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SECRETION BY THE MALPIGHIAN TUBULES OF *RHODNIUS PROLIXUS* STAL: ELECTRICAL EVENTS

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SUMMARY

(1) Transepithelial and intracellular potentials have been simultaneously recorded from *Rhodnius* upper Malpighian tubules before and after stimulation of fluid secretion. The transepithelial electrical response to the diuretic hormone mimic 5-hydroxytryptamine (5-HT) was triphasic; recordings of intracellular potential changes indicated that the three phases represented successive events at the apical membrane.

(2) Depolarizations produced by increasing the bathing medium potassium concentration indicated that the basal membrane was much more permeable to potassium than to sodium. Electrical responses to chloride-free saline were inconsistent with a significant basal membrane chloride permeability.

(3) Chloride movements across the basal membrane were opposed by an electrical gradient of about 65 mV. The results of experiments in which tubules were exposed to chloride-free saline or sodium-free saline suggested that chloride entry into the cells was linked to the entry of Na^+ and K^+. The effects of furosemide and bumetanide upon secretion and potential changes suggested that chloride crossed the basal membrane through co-transport with Na^+ and K^+. Chloride probably crosses the apical membrane into the lumen passively in response to a favourable electrical gradient of about 35 mV.

(4) Cations must be actively pumped into the lumen against an electrical gradient of 35 mV. Our results support previous evidence for an apical cation pump which actively transports Na and K into the lumen.

(5) A tentative model of ionic movements during fluid secretion is presented. It is suggested that the apical cation pump maintains sodium at low intracellular concentrations, thereby maintaining a favourable gradient for entry of Na^+ through the proposed basal co-transport step. The suggested stoichiometry is $Na^+ : K^+ : 2\,Cl^-$.

INTRODUCTION

During diuresis, the Malpighian tubules of *Rhodnius* secrete fluid at a very high rate, $3{\cdot}3\ \mu l\ min^{-1}\ cm^{-2}$. By comparison, the gall bladder of the dog secretes $1{\cdot}1\ \mu l\ min^{-1}\ cm^{-2}$ (Grim & Smith, 1957). The rapid secretion by the tubules is elicited by a diuretic hormone whose actions are mimicked by 5-hydroxytryptamine (5-HT;

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Maddrell, Pilcher & Gardiner, 1969). Recent studies have shown that water moves through the walls of the Malpighian tubules at high rates in response to an experimentally imposed osmotic gradient (O'Donnell, Aldis & Maddrell, 1982). It appears unlikely that the fluid moves through the lateral intercellular spaces (reviewed by Maddrell, 1980; O'Donnell & Maddrell, 1983*a*). Rather, the very high osmotic permeability of the tubule wall suggests that active and passive ion movements through the cells *in vivo* generate small osmotic gradients within the cells and the lumen, and that water moves transcellularly in response to these gradients (O'Donnell & Maddrell, 1983*a*). Other mechanisms which would act to couple water movements to solute transport cannot, however, be excluded.

The net ionic fluxes can be analysed through electrophysiological techniques similar in nature to those pioneered by Ussing and his collaborators (1960) in studies of frog skin. This paper reports the electrical events both across the Malpighian tubule epithelium as a whole, and also across the apical and basal plasma membranes, before and during rapid fluid secretion. Preliminary measurements of transepithelial potential differences (TEPs) in *Rhodnius* Malpighian tubules have been reported before (Maddrell, 1971). Experiments involving either alterations in the ionic composition of the bathing fluids, or the application of pharmacological agents, have provided information on the types of ion movements during fluid secretion and the membranes involved. These data are used to construct a model describing the ionic fluxes which support fluid secretion by *Rhodnius* Malpighian tubules. As is the case for many insect epithelia (reviewed by Harvey, Cioffi & Wolfersberger, 1983), it appears that an alkali ion pump situated in the apical cell membrane is involved in active cation movements during secretion. An important new finding is that chloride does not appear to enter the cells through passive channels in the basal membrane, as in the salivary gland and Malpighian tubules of blowflies (Berridge, 1969; Berridge, Lindley & Prince, 1975), nor is it actively pumped, as in the locust rectum (Hanrahan & Phillips, 1982, 1983). Rather, chloride appears to enter the Malpighian tubule cells of *Rhodnius* through co-transport with sodium and potassium.

MATERIALS AND METHODS

Malpighian tubules were dissected from 5th stage larvae of *Rhodnius prolixus* Stal as described previously (Maddrell, 1981). Each tubule consists of a single layer of squamous epithelial cells which form a blind-ended tube approximately 100 μm in diameter and 45 mm in length. Fluid secretion is confined to the upper two-thirds of the tubule's length; the work to be described here is restricted to a study of this region.

A dissected tubule was placed in a perfusion chamber which had three lateral compartments (Prince & Berridge, 1972; O'Donnell & Maddrell, 1983*b*). One compartment, containing saline, was separated by a paraffin-filled central compartment from the third compartment, through which saline flowed under gravity (Fig. 1). Different salines, each with its own stop valve, were connected to the perfusion compartment by a multiplexing non-return valve (Holder & Sattelle, 1972). Flow rates were sufficient to exchange the compartment's volume every 2–3 s.

The blind end of a dissected tubule was positioned in the perfused compartment, and the cut end of the tubule was pulled through a notch in the central paraffin-filled

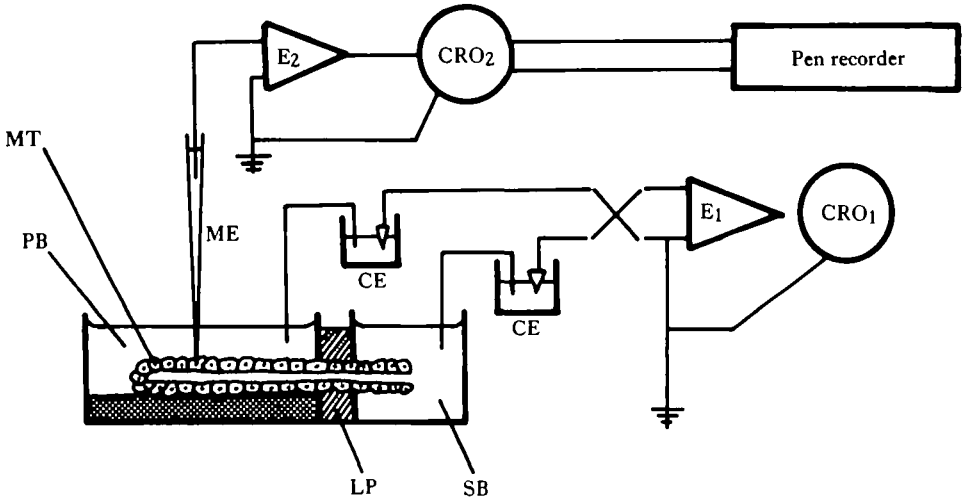

Fig. 1. Schematic diagram of the perfusion chamber used during simultaneous measurement of transepithelial and intracellular potentials. Malpighian tubules (MT) were placed on a bed of agar (stippled) in the perfused compartment (PB) of the chamber, pulled through a central compartment filled with liquid paraffin (LP) and secured to the wall of the non-perfused saline bath (SB) with a drop of silicone grease. The wall of the tubule was nicked with fine scissors so as to provide both an outlet for secreted fluid and to make the lumen of the tubule continuous with the non-perfused bath. For transepithelial potential measurements, agar bridges placed in each compartment were connected through calomel half-cells (CE) to an electrometer (E_1) whose output was displayed on an oscilloscope (CRO_1) and recorded on chart paper. Intracellular microelectrodes (ME) were connected to a high-impedance electrometer (E_2), and displayed on the second channels of the oscilloscope (CRO_2) and pen recorder. The apparatus was adapted from Prince & Berridge (1972).

compartment and secured in a drop of silicone grease to the far wall of the unperfused saline compartment. Displacement of the tubule by the flow of saline was prevented by wrapping the tubule around several fine glass pins embedded in a layer of agar on the floor of the perfused compartment. The wall of the unperfused portion of the tubule was nicked with ultrafine scissors so that the luminal fluid was continuous with the saline of the unperfused compartment; the basal (haemolymph) side of the tubule contacted the saline of the perfused compartment. The transepithelial potential could

Table 1. *Compositions of the experimental solutions (concentrations in mM)*

	Control	K-rich	Na-free	Cl-free
NaCl	129·0	—	—	—
KCl	8·6	137·6	137·6	—
$MgCl_2$	8·5	8·5	8·5	—
$CaCl_2$	2·0	2·0	2·0	—
$NaHCO_3$	10·2	10·2	—	10·2
NaH_2PO_4	4·3	4·3	—	4·3
Glucose	34·0	34·0	34·0	34·0
$KHCO_3$	—	—	10·2	—
KH_2PO_4	—	—	4·3	—
K_2SO_4	—	—	—	4·3
$CaSO_4$	—	—	—	2·0
$MgSO_4$	—	—	—	8·5
Na isethionate	—	—	—	129·0

then be measured simply by placing an agar bridge in each compartment and connecting the bridges through calomel half-cells to a Keithley electrometer (Fig. 1).

Basal or apical membrane potentials were measured relative to an indifferent electrode placed in the perfused or unperfused compartment, respectively (Fig. 1). Electrodes were pulled from 1 mm diameter thin-walled filamented glass capillary tubing and filled with 3 M KCl. Microelectrodes with fine tips, with resistances of 30–40 MΩ, were required to penetrate the basal membrane, which is thrown into a series of deep finger-like infoldings (M. J. O'Donnell, S. H. P. Maddrell, H. LeB. Skaer & J. B. Harrison, in preparation).

The compositions of the salines used in these experiments are given in Table 1. All experiments were done at temperatures in the range 22–25 °C. Tubules were stimulated to secrete by the addition of 10^{-6} M 5-HT. The drugs furosemide and bumetanide, which arrest sodium-dependent chloride transport in some vertebrate tissues (Frizzell, Field & Schultz, 1979*a*; Palfrey, Alper & Greengard, 1980), were applied at various concentrations in control saline.

Mean values are presented as mean ± S.E. Significance of difference between means was calculated by the *t*-test.

RESULTS

Potential changes during 5-HT stimulation

The rate of fluid secretion *in vitro* is stimulated up to 1000-fold by treatment with 5-HT (Maddrell *et al.* 1969). When tubules were exposed to 5-HT, the transepithelial potential changed in three distinct phases (Fig. 2), as observed previously (Maddrell, 1971). Measurements with intracellular microelectrodes showed only a small change in potential across the basal membrane in response to 5-HT, from -67 ± 2 mV ($N = 7$) before stimulation, to -66 ± 2 mV after stimulation. Although the basolateral membrane usually depolarized in response to 5-HT, it occasionally hyperpolarized (Fig. 2). Because the potential changes across the basal membrane were so small, the transepithelial 5-HT response is almost completely attributable to potential changes across the apical membrane (Fig. 3, Table 2). The apical PD and the TEP

Table 2. *Effects of 5-HT on transepithelial and apical potential changes (in mV)*

Potential differences	Transepithelial	Apical
Pre-stimulation	−8 ± 3 (22)	−53 ± 4 (23)
Phase 1	−23 ± 3 (22)	−38 ± 3 (23)
Phase 2	18 ± 3 (23)	−82 ± 4 (23)
Phase 3	−28 ± 3 (25)	−30 ± 3 (26)
Wash-off	20 ± 3 (14)	−79 ± 5 (15)
Changes in potential		
Phase 1–Pre-stimulation	−16 ± 2 (22)*	15 ± 2 (23)*
Phase 2–Pre-stimulation	27 ± 3 (22)*	−29 ± 4 (23)*
Phase 3–Pre-stimulation	−20 ± 3 (22)*	24 ± 4 (23)*

* Magnitudes of the indicated pairs of transepithelial and apical changes are not significantly different ($P < 0.05$).

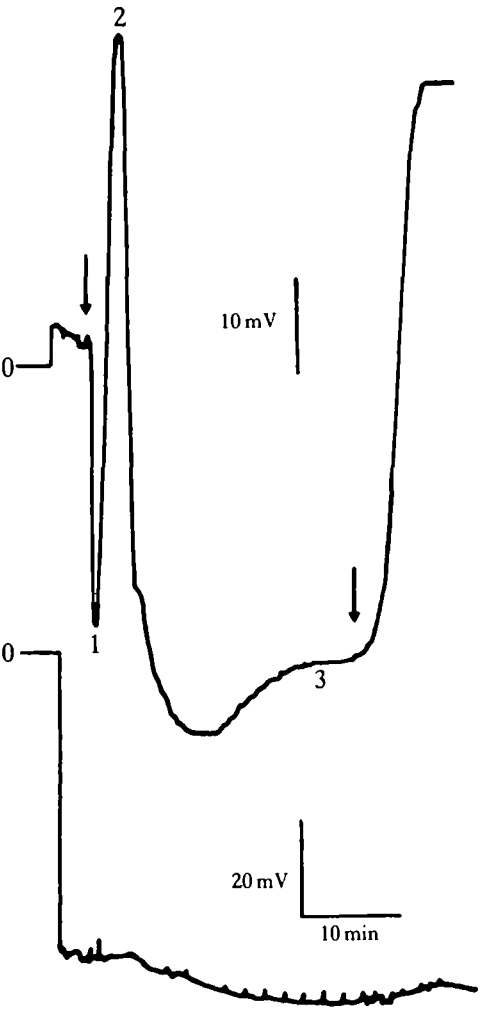

Fig. 2. Record of changes in transepithelial potential (upper trace) and basal membrane potential in response to stimulation of fluid secretion by addition of 5-HT. The direction of negative-going potential changes is downwards for both traces. Between the arrows the flow of control saline was replaced by control saline containing 10^{-6} M 5-HT. The significance of the three phases (numbered) of the response is discussed in the text.

are of opposite polarities because the TEP is measured relative to the bath and the apical PD is measured relative to the lumen.

The results of these experiments can be used to calculate the potential profile across the tubule wall. When the 5-HT response is complete, the lumen of the tubule is at a potential about 28 mV negative to the bath (Table 2), and the cell interior is at a potential about 66 mV negative to the bath. The tubule lumen, therefore, is as much as 38 mV positive to the cell. These figures indicate that anion movements towards the lumen are opposed by the potential difference across the basal membrane, whereas cation movements into the lumen are opposed by the potential difference across the apical membrane.

When the 5-HT stimulus was removed, the transepithelial potential rapidly became

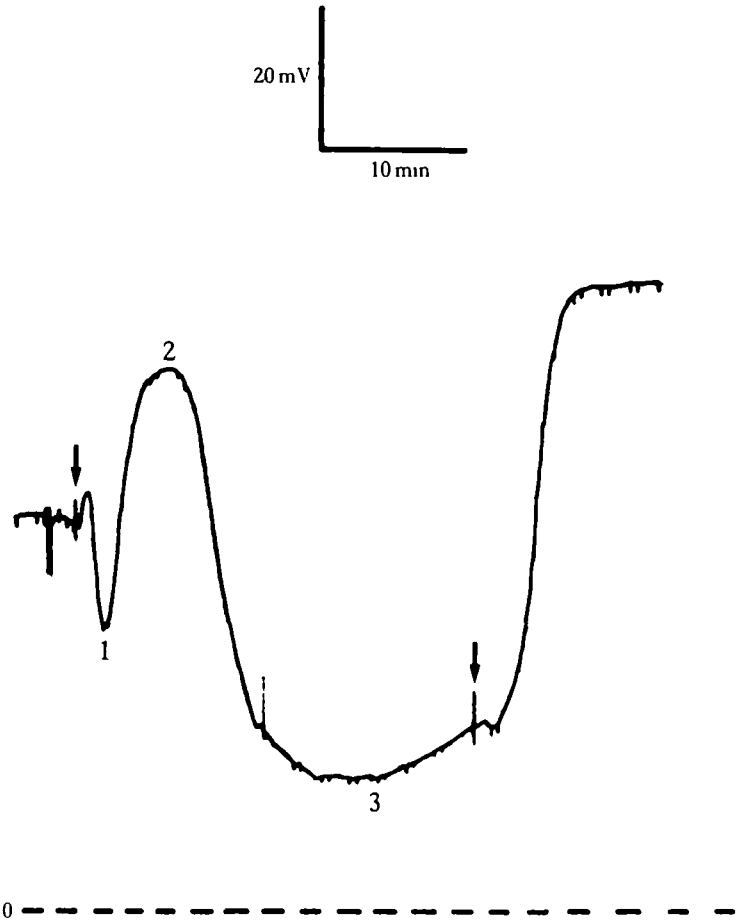

Fig. 3. Record of potential changes across the apical membrane in response to 5-HT. Between the arrows control saline was replaced by saline containing 10^{-6} M 5-HT. Negative-going potentials produced an upward deflection of the recording.

positive (Fig. 2). The change in TEP during 5-HT wash-off reflected a potential change of equal magnitude across the apical membrane (Fig. 3, Table 2). If 5-HT was reapplied within about 20 min the TEP rapidly changed back to a negative value of −20 to −30 mV. However, if the tissue was washed for 40–60 min in 5-HT-free saline, the TEP gradually changed from about 25–30 mV lumen positive to a negative value (Fig. 4). Under these conditions, the potential did not change monophasically to a negative value when 5-HT was reapplied; the triphasic pattern of potential change typical of previously unstimulated tubules was observed (Fig. 4). The ionic movements which produce the potential changes associated with the addition or removal of 5-HT are investigated below.

Selective permeability of the basal membrane to potassium

Intracellular recordings from unstimulated tubules showed a change in basal potential of 52 ± 4 mV ($N = 4$) when the bathing fluid potassium concentration w

anged from 8·6 mM to 137·6 mM. In stimulated tubules, however, exposure to K-rich (137·6 mM) saline changed the transepithelial potential by 66 mV, from -32 ± 2 mV ($N = 24$) to 34 ± 3 mV (Fig. 5); intracellular recordings indicated that this potential change was caused by a depolarization of 66 ± 5 mV ($N = 9$) across the basal membrane. The potential change predicted by the Nernst equation, assuming that the basal membrane had a permeability to potassium ions much higher than towards sodium ions, and that intracellular potassium concentration remained constant, was $E = -58 \log(137{\cdot}6/8{\cdot}6) = -70$ mV. The close fit to the Nernst value for stimulated tubules (Fig. 5) suggests that basal membrane potassium permeability is much higher than sodium permeability. That the effect is smaller in unstimulated tubules suggests that one effect of 5-HT stimulation is to increase the potassium permeability of the basal membrane. However, the concentrations of sodium and potassium in the fluid secreted by stimulated tubules are approximately equal (Maddrell, 1969). It would appear, therefore, that sodium may have an alternative route into the cell. This point will be discussed further in relation to the means by which chloride ions cross the basal membrane.

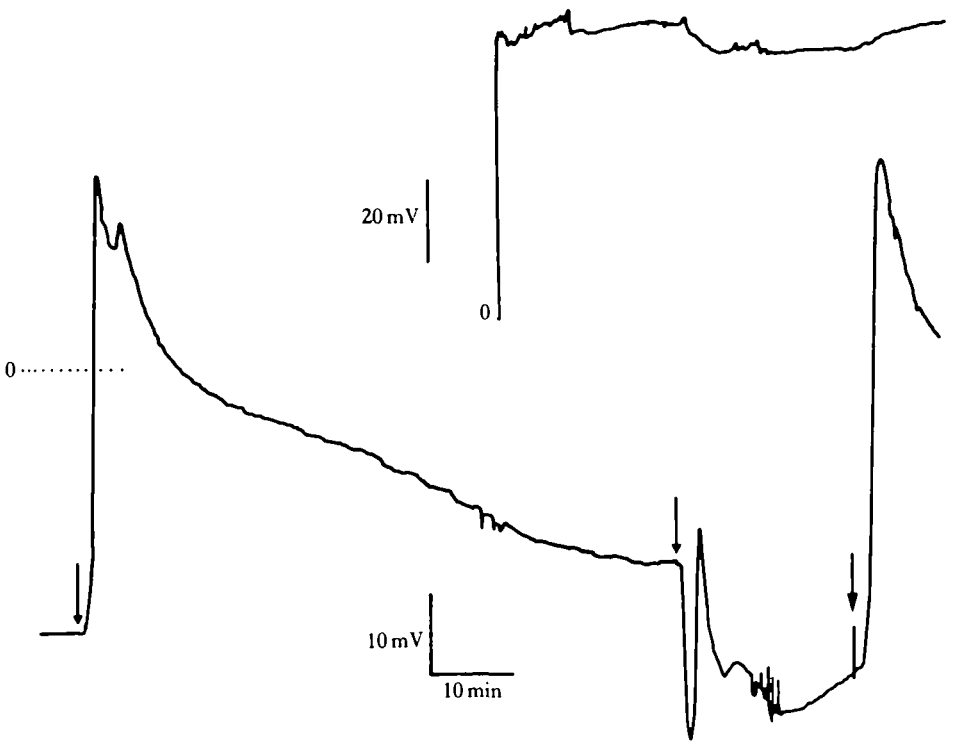

Fig. 4. Record of potential changes following removal of 5-HT from the saline bathing previously stimulated tubules. Lower trace: transepithelial potential. Upper trace: basal membrane potential. Negative-going potential changes produced upward deflections of the basal trace and downward deflections of the transepithelial trace. Salines were changed at the times indicated by the arrows. The tubule was bathed in control saline containing 10^{-6} M 5-HT for 30 min prior to the start of the lower trace, and also during the time between the middle and right-most arrow. At other times the tubule was bathed in control saline that did not contain 5-HT.

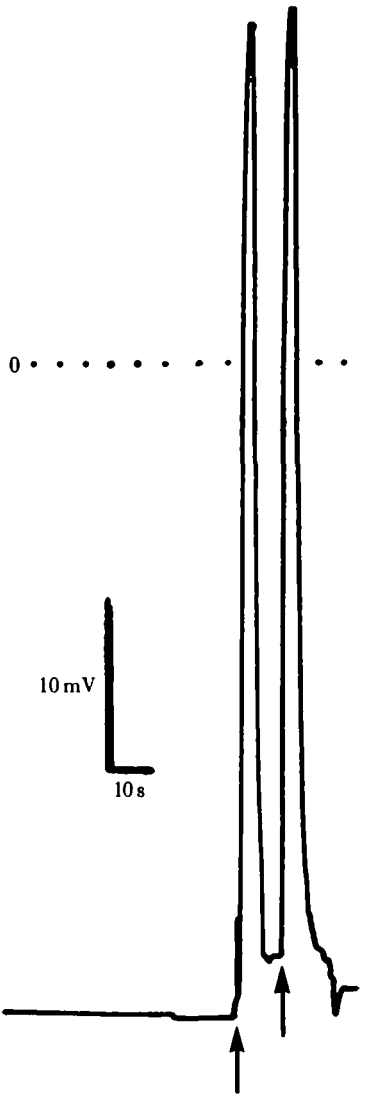

Fig. 5. Record of transepithelial potential changes produced across the basal membrane by high potassium (137·6 mM) concentrations in the perfusion fluids. At each spike, the perfusion fluid was changed from control saline to high potassium saline for 5 s.

Effects of sodium depletion

Treatment with potassium-rich, sodium-free saline caused a change in TEP of unstimulated tubules from -9 ± 4 mV ($N = 10$) to 32 ± 5 mV. This change could be attributed to the effects of the high potassium concentration (152 mM) of the bathing fluids upon the basal membrane potential. In contrast, stimulated tubules underwent a much larger biphasic change in TEP in potassium-rich, sodium-free saline (Fig. 6). Measurements with intracellular microelectrodes indicated that the initial very rapid change in potential was the basal membrane response to elevated potassium; TEP changed from -30 ± 3 mV ($N = 12$) to 25 ± 3 mV during this early depolarization.

exposure to sodium-free saline was continued for several more minutes, the TEP changed more slowly by a further 75 mV, reaching a stable value 131 ± 2 mV ($N = 8$) more positive than the potential in control saline with 5-HT. The net change in TEP was reduced dramatically by small concentrations of sodium; in saline containing only 0·5 mM sodium the change in TEP was reduced to 112 ± 3 mV ($N = 8$), and in 3 mM sodium the change was merely 80 ± 2 mV ($N = 10$).

The apical potential was insensitive to transient changes in bathing fluid potassium concentration; the effect on it of potassium-rich, sodium-free saline was therefore due to the absence of sodium *per se* (Fig. 7). The apical PD of stimulated tubules hyperpolarized by 60 ± 6 mV ($N = 16$) relative to the PD in control saline (−44 ± 5 mV).

Effects of chloride-free saline

The TEP in unstimulated tubules was relatively insensitive to bathing fluid chloride concentration; the TEP was 9 ± 4 mV ($N = 8$) in control saline, and 9 ± 5 mV in chloride-free saline. However, in stimulated tubules the TEP changed from −32 ± 3 mV ($N = 14$) in control saline to 49 ± 6 mV in chloride-free saline. This transepithelial change of about 81 mV was matched by an apical hyperpolarization of

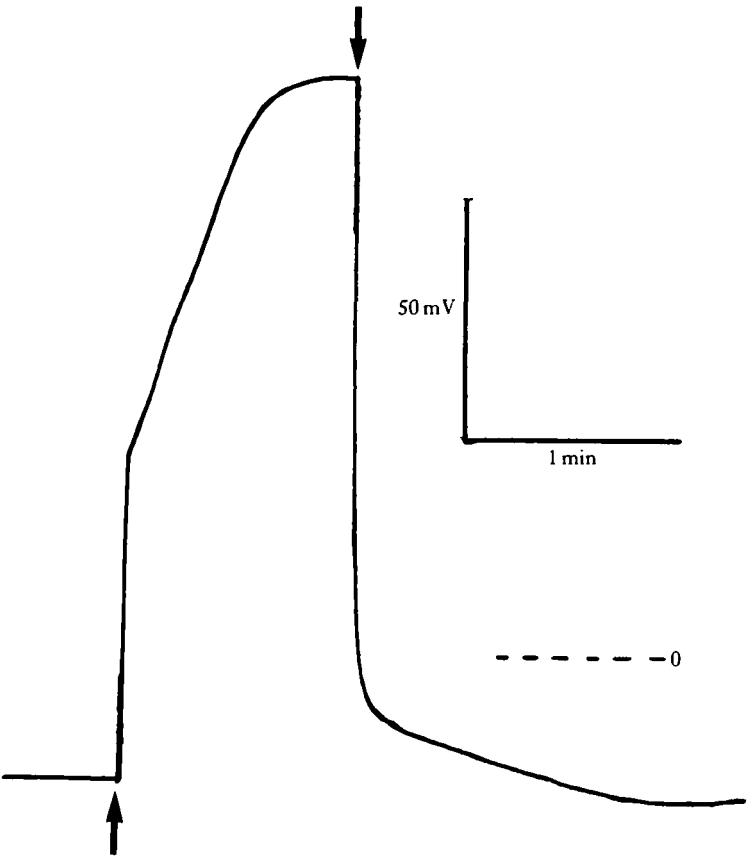

Fig. 6. Effects of sodium-free saline upon transepithelial potentials of stimulated tubules. Between the arrows, the perfusion fluid was changed from control saline containing 5-HT to sodium-free saline containing 5-HT.

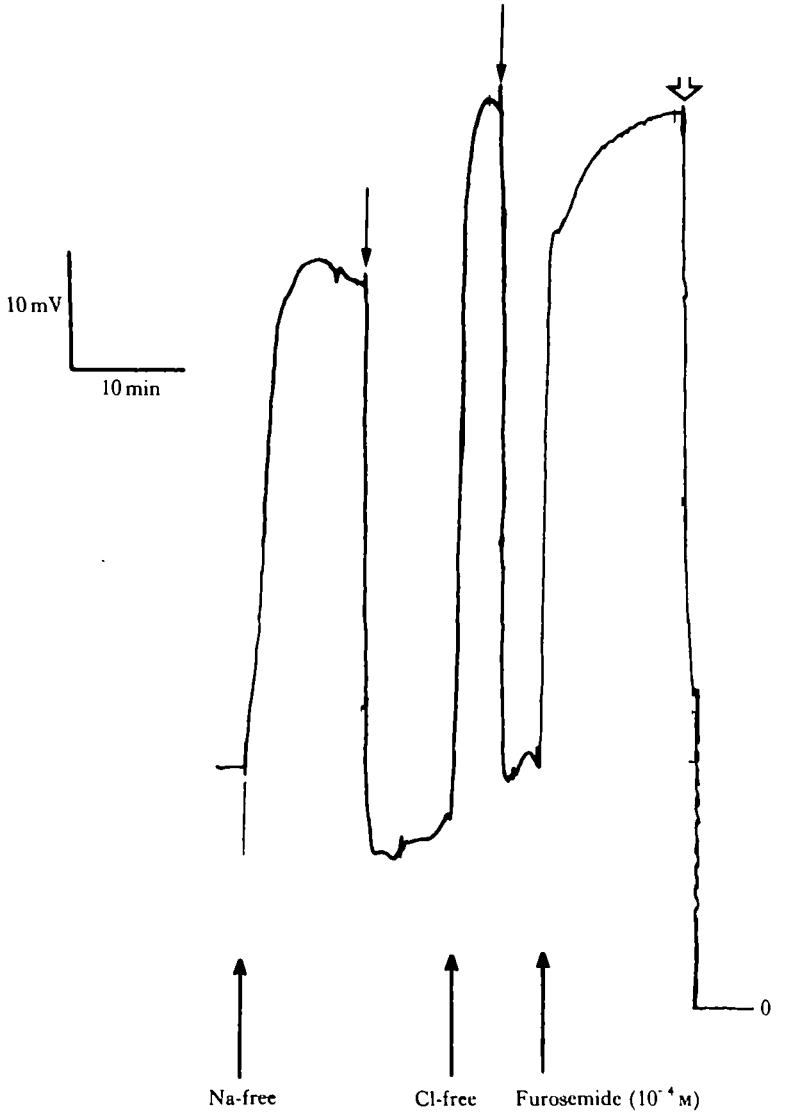

Fig. 7. Changes in apical membrane potential in response to sodium-free saline, chloride-free saline and control saline containing 10^{-4} M furosemide. All salines contained 10^{-6} M 5-HT. The flow of each of the indicated salines was initiated at the time indicated by the upward pointing arrows; the flow of control saline was restored at the times indicated by the downward pointing arrows. At the open arrow, the microelectrode was retracted from the cell and placed in the non-perfused bath (0 potential).

about 77 mV, from -47 ± 4 mV ($N = 16$) in control saline to -124 ± 6 mV in chloride-free saline (Fig. 7).

The basal membrane potential hyperpolarized by only 3 ± 2 mV ($N = 13$) in chloride-free saline, from -74 ± 3 mV ($N = 13$) to -77 ± 5 mV. These changes suggested that the basal membrane was not freely permeable to chloride; the change in potential was far too small and the polarity opposite to the change predicted by the Nernst equation for a chloride-selective membrane.

It is of interest to note that the effects of sodium depletion upon the apical membrane potential were very similar to the effects of chloride-free saline. This similarity, in conjunction with the polarity of the apical potential difference in stimulated tubules, suggested the following working hypothesis to explain ionic movements during fluid secretion. Because the apical potential difference opposes cation movements from cell to lumen, the previous suggestion of an energy-dependent apical cation pump (Maddrell, 1971, 1976) is supported. When tubules are bathed in chloride-free saline, chloride ions would become unavailable to act as counterions, either by passive diffusion or active pumping into the lumen. Under such conditions, continued pumping of potassium into the lumen would lead to the development of a lumen-positive potential difference across the apical membrane (Fig. 7). Moreover, the similar effects of sodium depletion and chloride-free saline upon the apical membrane potential suggest that chloride entry into the cell would be at least partly dependent upon the presence of sodium in the bathing saline. Under conditions of sodium depletion, less chloride would enter the cell, and continued pumping of potassium into the lumen would again tend to hyperpolarize the apical membrane as chloride became less available to act as a counterion. The observations that the basal membrane potential opposed chloride movements into the cell, and that the basal membrane did not appear to be significantly conductive to sodium or chloride, supported the possibility of some means of electroneutral sodium-dependent chloride transport across the basal membrane. This possibility was investigated further by exposing the tubules to pharmacological reagents which are known to inhibit sodium-dependent chloride transport in other tissues.

Effects of furosemide and bumetanide

Furosemide and bumetanide are drugs thought to block sodium chloride co-transport in some vertebrate epithelia, such as the flounder intestine (Frizzell *et al.* 1979*a*; Frizzell, Smith, Vosburgh & Field, 1979*b*), the rat ileum (Humphries, 1976) and the avian erythrocyte (Palfrey *et al.* 1980). Fluid secretion by Malpighian tubules was severely affected by these drugs; in saline containing 10^{-4} M furosemide, secretion rates dropped to $21 \pm 2{\cdot}8\%$ ($N = 6$) of their values in control saline. Bumetanide was even more effective; in experiments with twelve tubules, the rate of fluid secretion was reduced to 50% in 4×10^{-6} M bumetanide, and to 20% of its control rate, in saline containing 10^{-5} M bumetanide. The basis for the inhibition of secretion was suggested by the dramatic effect of furosemide on TEP and apical PD. TEP changed by 80 mV, from -30 ± 2 mV ($N = 40$) in control saline containing 5-HT, to 50 ± 3 mV in control saline containing 5-HT and 10^{-4} M furosemide. These changes were similar to the effects of chloride-free saline, suggesting that furosemide inhibited chloride entry into the cells.

The responses of the apical PD were somewhat smaller; the potential changed from -50 ± 4 mV ($N = 17$) to -116 ± 8 mV (Fig. 7) a difference of 66 mV. The change was significantly less ($P < 0{\cdot}05$) than the 80 mV change in TEP. The explanation for the difference can be accounted for by a small effect of furosemide on the potential across the basal membrane. In a separate series of experiments, the basal membrane potential declined significantly from -66 ± 5 mV ($N = 14$) in control saline to -57 ± 7 mV saline containing furosemide. Subtracting the latter value from the apical potentials

gave an expected transepithelial potential of 58 ± 8 mV ($N = 17$), which was not significantly different from the TEP (50 ± 3 mV) measured in the experiments described above.

DISCUSSION

The apical membrane

Fluxes of potassium, and also of sodium, across the apical membrane are against an electrical gradient of about 30 mV. The analyses of Gupta, Hall, Maddrell & Moreton (1976) and Maddrell (1976) indicate that intracellular concentrations of potassium and sodium are 0·91 and 0·94 times their respective concentrations in the secreted fluid, so trans-apical movements of Na and K are against a chemical gradient as well.

Cations, therefore, must be actively transported across the apical plasma membrane. Fluid secretion occurs at 90 % of the control rate in potassium-free saline, and at 40 % of the control rate in sodium-free saline; under the latter conditions, the secreted fluid contains predominantly KCl (Maddrell, 1969). A single type of cation pump with a higher affinity for sodium than potassium, could account for these observations and for the transport of both sodium and potassium by *Rhodnius* tubules in control saline (Dr J. L. Wood, personal communication cited by Maddrell, 1976). The higher sodium affinity has also been suggested by experiments with radioactive tracers (Maddrell, 1976) which showed that at low concentrations of the ions, Na is transported much faster than K. However, the amount of each species pumped will be dependent not just upon the pump's affinity for a given ion, but also upon the concentration of that species at the pumping sites. The intracellular potassium concentration is in fact much higher than that of sodium (Gupta *et al.* 1976). This may explain why, in spite of the apparently high affinity of the pump for sodium, potassium and sodium concentrations in the secreted fluid are both about 90 mM (Maddrell, 1969).

Apical chloride movements are likely to be completely passive. The favourable electrical gradient across the apical membrane (30 mV) in stimulated tubules could account for a 3·3-fold difference between cytoplasmic and luminal chloride concentrations. Concentration differences suggested by the results of Gupta *et al.* (1976) are three-fold or less.

The basal membrane

The effects of changes in the concentrations of potassium, sodium and chloride upon the basal membrane potential indicated that the basal membrane is permeable to potassium, but not to sodium or chloride. It is unlikely that there could be a significant passive influx of chloride into the cell because the basal membrane potential of -65 mV would require, in accordance with the Nernst equation, intracellular chloride concentrations to be 13-fold less than the concentrations in the bathing saline. However, the measurements of Gupta *et al.* (1976) indicate that intracellular chloride concentrations, relative to the bathing saline, are reduced only five-fold in the bulk cytoplasm and only two-fold in the region of the basal infoldings.

Because passive sodium and chloride movements across the basal membrane appeared unlikely, we proposed, as a working hypothesis, that sodium and chloride movements across the basal membrane are linked. The basis for this suggestion was the observation that a lumen-positive potential develops across the apical membrane when either sodium or chloride is removed from the bathing saline. Both changes could be explained by a depletion of intracellular chloride. Continued action of the apical cation pump in the relative absence of chloride to act as a counterion leads to an increase in the apical potential. Addition of low concentrations of sodium to the bathing saline greatly reduces the apical hyperpolarization.

Potential changes produced by exposure of the tubules to the drug furosemide were also similar to the effects of chloride deprivation. This observation provided further support for the suggestion of co-transport of sodium and chloride. In vertebrate epithelia, it has been proposed that a one-for-one neutral coupled entry mechanism across the mucosal membrane prompts all or most of the Cl transport across the epithelium. Furosemide, added to the mucosal solution, inhibits the coupled entry of NaCl, thereby reducing net transepithelial Cl flux (Frizzell *et al.* 1979*a*). In Malpighian tubules, as for many vertebrate epithelia, there appears to be a favourable electrochemical potential gradient for sodium entry into the cell, and an unfavourable gradient for chloride. A co-transport system on the basal membrane is, therefore, energetically advantageous; uphill chloride movements are achieved through dissipative sodium entry. The gradient is continuously maintained during stimulated fluid secretion by the apical cation pump, which removes sodium and potassium from the cell.

However, several lines of evidence suggest that chloride movements across the basal membrane can occur, to a limited extent, when the bathing fluids contain potassium but not sodium. As noted above, secretion in a sodium-free saline containing predominantly potassium and chloride occurs at 40 % of the control rate. Chloride must therefore have a mode of entry into the cell which is not dependent upon only sodium. Even during secretion in control saline, the secretion contains equal concentrations of sodium and potassium (90 mM), and chloride is the accompanying anion (180 mM). A one-for-one NaCl co-transport system could account only for chloride concentrations of 90 mM. In addition, the apical hyperpolarization produced in chloride-free saline was greater than that for sodium-free conditions. This observation suggested that some chloride was able to enter the cell even in the absence of sodium.

Our results can be interpreted, therefore, as evidence of chloride movements across the basal membrane through co-transport with both sodium and potassium; the suggested stoichiometry is $Na^+ : K^+ : 2\,Cl^-$. Co-transport mechanisms with this stoichiometry have been proposed for other tissues, including Ehrlich ascites tumour cells (Geck *et al.* 1980), duck erythrocytes (Haas, Schmidt & McManus, 1980), and avian retinal pigment epithelium (Framlach & Misfeldt, 1983). Inhibition of fluid secretion during exposure to bumetanide or furosemide is consistent with this type of mechanism. If chloride entered by any other route, and potassium entry were unaffected by furosemide, fluid transport should continue in the presence of furosemide. On the other hand, if furosemide blocks a $Na^+ : K^+ : 2\,Cl^-$ co-transport mechanism, anion entry is prevented and secretion stops. When the bathing saline contains sodium or

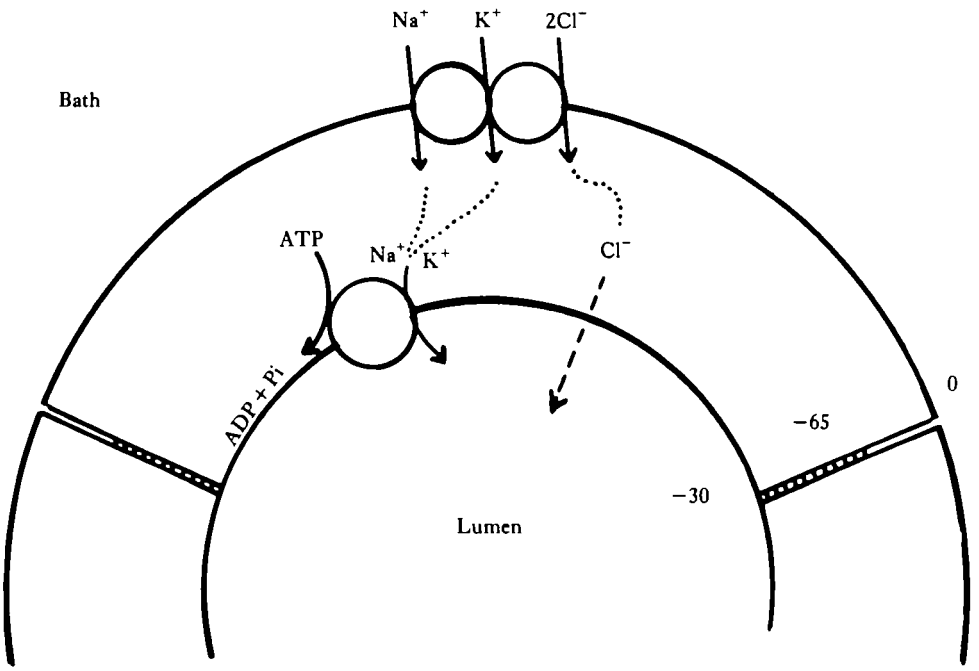

Fig. 8. Schematic diagram indicating movements of ions through a Malpighian tubule cell during 5-HT-stimulated fluid secretion. Passive ion fluxes are indicated by dashed arrows, and active or co-transport fluxes are indicated by solid arrows. It is suggested that the movements of water are a passive response to the osmotic gradient created by the net ionic movements into the cell and into the lumen of the tubules. Transepithelial and basal membrane potentials (mV) are indicated.

potassium, but not both, chloride entry may be possible if the co-transport mechanism accepts, though less readily, stoichiometries such as $2Na^+:2Cl^-$ or $2K^+:2Cl^-$. Secretion could then continue at a reduced rate, as observed.

Triphasic potential changes produced by 5-HT stimulation

The basal co-transport mechanism, the apical cation pump, and the apical chloride channels which we have suggested above can be incorporated into a speculative model of ionic movements during fluid secretion (Fig. 8). This model can be used to provide a tentative explanation for the effects of 5-HT upon the potential difference across the apical membrane. The first phase of the response to 5-HT is a depolarization of the apical membrane potential, suggestive of a movement of anions from the cell into the lumen. This movement could result from the activation of the co-transport mechanism proposed for the basal membrane. No change would be expected in the basal membrane potential, because the co-transport mechanism is electrically silent. However, the rate of chloride entry into the cell would increase, and the apical potential would then hyperpolarize slightly as chloride moved down the electrochemical gradient into the lumen, through channels whose conductance was low, but not negligible.

The second phase of the 5-HT response is a hyperpolarization of the apical membrane potential. This change suggests that cation movements into the lumen a

completely countered by anion movements. This situation could occur if activation of the apical cation pumps occurs more quickly or precedes an increase in the chloride conductance of the apical membrane. Under these conditions, cation pumping would tend to make the lumen positive with respect to the bathing saline.

During the third phase of the 5-HT response, the lumen gradually becomes negative with respect to the bathing saline. This change could be explained if the apical membrane chloride conductance increases at this time, thereby facilitating the movement of chloride into the lumen.

When a previously stimulated tubule is exposed to 5-HT-free saline, the TEP changes rapidly from a negative to a positive value, and then slowly declines to the value typical of unstimulated tubules. These observations are most easily explained by a rapid decrease in apical chloride conductance when 5-HT is removed from the bathing salines. If the activity of the apical cation pump declines more slowly in the absence of 5-HT, an imbalance between cation and anion entry into the lumen will develop, and the lumen will become positive, with respect to the bathing saline, until the rate of cation pumping declines to a low level.

It must be stressed that these explanations for the changes in potential produced by addition or removal of 5-HT remain speculative. The use of ion-sensitive intracellular microelectrodes in future work will greatly aid analysis of the ionic movements underlying the 5-HT response.

The proposed mechanism of fluid secretion is not dependent on the high potassium permeability of the basal membrane. Because the secreted fluid contains equimolar concentrations of sodium and potassium, and because chloride appears to enter through a co-transport step, potassium concentrations in the secretion can also be accounted for by potassium entry into the cell by the co-transport step. The basal membrane potassium permeability may, therefore, be involved in Malpighian tubule functions unrelated to diuresis, or it may be indirectly related to the secretion mechanism. The use of ion-sensitive microelectrodes will again be of use in clarifying the role of the basal membrane potassium permeability, and in testing the proposed basal co-transport mechanism.

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REFERENCES

BERRIDGE, M. J. (1969). Urine formation by the Malpighian tubules of *Calliphora*. II. Anions. *J. exp. Biol.* **50**, 15–28.

BERRIDGE, M. J., LINDLEY, B. D. & PRINCE, W. T. (1975). Membrane permeability changes during stimulation of isolated salivary glands of *Calliphora* by 5-hydroxytryptamine. *J. Physiol., Lond.* **244**, 549–567.

FRAMLACH, D. A. & MISFELDT, D. S. (1983). Furosemide-sensitive Cl transport in embryonic chicken retinal pigment epithelium. *Am. J. Physiol.* **244**, F679–F685.

FRIZZELL, R. A., FIELD, M. & SCHULTZ, S. G. (1979*a*). Sodium-coupled chloride transport by epithelial tissues. *Am. J. Physiol.* **236**(1), F1–F8.

FRIZZELL, R. A., SMITH, P. L., VOSBURGH, E. & FIELD, M. (1979*b*). Coupled sodium-chloride influx across brush border of flounder intestine. *J. Membrane Biol.* **46**, 27–39.

GECK, P., PIETRZYK, C., BURCKHARDT, B. C., PFEIFFER, B. & HEINZ, E. (1980). Electrically silent co-transport of Na^+, K^+ and Cl^- in Ehrlich cells. *Biochim. biophys. Acta* **600**, 432–447.

GRIM, E. & SMITH, G. A. (1957). Water flux rates across dog gallbladder wall. *Am. J. Physiol.* **191**, 555–5[illegible]

GUPTA, B. L., HALL, T. A., MADDRELL, S. H. P. & MORETON, R. B. (1976). Distribution of ions in a fluid transporting epithelium determined by electron probe X-ray microanalysis. *Nature, Lond.* **264**, 284–287.

HAAS, M., SCHMIDT, W. F., III & MCMANUS, T. J. (1980). Catecholamine-stimulated ion transport in duck red cells. Gradient effects in electrically neutral $[Na^+ + K^+ + 2Cl^-]$ co-transport. *J. gen. Physiol.* **80**, 124–147.

HANRAHAN, J. W. & PHILLIPS, J. E. (1982). Electrogenic, K^+-dependent chloride transport in locust hindgut. *Phil. Trans. R. Soc. Ser. B* **299**, 585–595.

HANRAHAN, J. W. & PHILLIPS, J. E. (1983). Mechanism and control of salt absorption in locust rectum. *Am. J. Physiol.* **244**, R131–R142.

HARVEY, W. R., CIOFFI, M. & WOLFERSBERGER, M. G. (1983). Chemiosmotic potassium ion pump of insect epithelia. *Am. J. Physiol.* **244**, R163–R175.

HOLDER, R. E. D. & SATTELLE, D. B. (1972). A multiway non-return valve for use in physiological experiments. *J. Physiol., Lond.* **226**, 2P–3P.

HUMPHRIES, M. H. (1976). Inhibition of NaCl absorption from perfused rat ileum by furosemide. *Am. J. Physiol.* **230**, 1517–1523.

MADDRELL, S. H. P. (1969). Secretion by the Malpighian tubules of *Rhodnius*. The movements of ions and water. *J. exp. Biol.* **51**, 71–97.

MADDRELL, S. H. P. (1971). The mechanisms of insect excretory systems. *Adv. Insect Physiol.* **8**, 199–331.

MADDRELL, S. H. P. (1976). Insect Malpighian tubules. In *Transport of Ions and Water in Animals*, (eds B. L. Gupta, R. B. Moreton, J. L. Oschman & B. J. Wall), pp. 541–569. London: Academic Press.

MADDRELL, S. H. P. (1980). Characteristics of epithelial transport in insect Malpighian tubules. In *Current Topics in Membranes and Transport*, Vol. 14, (eds F. Bonner & A. Kleinzeller), pp. 427–463. New York, London: Academic Press.

MADDRELL, S. H. P. (1981). Bioassay of diuretic hormone in *Rhodnius*. In *Neurohormonal Techniques in Insects*, (ed. T. A. Miller), pp. 81–90. New York: Springer-Verlag.

MADDRELL, S. H. P., PILCHER, D. E. M. & GARDINER, B. O. C. (1969). Stimulatory effect of 5-hydroxytryptamine (serotonin) on secretion by Malpighian tubules of insects. *Nature, Lond.* **222**, 784–785.

O'DONNELL, M. J. & MADDRELL, S. H. P. (1983*a*). Paracellular and transcellular routes for water and solute movements across insect epithelia. *J. exp. Biol.* **106**, 231–253.

O'DONNELL, M. J. & MADDRELL, S. H. P. (1983*b*). *In vitro* techniques for studies of Malpighian tubules. In *Measurement of Ion Transport and Metabolic Rate in Insects*, Springer Series in Experimental Entomology, (eds T. J. Bradley & T. A. Miller), Chapter 2, pp. 5–18. New York: Springer-Verlag.

O'DONNELL, M. J., ALDIS, G. K. & MADDRELL, S. H. P. (1982). Measurements of osmotic permeability in the Malpighian tubules of an insect, *Rhodnius prolixus* Stal. *Proc. R. Soc.* B **216**, 267–277.

PALFREY, H. C., ALPER, S. L. & GREENGARD, P. (1980). Protein phosphorylation and the regulation of cation co-transport. *J. exp. Biol.* **89**, 103–115.

PRINCE, W. T. & BERRIDGE, M. J. (1972). The effects of 5-hydroxytryptamine and cyclic AMP on the potential profile across isolated salivary glands. *J. exp. Biol.* **56**, 323–333.

USSING, H. H. (1960). *The Alkali Metal Ions in Biology*. Berlin: Springer-Verlag.