in the saline bathing the luminal surface of everted preparations, it was found that the rate of absorption of fluid depended on the chloride concentration (see Fig. 5). The results show that, in the presence of 143·5 mM sodium, 60 mM chloride is sufficient to support the maximum rate of absorption.

Osmotic concentration of the absorbed fluid

Experiments were made to compare the osmotic concentration of the luminal contents of the anterior midgut with that of the absorbed fluid. Preparations were injected with various dilutions of a saline of twice the normal concentration. These were then placed under liquid paraffin, where many of the preparations show

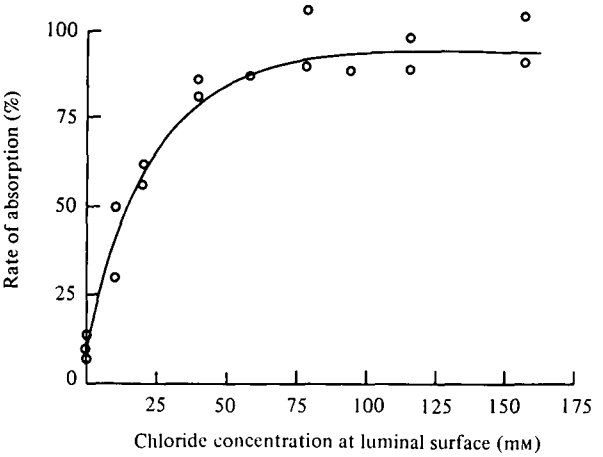

Fig. 5. The effects of changes in the chloride concentration at the luminal surface on the rates of fluid absorption shown by isolated midgut preparations. Chloride concentrations were varied by exchange with sulphate. The rates of absorption are expressed as percentages of that seen in standard saline solution.

vigorous peristalsis. Once sufficient fluid had accumulated between the basal surface and the liquid paraffin, the absorbate and the contents of the preparation were sampled and the melting points of these solutions were determined. (The time taken for sufficient absorbate to be produced varied with the osmotic concentration of the injected fluid; about 30 min in 40% saline and around 90 min in 180% saline. While no quantitative estimate of the rate of absorption could be made with these preparations, it seemed possible that an inverse relationship existed between the rate of absorption and the osmotic concentration of the contents of the anterior midgut.) Sixteen preparations were used to study the effects of five solutions, the osmotic concentrations of which ranged between 40% and 180% of that of normal saline. In all cases the osmotic pressure of the absorbed fluid was very similar to that of the contents of the isolated midgut (see Fig. 6).

Ionic composition of the absorbed fluid

In order to determine the ionic composition of the absorbed fluid, preparations injected with 70–80 μl of standard saline were kept under liquid paraffin for 3 h, after which time they had produced several μl of fluid at the basal surface. The fluid was collected and the concentrations of sodium, potassium and chloride in this solution were estimated. The results are presented in Table 1. Duplicate measurements were made for each determination.

These results showed that the absorbate had a higher concentration of sodium and lower concentration of potassium than that of the saline placed in the lumen. (By sampling and analysing the luminal contents of three preparations immediately after the injection of saline it was found that the concentrations of sodium and potassium were not significantly altered by contamination with the small remaining amount of contents of the anterior midgut. The results obtained for these controls are shown Table 1.)

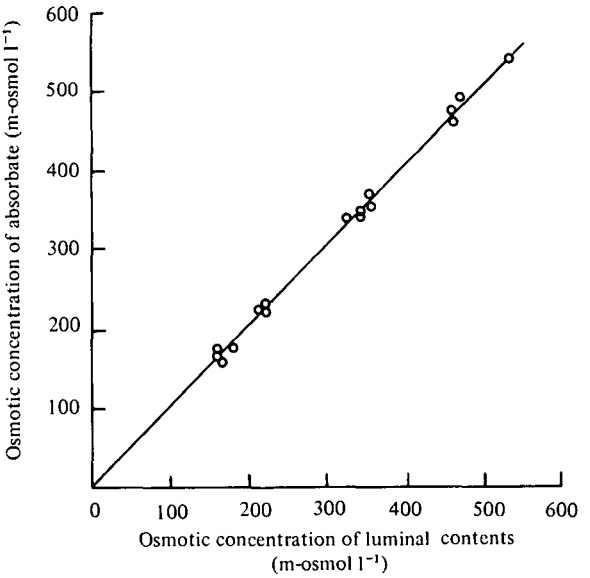

Fig. 6. Comparison of the osmotic concentrations of the absorbate and luminal contents when the latter is varied. The line drawn is the iso-osmotic line.

Table 1. *Ionic composition of the absorbed fluid*

	Na	K	Cl (mM/l)
Ringer			
Calculated composition of injected Ringer	43·5	8·6	158·6
Observed composition of midgut contents (mean of 3 determinations)	145·3	9·0	—
Observed composition of the absorbate (mean and standard error of 7 determinations)	178·4 ± 2·2	1·7 ± 0·2	157·5 ± 2·2
Low sodium			
Calculated composition of injected Ringer	50·0	102·0	158·5
Observed composition of the absorbate (mean and standard error of 6 determinations)	173·5 ± 2·9	2·8 ± 0·4	156·3 ± 1·9

The experiment was repeated using for the injection a saline in which the sodium concentration had been reduced to 50 mM by substitution with potassium. The composition of the absorbate produced by these preparations was not significantly different from that produced by preparations filled with normal saline (see Table 1). Thus it was shown that the tissue maintained the ionic composition of the absorbed fluid when the solution in the lumen had been altered so as to increase the concentration gradients of sodium and potassium across the epithelium.

It will be evident from Table 1 that the summed concentration of sodium and potassium in the absorbed fluid is higher than the chloride concentration; the identity of the other anion(s) that must have been present was not determined.

Effects of ouabain and K-free solutions

The cardiac glycoside, ouabain, is known to inhibit the activity of sodium–potassium exchange pumps in a variety of tissues (see, for example, Glynn, 1964). Application of this compound at a concentration of 10^{-3} M to the basal surface of five normal preparations, bathed in normal saline and stimulated with 10^{-5} M 5-HT, reduced the observed rate of absorption by a mean of 81 %. When the experiment was repeated using 10^{-5} M ouabain, the reduction in the rate of absorption was 66 % (mean of 5 determinations). A similar reduction in the rate of absorption was brought about by the use of potassium-free saline at the basal surface. In this case the rate of absorption could be fully restored by adding a drop of potassium chloride solution to the saline in the experimental chamber.

By contrast, application of 10^{-5} M ouabain or potassium-free saline to the luminal surface of the everted preparation had no effect on the rate of absorption.

Transepithelial potential differences

Determinations of transepithelial potential differences were made both before and after stimulation with 5-HT. Before stimulation, the transepithelial potential averaged $14{\cdot}3 \pm 0{\cdot}8$ mV ($n = 78$), the basal (haemolymph) surface being at a potential positive with respect to that of the luminal surface. Upon treatment with solutions containing more than 2×10^{-8} 5-HT, the basal surface always became still more positive with respect to the luminal face (the maximum value reached was $28{\cdot}1 \pm 1{\cdot}2$ mV ($n = 61$). Usually the potential then fell to a new level which was typically more positive than before stimulation, though in a few cases the potential settled at a level less positive than before. The results of such experiments are illustrated in Fig. 7.

That the rate of fluid absorption is Na-dependent, and that the haemolymph-facing side is at a positive potential with respect to the luminal face suggests the involvement

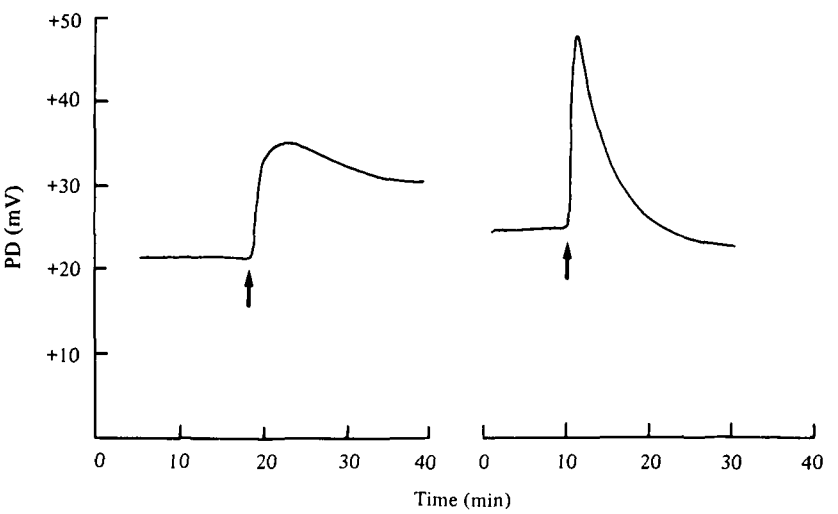

Fig. 7. Tracings of recordings of potential differences measured across isolated midgut preparations to show the effect of treatment with 5-HT (at times indicated by the arrows).

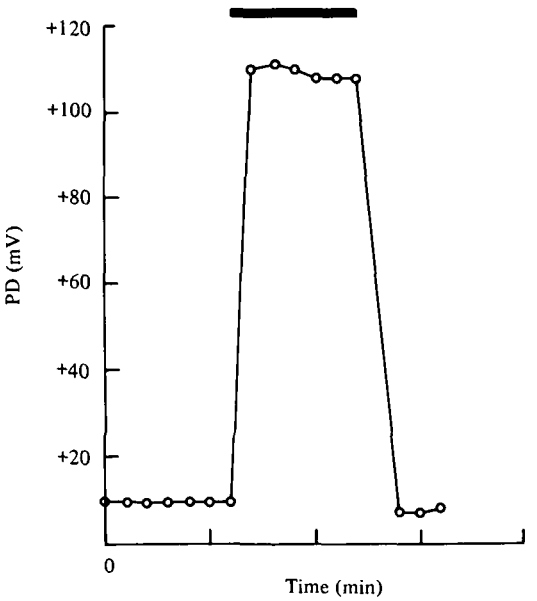

Fig. 8. The effect on transepithelial potential difference when the luminal face of a midgut preparation was bathed (——) in a sulphate-based saline containing no chloride ions.

of a haemolymph-directed active transport of sodium ions with chloride ions following passively. It was of interest, therefore, to test the effect of substituting chloride-free saline for normal saline at the luminal surface. This brought about a dramatic increase in the potential from $10 \pm 0{\cdot}6$ mV to 110 ± 3 mV (basal surface positive) within four minutes (mean and standard error of five preparations). A typical result is shown in Fig. 8.

Determinations of short-circuit current

Exposure to 5-HT affected the transepithelial potential difference relatively little compared with the increases in fluid transport rates of at least six times. This suggested that determinations of short-circuit currents (I_{sc}) might show larger changes. The time course of the potential difference, the short-circuit current, and the resistance shown by pieces of midgut, $12{\cdot}5$ mm^2 in area, on exposure to 5-HT is shown in Fig. 9. The large increase in I_{sc} shows that 5-HT induces a large increase in the active flux. This suggests that 5-HT acts to increase sharply an electrogenic active transport of sodium ions from the lumen of the gut. That such accelerated transport is not accompanied under open-circuit conditions by a concomitant increase in potential difference is seen to be the result of a substantial fall in the transepithelial electrical resistance. It remains to be shown (i) whether this is a consequence of an increase in permeability to sodium ions and/or chloride ions, and (ii) what fraction of the I_{sc} is carried by sodium ions.

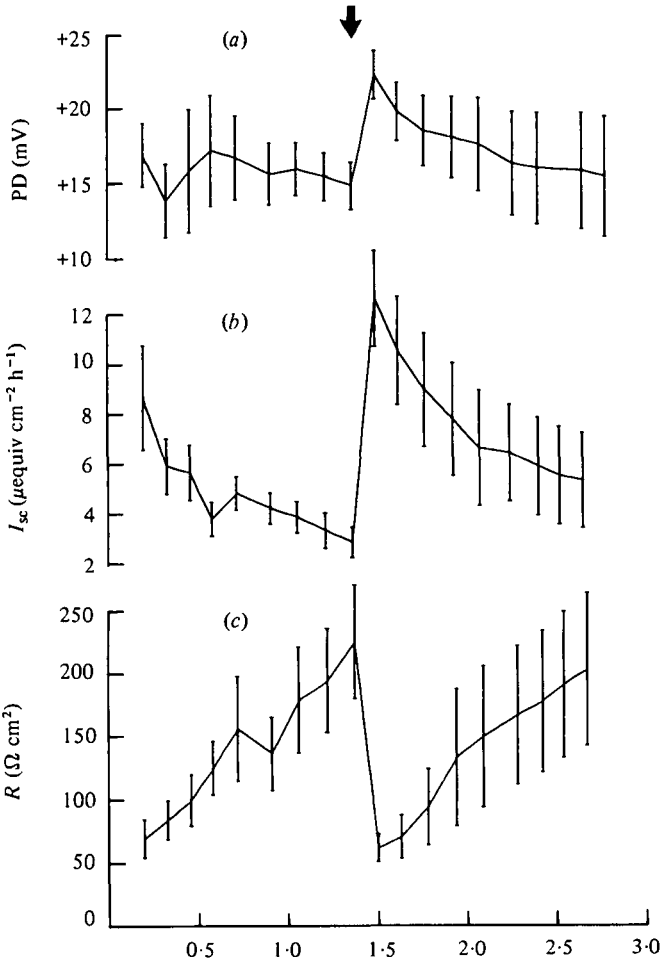

Fig. 9. The effect on (*a*), the transepithelial potential difference (PD), (*b*), the short-circuit current (I_{SC}), and (*c*), the specific resistance (R) of the epithelium of adding 5-HT (at 10^{-5} M) at the point indicated by the arrow. The vertical lines attached to the points represent ± S.E. ($n = 7$).

DISCUSSION

The results described in this paper show that the isolated midgut of *Rhodnius* carries out virtually iso-osmotic transport of fluid from its lumen. Sodium and chloride ions are the major constituents of the transported fluid. Transport goes on at a rate of about 20 nl min^{-1} in unstimulated preparations but this is increased at least sixfold in preparations treated with 5-HT or cyclic AMP. For the following reasons, it appears likely that transport is driven by active transport of sodium ions. First, the haemolymph side of the epithelium (towards which transport occurs) is always at a potential positive with respect to the lumen. The potential difference is greatly increased if the lumen contains only impermeant anions. Finally, the rate of fluid transport is strongly dependent on the concentration of sodium ions in the luminal fluid. Transport across the epithelium is much depressed by the presence of ouabain or absence of potassium

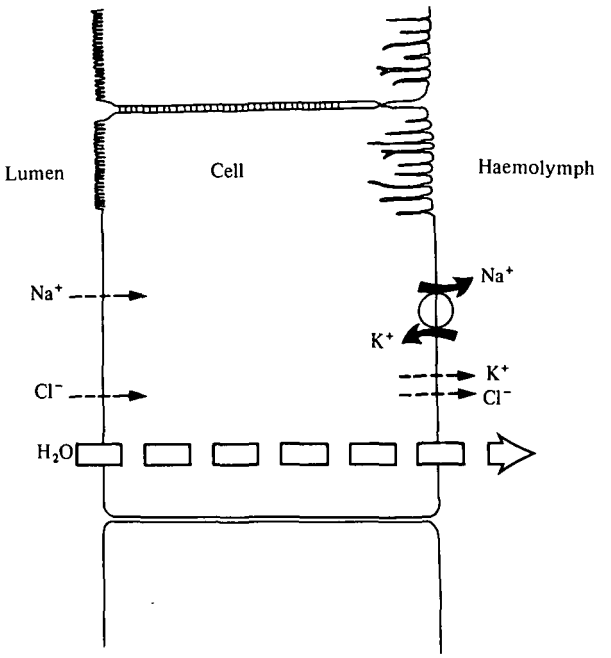

Fig. 10. Possible mechanism by which fluid transport by the anterior midgut of *Rhodnius* might occur. The structure of the cell membranes and the intercellular junction is indicated in the top part of the diagram. Passive processes are indicated by broken lines, active ones by solid lines. For further explanation, see text.

ions in the fluid bathing the haemolymph face. This suggests that an Na/K exchange pump may be centrally involved in the operation of the epithelium. A model of how transport might occur, consistent with the above findings, is shown in Fig. 10. Water flow is thought to be secondary to ion transport. This is because the transported fluid is almost iso-osmotic to the contents of the lumen and because the rate of fluid transport depends on the luminal concentrations of sodium ions and permeant anions. Just how the solute fluxes are coupled with water movements remains to be determined. In view of the difficulties in explaining such coupling in other epithelia that have been much more fully studied, any speculation based on the present results would be unprofitable. Although Fig. 10 shows water flow occurring by a cellular route, there is no evidence at present to prefer such a suggestion over one in which at least some of the water moves paracellularly, that is through the lateral cell–cell junctions and intercellular clefts. Sodium ions are shown in Fig. 10 to enter the cell passively from the luminal fluid. Preliminary results (unpublished results of S. H. P. Maddrell and L. Wakefield) have shown that the transepithelial potential difference is very little affected in the short term by changes in the relative concentrations of sodium and potassium ions in the luminal solution. This suggests that this face of the epithelial cells is about as permeable to sodium ions as it is to potassium ions. That the epithelium selectively transports sodium ions would then follow from the activity of the Na/K exchange pump on the cell membrane facing the haemolymph.

The ability of the anterior midgut of *Rhodnius* to transport *in vitro* an NaCl-base

[illegible]-osmotic fluid from lumen to haemolymph at high rates is entirely appropriate to its proposed role in fluid elimination after a blood meal *in vivo*. The insect needs rapidly to concentrate the nutritious part of the meal, the red blood cells and plasma proteins, and to eliminate the excess sodium and chloride ions and water from the plasma. *In vitro*, isolated preparations of the midgut epithelium transport fluid at up to 150 nl min^{-1}, yet, *in vivo*, fluid is eliminated at about 400 nl min^{-1} at the same temperature (Maddrell, 1964). The difference in rates is probably attributable to two factors. First, in our isolated preparations not all of the midgut epithelium is included, as some of it is cut away in preparing the tissue or excluded in the placing of fluid-tight ligatures which cannot be placed too near the cut edges of the tissue. Secondly, the tissue is detached from its tracheal air supply and bathed in an artificial bathing medium and this is, naturally, unlikely to allow the tissue to operate as rapidly as it can *in vivo*.

That fluid transport by *Rhodnius* midgut should be attributable to active transport of sodium ions is worth comment. It was the first known case in insects of sodium-dependent fluid transport; at the time of its discovery (Farmer, 1974), all other cases of fluid transport in insects were attributable primarily to movements of potassium ions. Since then it has been shown that fluid secretion by Malpighian tubules of *Glossina morsitans*, appropriately also a bloodsucker, also depend on sodium transport (Gee, 1976). When stimulated, *Rhodnius* Malpighian tubules secrete fluid rich in both sodium and potassium ions (Maddrell, 1969) and the mechanism is thought to involve active transport of both these ions (Maddrell, 1971; Gupta *et al.* 1976). Apart from these cases, fluid transport systems in insects thought to depend on active transport of potassium ions include the following: a wide range of Malpighian tubules (Ramsay, 1953; Maddrell, 1971); the salivary glands of *Calliphora* (Berridge, Lindley & Prince, 1976); the labial glands of Saturniid moths (Kafatos, 1968) and the secretion of moulting fluids in moths (Jungreis, 1979).

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