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CELLULAR MECHANISMS OF SALT SECRETION BY THE MALPIGHIAN TUBULES OF INSECTS (*)

by

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SUMMARY

Malpighian tubules secrete primary urine and the hindgut and rectum perform the fine regulation of its composition. The system plays an important role in the regulation of the salt and water content in insects : after eclosion and before flight some insects must lose a lot of water in order to reduce their weight ; or after a meal the blood sucking bug *Rhodnius,* for instance, or the female mosquito need to eliminate a high load of NaCl and water ; the mealworm and the desert beetle on the other hand use a cryptonephric system, built by the Malpighian tubules and the rectum, to reabsorb practically 100 % of the water present in the excreta.

Formica, the species described in this paper, is an omnivorous, continuously feeding species. K^+ secretion is essential in the formation of primary urine by the Malpighian tubules of *Formica.* In the present paper a model for this K^+ secretion is discussed in detail and a brief comparison is made with other species living in different conditions and/or exposed to different salt and water loads. The prime mover for salt secretion in the tubule is a V-type H^+ATPase in the luminal membrane in parallel with a Na^+/H^+ or K^+/H^+ antiporter. Uptake mechanisms for K^+ and/or Na^+ at the haemolymphal side may differ according to the species : in tubules of *Formica* uptake of K^+ through high conductance channels occurs in the presence of a high K^+ concentration. At lower K^+ concentrations a K/Cl and a Na/K/2Cl cotransporter also become functional in these tubules. In some species an appreciable Na^+ conductance is present (e.g. *Aedes aegypti*) or a Na^+/K^+-ATPase may play a role (e.g. *Rhodnius*). The pathway followed by the accompanying anion (mostly Cl^-) is still controversial.

Keywords : Malpighian tubule, electrophysiology, KCl secretion, intracellular measurements, cable analysis, *Formica.*

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INTRODUCTION

Insects can regulate the composition of their blood within narrow limits, although some species live in extreme and variable conditions and have widely varying diets (EDNEY, 1977; STOBBART and SHAW, 1974). A necessary link in this homeostatic regulation is the formation of primary urine by the blind ending Malpighian tubules. As blood pressure in insects is low, the urine is formed not through pressure filtration, but by secretion of salt osmotically followed by water. Metabolites and toxic components may diffuse passively or be secreted actively into the lumen (PHILLIPS, 1981). The primary urine is then voided into the intestine, just below the midgut and fine regulation is achieved by the rectum according to the animal's needs (PHILLIPS, 1977).

MADDRELL (1977; 1980) and PHILLIPS (1981) summarized facts and hypotheses on ion and water transport in the Malpighian tubules as they were known and imagined at that point in time. The facts and insights concerning the ionic basis of the fluid secretion were updated in 1993 by NICOLSON.

Briefly, fluid secretion by most insect tubules is driven by active transport of K^+ ions, while anions (mostly Cl^-) and water follow passively (see PHILLIPS, 1981). Exceptions are, on the one hand some so-called primitive species in the evolutionary trends in insects (*e.g., Libellula,* NICHOLLS, 1985) that rather use Na^+, an ion that is present in abundance in their haemolymph, and, on the other hand, blood-sucking species, that can switch from a mixed Na^+/K^+ in basal conditions to a preferentially Na^+ driven fluid secretion (*e.g. Rhodnius,* MADDRELL, 1980; *Aedes,* BEYENBACH and PETZEL, 1987) or that always preferentially transport Na^+ (*e.g. Glossina,* GEE, 1976). The reason for this shift from NaCl to KCl secretion in modern insects may be that most ancestral insects (like locusts, cockroaches and houseflies today) had a $NaCl^-$rich, low K^+ haemolymph (SUTCLIFFE, 1963), but fed on succulent plant material with the opposite ionic ratios. Fresh lettuce for example contains (in mmol per kg tissue water) 110 K^+, 14 Na^+ and 35 Cl^- (PHILLIPS, 1977). So the need to retain Na^+ and eliminate excess K^+ may have been solved in insects by using K^+ secretion in the primary urine production process. Blood-sucking species have then switched back to a predominantly Na^+ driven secretion at least in stimulated conditions, *i.e.* just after a blood meal. In the latter species the weight may increase several times after feeding, making the mosquito for instance vulnerable to predators. A rapid loss of weight is realized by increasing the rate of primary urine formation by the Malpighian tubules of blood feeders (NIJHOUT and CARROW, 1978; PETZEL *et al.*, 1987, MADDRELL, 1963). A similar stimulation of salt and water secretion by the tubules of butterflies causes loss of fluid (and of weight) after eclosion and before flight as was shown by RYERSE (1978) and NICOLSON (1976; 1980).

Other insects may need to retain as much water as possible. An extraordinary structure present in many Coleoptera and some Lepidoptera is the cryptonephric rectal complex. The structure consists of the blind end of the Malpighian tubules being closely apposed to the rectum, the whole being enveloped by a perinephral membrane. The extremely high concentrations of KCl (over 3 M, MACHIN and

O'DONNELL, 1991 ; O'DONNELL and MACHIN, 1991) realized in the lumen of the Malpighian tubules in some species like the mealworm (*Tenebrio molitor*) and the desert beetle (*Onymacris*) allow the system to reabsorb almost 100 % of the water from the excreta present in the rectum and to take up water vapour directly from the atmosphere (reviewed by O'DONNELL and MACHIN, 1988).

In the present paper we want to concentrate on a model for KCl transport across the Malpighian tubule cell in unstimulated conditions, based on recent information obtained with electrophysiological techniques on Malpighian tubules of the forest ant *Formica polyctena*. The forest ant is an omnivorous, continuously feeding species. So a diet containing both K^+ and Na^+ will be taken in, but the animal will not normally be exposed to extreme conditions of salt and water load as is the case in blood feeders. Adults in contrast with the larval stage (FLORKIN and JEUNIAUX, 1974) have a haemolymph with a high Na^+ over K^+ content (see VAN KERKHOVE *et al.*, 1989). The preference of the Malpighian tubule was found to be for KCl secretion however (VAN KERKHOVE *et al.*, 1989). This is in agreement with the findings for most modern insects (PHILLIPS, 1981). It was shown that the transepithelial K^+ transport always occurred against a large electrochemical gradient. K^+ transport must thus be transcellular. Cl^- transport is mostly passive. The Cl^- transport pathway could be either across the cells or paracellularly, across the shunt. Data will be described in detail allowing the construction of a model for the different steps of the K^+ transport.

From the model derived for this tissue, differences with other species living in different conditions and/or exposed to different salt and/water loads will be briefly commented on.

MATERIAL AND METHODS

The methods used in this study have been described in detail elsewhere, but will be briefly summarized.

Dissection and experimental set-up (see also VAN KERKHOVE *et al.*, 1989)

After decapitation the ventral abdominal sternites were removed and the midgut with the Malpighian tubules attached to it was removed. One Malpighian tubule was cut off, as close to the midgut as possible and transferred to a bathing droplet of about 50 to 100 µl covered with paraffin oil. *Formica* Malpighian tubules are short (2 to 3 mm) so the Ramsay method (RAMSAY, 1953) for measurement of fluid secretion had to be adapted. The cut end of the tubule was sucked into a holding pipette and partly pulled out of the bathing droplet. The part in the oil was nicked and secretory fluid leaving the tubule was collected every 10 min with a collecting pipette. Fluid secretion rates in control Ringer, containing 51 mM K^+ and 143 mM Cl^-, were typically between 100 and 200 pl/min. The bathing droplet could be continuously refreshed with the help of a perfusion and a suction pipette and the com-

position of the bathing solution could be easily changed in order to study the effect of ion concentrations or of different drugs.

Intracellular and intraluminal measurements with double barrelled ion-sensitive or conventional microelectrodes

In order to be able to puncture the cell (or the lumen) with a microelectrode both ends of the tubule were fixed in a holding pipette. The preparation of the double-barrelled ion-sensitive (K^+, H^+ or Cl^- sensitive) or of the conventional electrodes has been described extensively by ZHANG *et al.* (1994), LEYSSENS *et al.* (1992, 1993a and b) and DIJKSTRA (1993).

Cable analysis

The electrical properties of the epithelium and of its basal (i.e. bath side) and luminal cell membrane can be studied by perfusing the lumen of an isolated tubule with Ringer solution of a known composition and by sending a small electrical current across the cell layer. At the same time the transepithelial and transmembrane voltage deflections can be measured as caused by the passage of this current. This allows the calculation of the transepithelial and the transmembranal resistances. Also, if the luminal and bath perfusion solutions are symmetrical, the equivalent active short circuit current can be calculated. The theoretical considerations and the cable equations in the study of *Formica* Malpighian tubules have been described in WELTENS *et al.* (1992) and DIJKSTRA *et al.* (1994a).

MODEL AND DISCUSSION

The effects of different drugs and changes in the ion composition of the bathing fluid were studied on fluid secretion, on the cellular and luminal ion concentrations and on the electrophysiological parameters of the tubule. From the data a model emerged unraveling the different mechanisms that may be involved in the transfer of K^+, H^+ and Cl^- across the epithelium. Fig. 1 gives an overview of the mechanisms present for which evidence was found (see Table 1 and Fig. 2). The consequences and interpretation of these characteristics for KCl transport will be discussed.

The effect of bath K^+ (and Na^+) concentration on fluid secretion

Malpighian tubules of *Formica polyctena* show a strong dependence on the presence of K^+ in the bathing fluid (Table 1 and 2). In a K^+ free, high Na^+ medium no fluid is secreted (VAN KERKHOVE *et al.*, 1989). If no Na^+ is present but the K^+ concentration is high, fluid secretion reaches a high rate. So fluid secretion is very sensitive to the K^+ concentration in the bathing medium. No Na^+ needs to be present to sustain the fluid secretion, at least in high K^+ conditions.

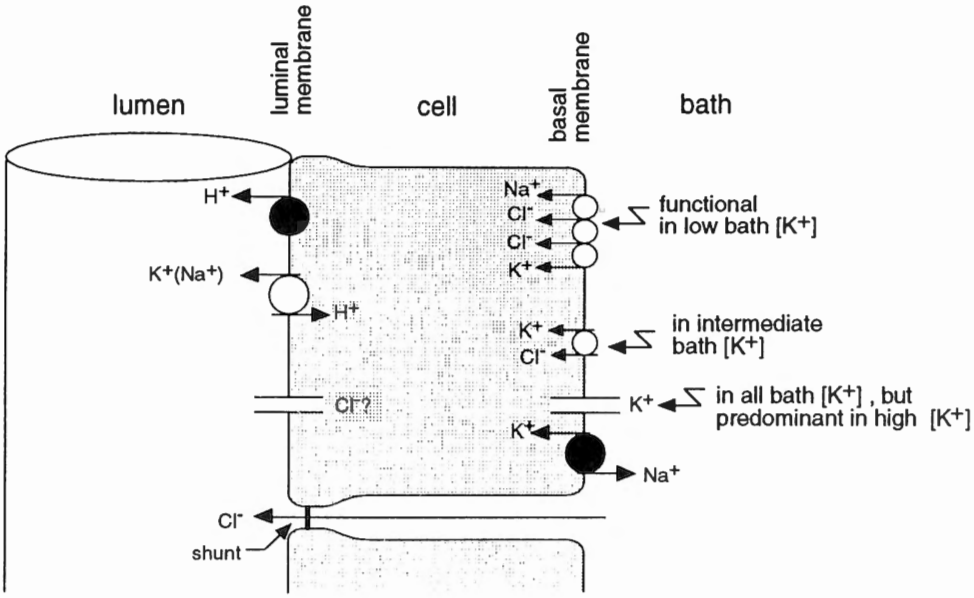

Fig. 1. — Overview of the transport mechanisms that may play a role in the salt secretion by the Malpighian tubules of *Formica* in unstimulated conditions. The relative importance of the Na/K/2Cl and the K/Cl cotransporter for basal K^+ uptake decreases with increasing bath K^+ concentration. In the presence of a high K^+ concentration uptake takes place primarily through the conductive K^+ channel. A Na/K pump was found with immunocytochemical techniques, but does not necessarily play a role in KCl secretion. A V-type H^+ATPase creates a proton concentration gradient across the luminal membrane. This gradient is sufficiently high to drive K^+ extrusion via an electroneutral H^+/K^+ antiporter. The pathway for Cl^- is still controversial.

Electrochemical gradients for K^+ and Cl^- across the epithelium and across the basal and luminal cell membrane

Data from LEYSSENS *et al.* (1993a) and DIJKSTRA (1993) allow us to show the profile of the electrochemical gradients for the two ions that are preferentially transported by the Malpighian tubule of *Formica* (see Fig. 2). The data show that both K^+ and Cl^- are concentrated in the lumen. K^+ however is secreted not only against a concentration but also against an electrical gradient, the lumen being positive with respect to the bath. So K^+ transport is active and must be transcellular. Cl^- on the other hand is transported against a concentration gradient representing an opposing force of about 12 mV. But the luminal positive potential of 39 mV is large enough to attract Cl^- into the lumen against its concentration gradient (total force being 39 mV − 12 mV = 27 mV).

When estimating the electrochemical gradients across the basal membrane (bath side) K^+ seems to be passively distributed, the concentration and the electrical potential force exactly opposing each other. The large active step for K^+ resides

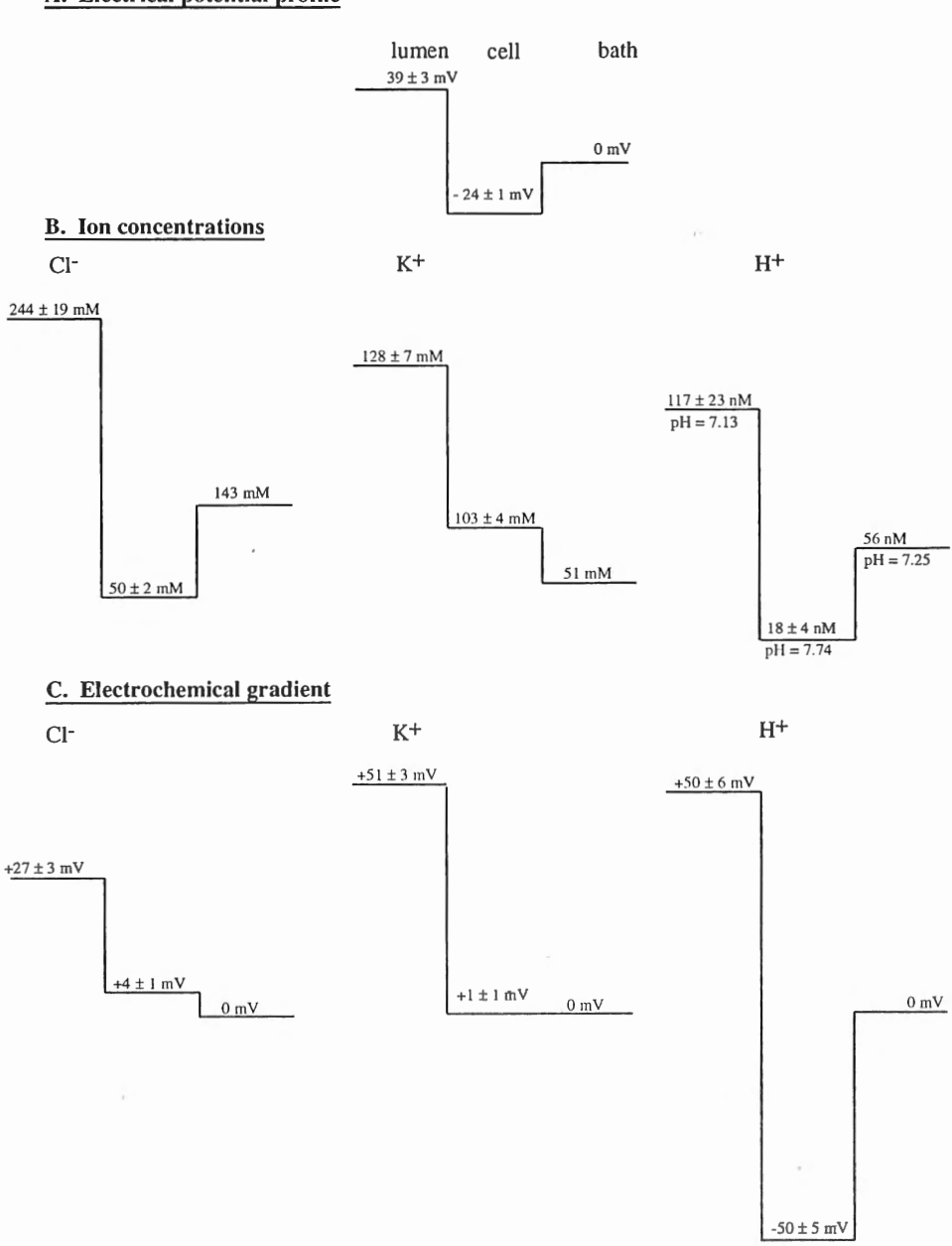

Fig. 2. — Profiles across the Malpighian tubule of *Formica* in the presence of 51 mM K^+, 63 mM Na^+, 143 mM Cl^- and pH 7.2. Summary of data from LEYSSENS *et al.* (1993a and b) and DIJKSTRA (1993). The data for K^+, Cl and H^+ were obtained from different series of tubules. Mean values ± SE (n = 6 to 63).

almost completely in the luminal membrane. The mechanism for this K^+ extrusion will be discussed below. The thermodynamic electrochemical gradient for Cl^- movement is in the direction of secretion both across the basal (4 mV) and the luminal membrane (23 mV).

K^+ uptake mechanisms across the basal membrane

K^+ channels

The basal membrane (bath side) has a high K^+ selectivity and a very low resistance ($4\Omega.cm^2$, see WELTENS *et al.*, 1992 ; LEYSSENS *et al.*, 1992 ; DIJKSTRA *et al.*, 1994a) and, as shown above, the electrochemical gradient for K^+ is very small. If K^+ was indeed distributed passively across the basal membrane, no net passive movement of K^+ would occur across this membrane. It is possible however that the conductance of this membrane is so high that an inward electrochemical driving force of 1 mV is large enough to drive sufficient K^+ into the cell to sustain the observed net K^+ transport (LEYSSENS *et al.*, 1993a). Net K^+ secretion in control conditions is calculated to be $0.2\ mA/cm^2$. If all the K^+ uptake across the basal membrane (bl) was via the conductive K^+ channels with conductance, g_{K+}, we can write :

$$\text{Net } K^+ \text{ secretion} = I^{bl}_{K+} = g^{bl}_{K+}.\ (\text{electrochemical } K^+ \text{ gradient in mV})$$
$$= 1/R^{bl}_{K+}.\ (\text{electrochemical } K^+ \text{ gradient in mV})$$
$$= 0.2\ mA/cm^2 = (1/5\Omega.cm^2).1mV$$

The value for the basal membrane resistance derived from the data was $4\Omega.cm^2$. When the resistance is that low an electrochemical gradient of 1 mV would indeed be large enough to explain the total K^+ uptake necessary to sustain the transepithelial K^+ secretion.

(A) Electrical potential differences across the epithelium (Vte), *i.e.* lumen with respect to the bath, and across the basal membrane (V_{bl}), *i.e.* cell with respect to bath. The electrical potential difference across the luminal membrane can be derived as follows : $V_{lu} = -[V_{te} - V_{bl}] = -[39\ mV - (-24\ mV)] = -63$ mV (cell negative with respect to the lumen).
(B) Ion concentrations : Cl^- and K^+ in mM and H^+ in nM. The ratio of the luminal over intracellular H^+ concentration is large enough (6.5) to drive K^+ extrusion across the luminal membrane against its concentration gradient (ratio for $K^+ = 1.2$).
(C) Electrochemical gradient across the epithelium (V^{te}) and across the basal membrane (μ_{bl}) for Cl^-, K^+ and H^+ in mV. The electrochemical gradient (μ) for an ion (i) is calculated as [the electrical potential difference (see A) across the barrier minus the equilibrium potential for the ion across the same barrier]. The equilibrium potential is calculated from the ion concentrations (see B). The electrochemical gradient (μ) for ion (i) transfer from cell to lumen across the luminal membrane is $[\mu_{te}i - \mu_{bl}i]$. It can be seen that the large active step for K^+ secretion (about 50 mV) is situated entirely across the luminal membrane. The electrochemical gradient across the basal membrane is not statistically different from zero. Driving forces for Cl^- movement are in the direction of secretion across all barriers. Protons could passively enter the cells from the bath side, if a H^+ channel or carrier was present. They are actively pumped out of the cell into the lumen against both a concentration and an electrical gradient.

TABLE 1

Effect of drugs or ion composition of the bathing solution on fluid secretion rate in isolated Malpighian tubules of *Formica**

Effect of	Bath K^+ and Na^+ concentration			mechanism
	5 or 10 mM K^+ 108 or 113 mM Na^+	51 mM K^+ 62 mM Na^+	113 mM K^+ Na^+ free	
Fluid secretion in control solution	slow	intermediate	fast	
$Ba2^+$ (6 mM)	↓↓	↓↓	↓↓	K^+ channels
Omission of Na^+	↓	no effect	no effect[a]	K/Cl or Na/K/2Cl cotransporter
Cl^- substituted by Br^-	↓	↑		
Bumetanide 10^{-5} M	↓	no effect		
10^{-4}.M	↓↓	↓	no effect	
Quabain 10^{-3} M	no effect			Na/K pump
Schering 28080 10^{-4} M	no effect	no effect		K/H pump
Vanadate 10^{-3} M		↓↓	↓↓[b]	Na/K pump or H^+ pump
Bafilomycin A1 5.10^{-6} M		↓↓		H^+ pump
NEM** 5.10^{-4} M		stops		H^+ pump

* *Summary of results from* WELTENS *et al.* (1992), DIJKSTRA (1993), LEYSSENS (1993) and LEYSSENS *et al.* (submitted). Symbols : moderate (↓), severe (↓↓) or complete (stops) inhibition of fluid secretion ; moderate (↑) stimulation or no change (no effect) in secretion.
** NEM : N-ethyl-maleimide.
[a] Omission of 3.5 mM Na^+ (*i.e.* by titrating pH of solution with KOH instead of NaOH) did not result in a change in fluid secretion rate.
[b] Note that vanadate inhibits fluid secretion in the absence of Na^+.

Such a gradient is very difficult to detect as it is within the technical limitations of the experimental methods (see Fig. 2 : the 'measured' gradient was of the order

of 1 mV and not significantly different from zero). The fact however that fluid secretion was drastically slowed down (see Table 1) and the basal membrane hyperpolarized in the presence of Ba^{2+}, a well known K^+ channel blocker, led us to believe that the blocked K^+ movement was an inward K^+ current, providing at least part of the K^+ ions needed to sustain the K^+ extrusion across the luminal membrane (LEYSSENS *et al.*, 1993a, WELTENS *et al.*, 1992).

Electroneutral K/Cl and K/Na/2Cl cotransport

K^+ channels seem to be the main pathway for K^+ uptake in a high K^+ concentration in the bath. Bumetanide, a blocker of the K/Cl or Na/K/Cl cotransporters, had no effect on fluid secretion in a high bath K^+ concentration (see Table 1). When K^+ is lowered (and Na^+ raised) however cation/anion cotransporters seem to gain in relative importance. In these media the fluid secretion is much slower, but part of the K^+ uptake is now realized by the mentioned cotransporters. In low bath K^+ the cell inward electrochemical gradient for K^+ decreases (LEYSSENS *et al.*,1993a) and clearly, less K^+ is available for uptake through channels. The necessary concentration gradients are present on the other hand for the cotransporters to be functional : experiments measuring the intracellular Cl^- and K^+ concentration showed that in an intermediate bath K^+ concentration (51 mM) the cell inward gradient for Cl^- is large enough to drive K^+ uptake via a K/Cl cotransporter, in 5 or 10 mM K^+ the Na^+ inward gradient is needed to drive a K/Na/2Cl cotransporter (LEYSSENS *et al.*, 1993a ; DIJKSTRA, 1993). Experiments using specific blockers (bumetanide) or ion substitutions (omission of Na^+ or substition of Cl^- by Br-) corroborate the above hypothesis (see Table 1 and DIJKSTRA, 1993 ; LEYSSENS *et al.*, 1994) : omission of Na^+ from the bathing solution (replaced by N-methyl-D-glucamine) caused a partial inhibition of fluid secretion, but only in a low K^+ concentration (10 mM), not in 51 mM. In other tissues a concentration of 10^{-5} M bumetanide is enough to block the Na/K/2Cl cotransporter, a higher dose is necessary before the K/Cl cotransporter is affected (see ELLORY and HALL, 1988 ; PALFREY and O'DONNELL, 1992). A concentration of 10^{-5} M partially blocked fluid secretion of the Malpighian tubule in 5 mM bath K^+, but not in 51 mM. In the latter K^+ concentration a dose of 10^{-4} M bumetanide was needed before a partial inhibition was observed (see Table 1). Also Br^-, that is known to inhibit the Na/K/2Cl but to stimulate the K/Cl cotransporter (ELLORY and HALL, 1988), had opposite effects on fluid secretion in 10 mM K^+ (inhibition) and in 51 mM K^+ (stimulation) (Table 1). The evidence strongly suggests that part of the K^+ uptake occurs via a K/Cl cotransporter in 51 mM K^+ or via a Na/K/2Cl cotransporter in a lower K^+ concentration (5 to 10 mM) in the bath.

Active pumps

No conclusive physiological evidence was found for a role of a Na/K ATPase (no effect of ouabain) or a K/H ATPase (no effect of Schering compound 28080) in sustaining the fluid secretion of the tubules of *Formica* (Table 1 ; LEYSSENS *et al.*, 1994). Immunocytochemical techniques however revealed that a Na/K pump may

be present in the basal membrane of *Formica* Malpighian tubules (GARAYOA, personal communication).

Also a high dose of vanadate, a blocker of P-type ATPases like the Na/K pump, inhibited fluid secretion (see Table 1). An effect on a Na/K pump can not be excluded (DIJKSTRA *et al.*, 1994b). But vanadate also inhibited fluid secretion in the absence of Na (see Table 1) and it depolarized the luminal membrane. It is possible therefore that the luminal H^+ pump is affected by vanadate (LEYSSENS *et al.*, submitted) as was shown in yeast cells by BELTRÁN and NELSON (1992).

K^+ extrusion across the luminal membrane, the role of luminal H^+ turnover

For a long time the K^+ extrusion was thought to be the result of an electrogenic K^+ ATPase, hypothesized by HARVEY and NEDERGAARD in 1964 in the midgut of a silkworm. In 1986 however WIECZOREK and coworkers found that the luminal membrane of the goblet cells of the *Manduca sexta* midgut contained a vacuolar type H^+ ATPase. The same group (WIECZOREK *et al.*, 1989 and 1991) presented evidence that the K^+ extrusion is the result of an exchange of cellular K^+ for luminal H^+. Depending on the function of the epithelium and the stoichiometry of the antiporter the H^+ ATPase needs to build up either a large electrical potential difference to extrude K^+ (if one K^+ is exchanged for two H^+ as in *Manduca sexta* for instance, AZUMA and WIECZOREK, 1993), or a large H^+ concentration gradient (if the antiporter is electroneutral).

Up to now data obtained for the *Formica* Malpighian tubule all indicate that a vacuolar type H^+ ATPase may be present. Evidence was presented by WELTENS *et al.* (1992) and DIJKSTRA *et al.* (1994b) : bafylomycin A1 and NEM (N-ethylmaleimide) both known to block the V-type H^+ ATPase inhibited fluid secretion and depolarized the luminal membrane of the tubule cell. ZHANG *et al.* (1994) found that an appreciable proton concentration gradient is built up across the luminal membrane (see also Fig. 2). Also DIJKSTRA *et al.* (1994b) showed that acidifying the lumen of luminally perfused tubules or applying bafilomycin A1 blocked the active equivalent short circuit current.

The H^+/K^+ exchanger in parallel with the proton pump is probably an electroneutral one for one exchanger in *Formica* tubules : the luminal proton concentration gradient is always cell inward and larger than the K^+ gradient in conditions where the tubule secretes fluid (Fig. 2 ; ZHANG *et al.*, 1994) ; when fluid secretion is slowed down (in low bath K^+ concentration for instance or in the presence of Ba^{2+}) or when it is completely blocked by DNP the ratio of the proton over the K^+ concentration gradient reaches a value close to one (ZHANG *et al.*, 1994 ; Leyssens *et al.*, 1993b).

Intrinsic regulation of K^+ secretion by K^+. Cross talk between the basal and luminal membranes

In an epithelium in steady state the quantity of ions or other substances transferred across the basal and luminal membranes must always be closely matched. Otherwise the cells would accumulate or lose material and swell or shrink.

In Malpighian tubules of *Formica* the rate of transport increases with the bath K^+ concentration : more K^+ is taken up across the basal membrane per unit time, more K^+ must therefore leave the cell across the luminal membrane via the H^+/K^+ antiporter. Factors determining the rate of turnover of the H^+/K^+ exchanger are : (1) the rate at which the protons can be recycled by the active electrogenic proton pump (see Fig. 1), (2) the affinities of H^+ and K^+ for the exchanger and (3) the relative ratios of luminal over intracellular $[H^+]$ and $[K^{++}]$: $\{[H^+]_{lu}/[H^+]_{cell}\}/\{[K^+]_{lu}/[K^+]_{cell}\}$. The latter condition holds if the exchanger is electroneutral as is probably the case in *Formica* Malpighian tubules.

The rate of recycling of H^+ by the electrogenic proton pump will depend on the luminal electrical potential difference (V_{lu}) against which the pump must transfer the protons into the lumen. The electrophysiological properties of the *Formica* Malpighian tubules provide an intrinsic regulation of V_{lu} : the luminal membrane has a much higher resistance than the basal membrane (about 50 times, WELTENS *et al.*, 1992 ; DIJKSTRA *et al.*, 1994a). This means that the electromotive forces that determine the basal membrane potential (V_{bl}), *i.e.* mainly the K^+ diffusion potential in the case of the *Formica* Malpighian tubule (LEYSSENS *et al.*, 1992), will be 'imposed' on the luminal membrane (for theoretical considerations, see WELTENS *et al.*, 1992). As a consequence, when bath K^+ is raised, V_{bl} depolarizes, reducing at the same time V_{lu}. So the electrical gradient against which the H^+ pump must secrete the protons decreases and proton cycling can occur at a much faster rate.

A second phenomenon is that, the intracellular K^+ concentration of the cells increases and reaches a new steady state value when the bath K^+ is raised (LEYSSENS *et al.*, 1993a). Total cation concentration in the cell will remain constant, but K^+ is presumably replacing Na^+. Clearly, if $[K^+]_{cell}$ increases the turnover of the H^+/K^+ exchanger will be facilitated.

The pathway for passive Cl^- secretion

This pathway is still controversial. It may be transcellular (*i.e.* across both cell membranes) and/or paracellular (*i.e.* across the shunt). In *Formica* Malpighian tubule only one cell type is present (GARAYOA *et al.*, 1992). So a cell type specialized in Cl^- transport as in frog skin for instance does not seem likely in these tubules.

A rough estimate can be made of the relative importance of the transcellular or shunt pathways. If chloride travels across the epithelium via the cellular pathway, a basal carrier transport for chloride has to be present since no evidence was found for an appreciable Cl^- conductance across this membrane (LEYSSENS *et al.*, 1992). Experimental results showed that K/Cl and Na/K/Cl cotransporters may be present (see Fig. 1 and Table 1), but from the data it is clear that these cotransporters

sustain but part of the fluid secretion and that they function only in the presence of an intermediate or low bath K^+ concentration. At the luminal side a Cl^- channel could be present as is the case in many NaCl and KCl transporting epithelia. As explained above the movement (I) of ion (i) across a conductive membrane depends (1) on the electrical potential difference (V) across the barrier, (2) on the concentration gradient, expressed as the equilibrium or Nernst potential, E_i, and (3) on the specific conductance of the barrier for the ion, g_i. $I_i = g_i . (V - E_i)$.

We expect the Cl^- conductance (g_{Cl}) to be low since the total conductance of the luminal membrane is low (50 times lower than the basal membrane conductance, WELTENS *et al.*, 1992 ; DIJKSTRA *et al.*, 1994a). Therefore the driving force ($V - E_{Cl}$) should be rather large to obtain an appreciable Cl^- current across this membrane.

In low bath K^+ fluid secretion is slow (see Table 1), but V_{lu} is relatively high (LEYSSENS *et al.*, 1992). As stated above in low bath K^+ the relative importance of Cl^- uptake into the cell via the cation/anion cotransporters increases too. So part of the Cl^- secretion may pass through the cell. V_{te}, *i.e.* the driving force for Cl^- across the shunt on the other hand is small, this means that the contribution of the shunt in Cl^- transfer may decrease.

In high bath K^+ the opposite is true. V_{lu} decreases and V_{te} increases : Cl^- movement across the shunt seems to be favoured.

A detailed investigation of the specific Cl^- conductances and driving forces across all barriers is needed, before a final conclusion can be drawn, but the data seem to suggest that in high K^+, when secretion is fast, most of the Cl^- movement will be across the shunt. K^+ uptake across the basal membrane will be via the K^+ channel, independent of Cl^- and Cl^- uptake by the cells may be small. When bath K^+ is lowered and secretion slows down, some Cl^- (together with part of the K^+) may be taken up by the cell via the cation/anion cotransporters. This amount of Cl^- may leave the cell through a Cl^- channel in the luminal membrane be it at a relatively slow rate (due to the low Cl^-conductance), but taking advantage of the large electrical driving force (V_{lu}) present in low K^+ conditions. To complete the picture the E_{Cl} in all K^+ concentrations should be known as well.

Cl^- is far from equilibrium both across the shunt and across the luminal membrane (see Fig. 2 in the presence of 51 mM K^-). Therefore Cl^- may be a rate limiting step in the salt transport and a site for regulation. When fluid secretion was increased by the second messenger cAMP, it was found that the basolateral Na/K/2Cl cotransporter is stimulated (DE DECKER, 1993), accelerating Cl^- entry into the cell. In these circumstances the luminal (and transepithelial) electrical potential difference approaches the Cl^- equilibrium potential (DIJKSTRA, 1993), suggesting that the conductance for Cl^- (luminal and shunt ?) may have increased. More data are required however before it can be decided how much of this Cl^- is passing either through the shunt or through the cell.

BRIEF COMPARISON WITH OTHER SPECIES

The high K^+ dependence of fluid secretion of *Formica* Malpighian tubules is in sharp contrast with for instance tubules of the tsetse fly, *Glossina morsitans*, where fluid secretion is primarily dependent on $Na+$, although a minimal amount of K^+ needs to be present in a high Na^+ medium in order to keep the fluid secretion going at a high rate (GEE, 1976). In the ant only in low K^+ high Na^+ medium Na^+ becomes of importance in sustaining part of the fluid secretion (LEYSSENS *et al.*, unpublished). In *Musca* for instance a minimal amount of Na^+ is needed to maintain the fast fluid secretion rate in high K^+. Table 2 gives an overview of possible patterns of dependence on K^+ and Na^+ in a few species.

TABLE 2

Fluid secretion rate by isolated Malpighian tubules as a function of the Na^+ and K^+ concentration in the bath.

Species	Composition of bathing solution				References
	K^+ free high Na^+	low K^+ high Na^+	high K^+ low Na^+	high K^+ Na^+ free	
Formica	0	+	+++	+++	VAN KERKHOVE *et al.* (1989)
Carausius	0	+	+++	+++	PILCHER (1970)
Teleogryllus	§	+	+++	+++	MARSHALL *et al.* (1993)
Pieres	+	+	+++	+++	NICOLSON (1976)
Musca	(+)	++	+++	(+)	DALTON and WINDMILL (1980)
Glossina	0	+++	+	0	GEE (1976)
Libellula	+++	+++	+	0	NICHOLLS (1985)

Symbols : +++ (= high secretion rate), ++ or + (= intermediate), (+) (=low) and 0 (= secretion stops).
§ Data not available.

One explanation for the observed differences is that the importance of K^+ versus Na^+ channels may vary. Na^+ channels for instance are more important in the mosquito especially when the tubule is stimulated (see SAWYER and BEYENBACH, 1985). This makes sense in an animal that has to be able to get rid of a high NaCl load after the female mosquito has taken a blood meal. The animal is primarily interested in ingesting the blood proteins in order to prepare its eggs. The NaCl and water that are taken in at the same time have to be excreted as fast as possible to allow the animal to lose the extra weight that prevents it from flying away and make it an easy prey for predators.

The relative role played by the cation/anion cotransporters may also be different. Inhibitors of the anion/cation cotransporters (furosemide, bumetanide) were

reported to reduce fluid secretion in *Rhodnius*, (O' DONNELL and MADDRELL, 1984), in *Aedes*, at least in stimulated conditions (HEGARTY *et al.*, 1991), in *Locusta* (BALDRICK *et al.*, 1988) in *Glossina* (ISAACSON and NICOLSON, unpublished, cited in NICOLSON, 1993) and in *Drosophila* (WESSING *et al.*, 1986). It would be interesting to make a comparative study of the kinetics and ion affinities of these systems in the different species.

A role for a Na/K pump is mostly studied using cardiac glycosides, well known inhibitors of this pump in vertebrate tissue. Insects may have developed resistance against this plant alkaloid (Malpighian tubules of some species can even secrete it, MEREDITH *et al.*, 1984) and the effect of ouabain or similar compounds on fluid secretion of Malpighian tubules is variable (ANSTEE and BOWLER, 1979). In *Rhodnius* ouabain even stimulated fluid secretion, possibly because the cells become rich in Na^+ (MADDRELL and OVERTON, 1988).

Evidence for the presence of a proton pump in the Malpighian tubule of insects was first obtained by BERTRAM *et al.* (1991), when they showed that bafilomycin A1 stopped fluid secretion in tubules of *Drosophila*. ISAACSON and NICOLSON (unpublished, cited in NICOLSON, 1993) for *Onymacris* and *Glossina* and PANNABECKER and BEYENBACH (1993) for *Aedes* observed the same phenomenon.

Preferential secretion of either a K^+ or a Na^+ rich fluid may be due to differences in the affinity of the luminal proton/cation exchanger for either K^+ or Na^+. MADDRELL and O'DONNELL (1993) controlled the ion composition of the intracellular milieu using gramicidin that inserts into the basal membrane as non-selective cation permeable channel. They showed that in the blood sucking insect, *Rhodnius*, the tubules secrete a Na^+ rich fluid even when the cellular milieu contains equal concentrations of K^+ and Na^+. The system [proton pump plus proton/cation antiporter] clearly shows a preference for Na^+ over K^+.

Inhibition of fluid secretion by amiloride (reviewed by NICOLSON, 1993), even in the absence of Na^+ (LEYSSENS and ZHANG, unpublished results) is in favour of the presence of a luminal proton/cation antiporter.

In conclusion Malpighian tubules of different species seem to possess similar tools to transport KCl and/or NaCl, i.e. ion channels, cation/anion cotransporters, a Na/K pump in the basal membrane on the one hand, a proton pump and proton/cation antiporters (and Cl^- channels ?) in the luminal membrane on the other. Differences in the kinetics and in the relative importance (*e.g.* silent or active in control versus stimulated conditions) of these systems may allow the tubule to perform its function according to the specific needs of each species.

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