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INSECT-LIKE CHARACTERISTICS OF THE MALPIGHIAN TUBULES OF A NON-INSECT: FLUID SECRETION IN THE CENTIPEDE *LITHOBIUS FORFICATUS* (MYRIAPODA: CHILOPODA)

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Summary

Fluid secretion by isolated upper and lower portions of Malpighian tubules in the centipede *Lithobius forficatus* L. was studied. Ion requirements, cellular and transepithelial potentials, dependence on external osmolality and the effects of an insect diuretic factor and transport-active drugs were investigated. Unlike many insects, *L. forficatus* exhibited strongly Na^+-dependent, K^+-independent urine formation. However, as in many insects, upper and lower tubule portions from *L. forficatus* produced a K^+-enriched, hypertonic fluid, and the transepithelial potential was positive with respect to the haemolymph. Furthermore, furosemide (5×10^{-4} mol l^{-1}) reversibly inhibited urine formation. Ouabain, even at 10^{-3} mol l^{-1}, had little effect on urine flow rate in upper tubules but inhibited secretion in lower tubules, albeit not completely. Locust diuretic hormone (at 10^{-7} mol l^{-1}) enhanced fluid secretion in *L. forficatus*, but its action was not mimicked by dibutyryl cyclic AMP. The results suggest that some characteristics attributed exclusively to insects are common to non-insect arthropods.

Introduction

Since the pioneer work of Ramsay (1954) on isolated insect Malpighian tubules, the transport mechanisms of the Malpighian tubules of a number of insect species have been characterized (see reviews by Maddrell, 1980; Phillips, 1981; Bradley, 1985). Knowledge of transport characteristics of Malpighian tubules in other tracheates derives from a single study on a myriapod, the diplopod *Glomeris marginata* (Farquharson, 1974*a,b,c*). The differences from insect Malpighian tubules were attributed to evolutionary specialization (e.g. Phillips, 1981; Nicholls, 1985). To evaluate the specificity of fluid secretion mechanisms in insects and other tracheates, however, more data are needed.

This study describes the fluid secretion of the Malpighian tubules of another

Key words: *Lithobius forficatus*, urine formation, Malpighian tubules, fluid secretion.

myriapod, the chilopod *Lithobius forficatus* L. Each of its two Malpighian tubules is almost twice as long as the animal itself (Rilling, 1968). Each consists of a single, uniform cell layer without a muscular sheath (Füller, 1966). Micropuncture studies on whole animals showed that the tubules release a potassium-enriched, hypertonic fluid into the rectum (Wenning, 1978, 1979).

Mechanisms of urine formation in *L. forficatus* were examined in isolated Malpighian tubules with respect to ion requirement, cellular and transepithelial potentials, dependence on external osmolality and pharmacology of transport-active drugs and insect diuretic factors. The results are discussed with respect to the specificity and evolution of fluid secretion mechanisms in the Malpighian tubules of tracheates. Some of the results have appeared in abstract form (Wenning, 1989).

Materials and methods

Lithobius forficatus (average body mass 130 mg) were collected locally. The animals were kept in containers filled with leaves and moist soil in a room at 16 °C at high relative humidity and a light:dark cycle of 12 h:12 h.

Isolation of the Malpighian tubules and urine flow measurements

Preparation of the tubules and flow measurements were carried out at room temperature (20–24 °C). Animals were decapitated and dissected in artificial haemolymph. The two Malpighian tubules were freed from the few tracheoles holding them in place and severed near the ampulla. To test for regional differences in the Malpighian tubules, all experiments were carried out on upper and lower tubule portions separately. Each tubule was cut into two portions – an 'upper tubule' (distal portion), containing crystals in its lumen, and a 'lower tubule' of wider diameter (proximal to the ampulla), containing clear fluid. Upper and lower tubules were of approximately equal length. Preparation time was 30–45 min.

The experimental design for flow measurements was that originally described by Ramsay (1954). In brief, the upstream end of the respective tubule portion was tied with superthin surgical thread (Leonhard Klein, Germany), submerged in a droplet (200 μl) of bathing medium in a wax-lined dish and covered with liquid paraffin. The open end was drawn into the oil. Fluid production was calculated by measuring the diameter of urine droplets with a calibrated eyepiece graticule. Fluid production is given in nanolitres per unit time and millimetres effective tubule length, i.e. the tubule part submerged in the bathing medium. In some experiments, the tubule fluid was analyzed for its osmotic and ionic concentration.

Solutions

The composition of the artificial haemolymph was based on data of Wenning (1978): (mmol l^{-1}) Na^+, 190; K^+, 6; Cl^-, 204; Ca^{2+}, 2; Mg^{2+}, 2; trehalose, 10; buffered with 10 mmol l^{-1} Hepes (pH 7.0). Osmolality was 410 mosmol kg^{-1} H_2O.

To test for its effects on urine production, the osmolality was decreased by diluting artificial haemolymph with Hepes buffer (10 mmol l^{-1}; pH 7.0) or increased by adding sucrose or NaCl. Decreases in NaCl concentration without changes in osmolality were achieved by replacing NaCl with isotonic sucrose solution. [Na^+] was decreased by replacing NaCl with choline chloride, keeping [Cl^-] constant. Higher K^+ concentrations were achieved at the expense of NaCl.

Analytical methods

Osmolality was determined with a Clifton nanolitre osmometer (Clifton Technical Physics, New York, USA). The error was ±3 mosmol kg^{-1} H_2O. Na^+ and K^+ concentrations were determined using an ultramicro double-beam flame spectrophotometer (W. Hampel, Frankfurt; developed by Müller, 1958; modified by Malnic *et al.* 1964). The error was ±3 mmol l^{-1} Na^+ and ±0.3 mmol l^{-1} K^+.

Electrophysiology

For electrophysiological recordings, a chamber of 1.6 ml was used. The downstream end of the tubule was placed in a Vaseline trough filled with oil. The reference electrode (chlorided silver wire) was in the bath. To determine the cellular potential (basolateral E_m), the cells were impaled with glass microelectrodes (35–45 MΩ, filled with a mixture of 4 mol l^{-1} potassium acetate and 20 mmol l^{-1} KCl). To determine the transepithelial potential (E_{TEP}), a low-resistance (≈10 MΩ) glass microelectrode was compensated for its junction potential in the bath and then moved into the urine droplet.

Experimental protocol and statistics

Absolute flow rates among individual tubules varied. A control rate was established for each tubule by averaging the flow rate in artificial haemolymph measured for 60–120 min at intervals of 15–20 min. This rate was taken as 100 %. The bathing medium was then exchanged for the test solution. The new flow rate was determined for 60–80 min and expressed as a percentage of the control rate. To test whether effects were reversible, the bathing medium was replaced with artificial haemolymph and the flow rate was again determined for 60 min. Values are given as mean±S.E.M., with the number of tubules indicated for each set of experiments. Appropriate *t*-tests (paired, unpaired) were used to assess the significance of different treatments.

No time dependence of flow rate was observed (see Fig. 1), indicating that the difference between maintenance and experimental temperature (see above) did not affect the results.

Results

Flow, osmolality and ionic composition of urine in artificial haemolymph and electrical properties of the tubules

Although the ultrastructure of the epithelium of the Malpighian tubules of

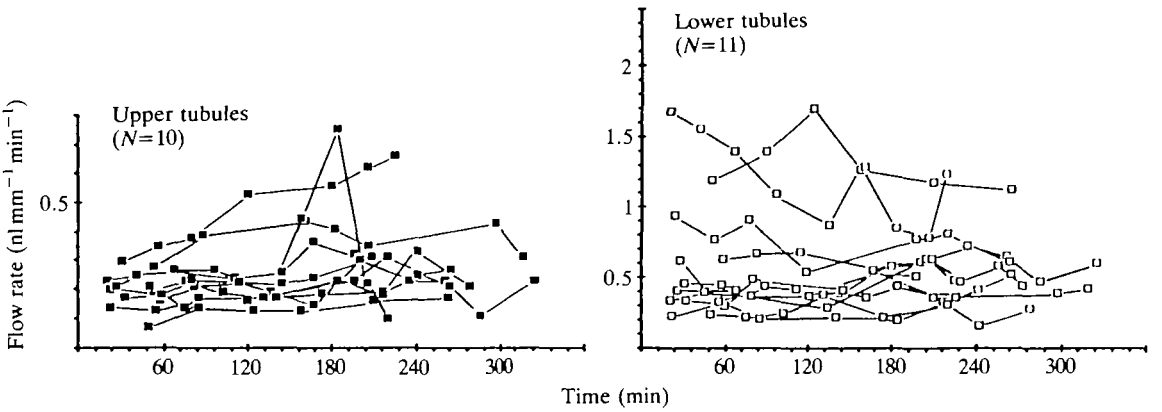

Fig. 1. Rates of urine flow in isolated upper and lower Malpighian tubule portions of *Lithobius forficatus* vary individually and do not decrease during the course of an experiment.

L. forficatus is uniform (Füller, 1966), the upper and lower parts of the tubules differed in appearance owing to the presence of crystals in the lumen of the 'upper' tubule. The 'lower' tubule was more transparent. As in some insects (Maddrell and Phillips, 1975; Cooper *et al.* 1989), upper and lower portions exhibited differences in their physiological properties.

The outer diameter was about 50 μm at the proximal end of the tubule and widened to about 90 μm at the entrance to the ampulla. The flow rate in the lower part of a tubule was significantly higher than in the upper part (Fig. 1, $P=0.022$, paired t-test; see also Table 1). Isolated tubules continued to produce urine for 24 h.

Both upper and lower tubules secreted a potassium-enriched fluid but the K^+ concentration was significantly higher in the fluid in the lower tubule. Na^+ was the main cation and its concentration was similar in the fluid in both tubule portions (Fig. 2; Table 1). At a concentration of 170–180 mmol l^{-1}, Cl^- was the main anion in the fluid released from the ampullae *in vivo* (Wenning, 1978) and from isolated whole tubules (A. Wenning, unpublished results). The tubule fluid was hypertonic to the bathing medium in upper tubules by 9.4 mosmol kg^{-1} H_2O and in lower tubules by 12 mosmol kg^{-1} H_2O (Fig. 2). Apparently, little or no reabsorption occurred along the tubules as it does, for example, in *Rhodnius prolixus* (Maddrell and Phillips, 1975).

Basolateral E_m was similar in upper and lower tubules (-39.2 ± 1.8 mV and -38.1 ± 1.7 mV, respectively), while E_{TEP} was significantly more positive in lower than in upper tubules ($+12.3\pm2$ mV *versus* $+5.5\pm0.8$ mV, $P=0.006$) (Fig. 2). The method used to measure E_{TEP} might diminish its true value, owing to leakage pathways (discussed by Aneshansley *et al.* 1989), although with tubule portions 10–14 mm long and with 2 mm between urine droplet and haemolymph, electrical isolation should be adequate.

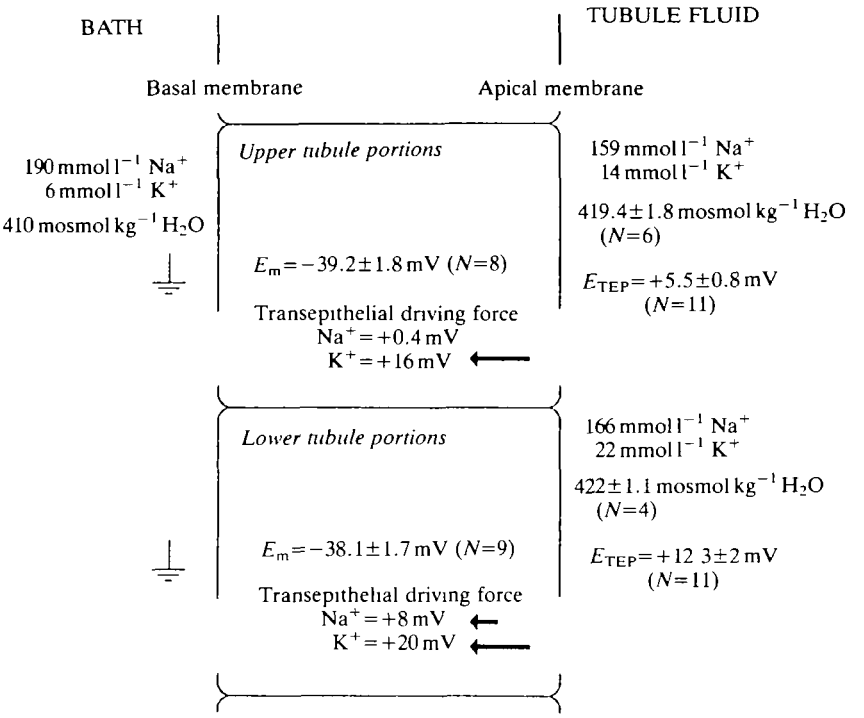

Fig. 2. Ion concentrations and electrical properties of the bath and tubule fluid. Values are mean±S.E.M. The electrochemical transepithelial potentials for Na^+ and K^+ of upper and lower tubules were calculated using the Nernst equation. Note that Na^+ may move paracellularly in upper tubules, but that electrochemical gradients oppose Na^+ transport in lower tubules and K^+ transport in upper and lower tubules. Arrows signify the direction of the transepithelial driving force.

Ion requirements and dependence of the flow rate on external osmolality

Flow rate in isolated Malpighian tubules was inversely correlated with external osmolality (Fig. 3), but upper and lower tubules differed significantly in their sensitivity. With decreasing external osmolality, the flow rate in upper tubules was more enhanced than that in lower tubules. With increasing external osmolality, achieved by adding NaCl, the flow rate in upper tubules was unaffected, whereas it was greatly reduced in lower tubules (Fig. 3, squares). An increase in osmolality at constant ionic strength caused a similar reduction of flow rate in upper and lower tubules (Fig. 3, diamonds).

The increase of flow rate in dilute media was due to hypotonicity rather than to the concomitant decrease in ionic strength, because a decrease in external $[K^+]$ or $[Na^+]$ at constant osmolality resulted in a decrease in flow rate (Fig. 4A,B). At 50 mmol l^{-1} Na^+ and at constant osmolality, fluid production ceased in both tubule portions (Fig. 4A). However, a comparison of the decline of flow rates in upper and lower tubules (Fig. 4A) shows that upper tubules were less affected when $[Na^+]$ was lowered to 140–170 mmol l^{-1}.

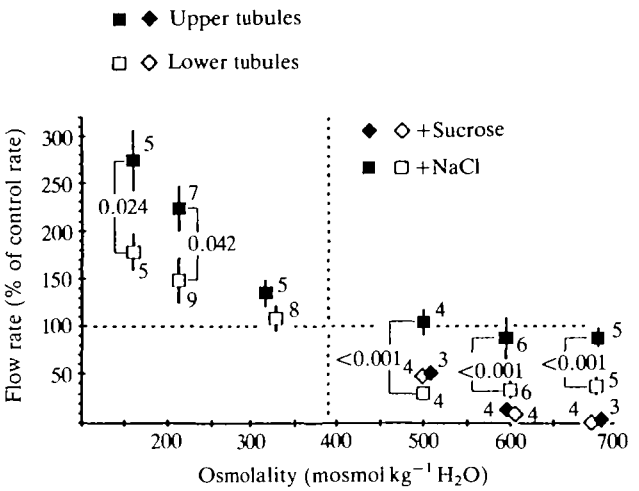

Fig. 3. Flow rates of fluid in upper and lower tubules are inversely related to external osmolality. Lower osmotic concentrations were achieved by dilution with Hepes buffer, higher osmotic concentrations by adding NaCl (squares) or sucrose (diamonds). Compared with lower tubules, upper tubules produce significantly more urine in dilute media and at elevated NaCl concentrations (P values indicated at each point; unpaired t-test). Dashed lines represent flow rate (horizontal) and bath osmolality (vertical) during the control period. Each point represents the average flow rate during the experimental time (60 min) and is expressed as a percentage of the control rate. Values are mean±S.E.M. The number of tubules used is indicated next to each point.

The strong inverse relationship between flow rate and osmolality in upper tubules (Fig. 3) seemed to be due to their relative insensitivity – compared with lower tubules – to a decrease in external Na^+ concentration. This was demonstrated by lowering either [NaCl] or [Na^+] (Fig. 4A). Lower tubules were more sensitive to decreases in Na^+ concentration in the bathing medium (Fig. 4A) and the increase in flow rate with decreasing osmolality (Fig. 3) was therefore not as steep as that in upper tubules.

Although sensitive to hypertonicity at constant ionic strength (Fig. 3, diamonds), fluid production in upper tubules did not decrease with increasing external [NaCl] (Fig. 4A), indicating an antagonistic effect of the increases in NaCl and osmolality. In lower tubules, a decrease in flow rate occurred regardless of whether sucrose or NaCl was added (Fig. 4A), illustrating the marked effect of hypertonicity.

Lowering [K^+] from 6 to 3 mmol l^{-1} caused the fluid production to drop to 30 % of the control rate in lower tubules; upper tubules were unaffected (Fig. 4B). Fluid production continued in nominally 0 mmol l^{-1} K^+. The increase in [K^+] at the expense of [Na^+], however, had little effect on fluid production in upper tubules, but decreased it in lower tubules (Fig. 4B). This was not due to the simultaneous decrease in external [Na^+], since an increase in [KCl] with no change in [NaCl] had the same effect (not illustrated).

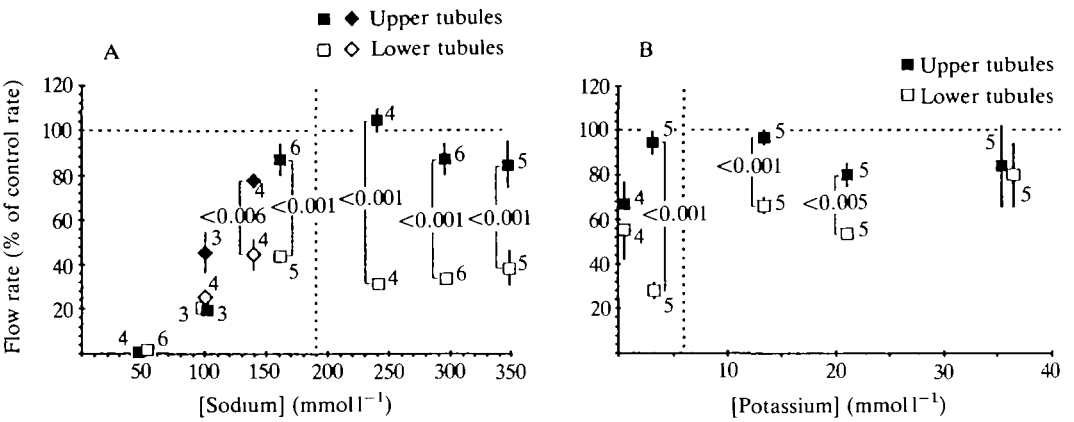

Fig. 4. Ion dependence of fluid production in isolated tubules. (A) Flow rates in upper and lower tubules depend on the presence of Na^+ at constant osmolality (left-hand part of the figure; squares, NaCl replaced by sucrose; diamonds, NaCl replaced by choline chloride). At increased NaCl concentrations, and therefore elevated osmolality, fluid production is unaffected in upper tubules, but is reduced in lower tubules. (B) Urine formation continues at nominally 0 mmol l^{-1} K^+ and at elevated K^+ concentrations, albeit at a reduced rate. Dashed lines represent flow rates (horizontal) and bath ion concentrations (vertical) during the control period. Each point represents the average flow rate during the experimental period (60 min) and is expressed as a percentage of the control rate (P values are indicated; unpaired t-test). Values are mean±S.E.M. The number of tubules used is indicated next to each point.

Pharmacology

Furosemide, an inhibitor of $Na^+/K^+/2Cl^-$ cotransport in a variety of transporting epithelia (reviewed by Greger, 1985), blocked fluid production completely in upper and lower tubules at 5×10^{-4} mol l^{-1} (Fig. 5). When furosemide was removed, the flow rate did not return to its control value.

Ouabain, an inhibitor of Mg^{2+}-dependent Na^+/K^+-ATPase, did not block fluid production completely (Fig. 6). Sensitivity differed in upper and lower tubules. At concentrations between 5×10^{-7} and 5×10^{-5} mol l^{-1}, ouabain was ineffective in upper tubules and had only minor effects in lower tubules (Fig. 6, and unpublished data). At 5×10^{-5} mol l^{-1}, fluid production was inhibited after wash-out in lower tubules and was stimulated in upper tubules (Fig. 6). At 10^{-3} mol l^{-1}, ouabain clearly inhibited urine formation in lower tubules, whereas the effects on upper tubules varied (Fig. 6).

Dibutyryl cyclic AMP (dbcAMP), applied in combination with the phosphodiesterase blocker isobutyl methylxanthine (IBMX) (both at 10^{-3} mol l^{-1}), caused a marked decrease in flow rate (Fig. 7), which returned to its normal value in upper tubules after wash-out.

Synthetic arginine-vasopressin-like diuretic hormone (DH) of *Locusta migratoria* (Proux *et al.* 1987) increased fluid production in isolated *L. forficatus* tubules at 10^{-7} mol l^{-1} (Fig. 8), which is 100 times the effective dose for *L. migratoria*

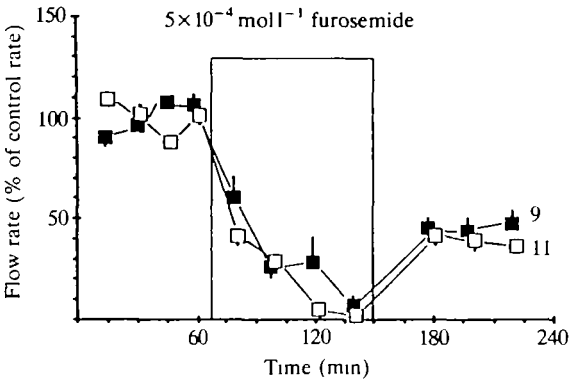

Fig. 5. Effects of 5×10^{-4} mol l^{-1} furosemide on urine flow rate in isolated tubules. The rectangle indicates the time of drug application. (■ upper tubules, □ lower tubules). Values are mean±S.E.M. The number of tubules used is indicated.

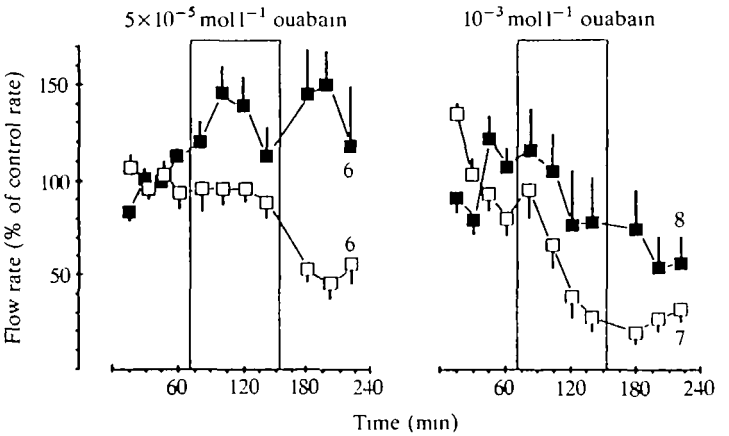

Fig. 6. Ouabain does not completely inhibit fluid production in isolated tubules. Note that, upon ouabain application, relative flow rates are higher in upper (■) than in lower (□) tubules. Other details as in Fig. 5.

(Proux *et al.* 1988). Locust DH at 10^{-8} mol l^{-1} was less effective (not shown). The response to peptides was, however, transient and lasted for 20 min. The locust diuretic hormone (DH) is the antiparallel dimer of a nonapeptide (F1) (Proux *et al.* 1987). F1 at 10^{-5} mol l^{-1} enhanced the flow rate in lower and upper tubules (Fig. 8).

Na^+/K^+ ratio in stimulated and unstimulated tubules

The question of whether Na^+ and/or K^+ transport increase with increased fluid production was investigated by measuring Na^+ and K^+ concentrations in the tubule fluid before and after they had been stimulated either by locust DH or by

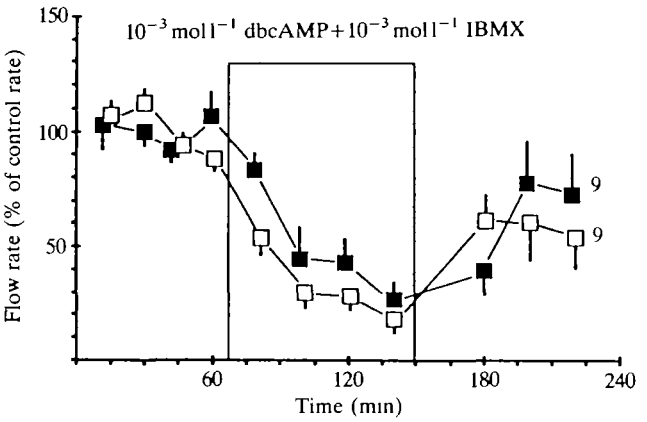

Fig. 7. Dibutyryl cyclic AMP (dbcAMP) and isobutylmethylxanthine (IBMX), both at 10^{-3} mol l^{-1}, inhibit fluid production in isolated upper (■) and lower (□) tubules. Other details as in Fig. 5.

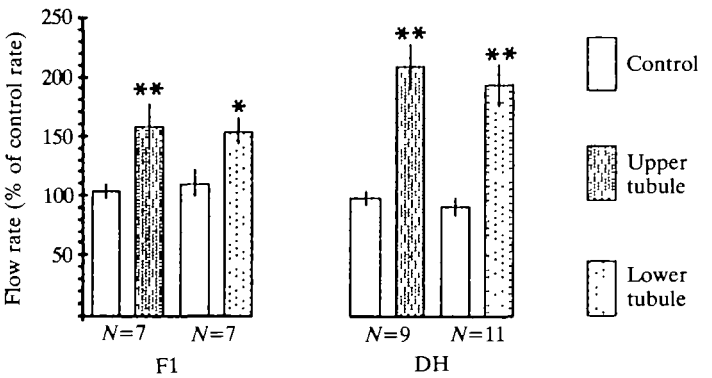

Fig. 8. Effects of two peptides isolated from *Locusta migratoria* on the mean flow rate (±S.E.M.) of fluid in isolated tubules from *Lithobius forficatus* immediately before (control) and 10–20 min after adding the peptides (DH, 10^{-7} mol l^{-1}; F1, 10^{-5} mol l^{-1}). DH, arginine-vasopressin-like locust diuretic hormone; F1, amidated monomer of DH (**$P<0.01$, *$P<0.03$; paired t-test).

lowering the osmolality (Table 1). Na^+ and K^+ secretion rates were calculated using flow rates determined simultaneously. Application of DH had no effect on Na^+ and K^+ concentrations of the tubule fluid in either portion, and consequently did not affect the Na^+/K^+ ratio. Absolute Na^+ and K^+ secretion rates increased significantly in the upper tubules, owing to the stimulation of fluid production. A slight increase was observed in the lower tubules. Upon lowering the external osmolality to 160 mosmol kg^{-1} H_2O, Na^+ and K^+ concentrations in the tubule fluid decreased. The Na^+/K^+ ratio was unaffected in lower tubules but decreased significantly in upper tubules, indicating that K^+ transport was stimulated more than Na^+ transport in the upper tubules.

Table 1. *Na^+ and K^+ secretion rates, concentrations and ratios (determined individually) and urine flow rates in upper and lower tubules before and after stimulation of fluid transport*

	Before stimulation	20 min after stimulation by locust DH (D1;10^{-7} mol l^{-1})	30 min after stimulation by low osmolality (160 mosmol kg^{-1} H_2O)
Upper tubules	N=19	N=10	N=9
Flow			
(nl mm^{-1} min^{-1})	0.13±0.03	0.23±0.04**	0.43±0.1**
Na^+			
(nmol mm^{-1} min^{-1})	20.9±4.05	35.9±5.7**	30.7±7**
(mmol l^{-1})	159±3	163±5.6	72±2.3**
K^+			
(nmol mm^{-1} min^{-1})	1.9±0.47	3.3±0.77**	4.7±1.23**
(mmol l^{-1})	13.9±0.7	14±1.2	10.7±0.9**
Na^+/K^+ ratio	12.6±1	12.4±1.4	7.2±0.6**
Lower tubules	N=19	N=7	N=12
Flow			
(nl mm^{-1} min^{-1})	0.44±0.07†	0.72±0.09*,†	1.28±0.37*,†
Na^+			
(nmol mm^{-1} min^{-1})	73.9±11.8	119.9±18.8	91.2±22.7
(mmol l^{-1})	166±3.4	167±8.8	78.4±3.1**
K^+			
(nmol mm^{-1} min^{-1})	10.5±2.15	13.5±3.4	15±4.2
(mmol l^{-1})	22.3±2.1†	18.8±3.8	13±2.2**
Na^+/K^+ ratio	9.1±1	11.2±2.2	7.5±0.9
Bath			
Na^+/K^+ ratio	33	33	33

Values given as mean±S.E.M.
** $P<0.008$, * $P<0.02$; paired t-test, before *versus* after stimulation.
† $P<0.001$; unpaired t-test, upper *versus* lower tubules.

Discussion

Ion dependence of urine formation

The luminal fluid in isolated upper and lower portions of the Malpighian tubules of the centipede *L. forficatus* is K^+-enriched, slightly hypertonic and electropositive with respect to artificial haemolymph (Fig. 2; Table 1), indicating active urine formation. This is also true for most insects. In contrast, fluids produced by the Malpighian tubules of the millipede *Glomeris marginata* and the dragonfly *Libellula quadrimaculata* remain similar in composition to the bathing fluid over a wide range of external Na^+ and K^+ concentrations, and the transepithelial potential was reported to be 0 mV (Farquharson, 1974*b*; Nicholls, 1985).

It has been established that an apical cation pump is the driving force for urine formation in insect Malpighian tubules (Berridge, 1968). This pump enriches th

tubule fluid with K^+, which is the major intracellular cation, but can also transport Na^+, as was first shown in a blood-sucking insect during diuresis (Gee, 1975). As a result of its ability to transport either Na^+ or K^+, depending on their respective intracellular concentrations, the pump was named the 'common cation pump' (Maddrell, 1977). Both Na^+ and K^+ are actively transported across most insect Malpighian tubules (see O'Donnell and Maddrell, 1984), generating a lumen-positive transepithelial potential (Sawyer and Beyenbach, 1985). Although the tubule fluid is enriched with K^+ in the chilopod *L. forficatus*, K^+ is not the prime mover of fluid secretion and an increase in $[K^+]$ does not enhance secretion (Fig. 4 A), as it does in most insects (Bradley, 1985; Morgan and Mordue, 1981; Baldrick *et al.* 1988; Van Kerkhove *et al.* 1989).

In contrast, Na^+ is essential for fluid secretion in both upper and lower portions of isolated Malpighian tubules of *L. forficatus* (Fig. 4A), in the pill millipede (Farquharson, 1974*b*) and in a dragonfly (Nicholls, 1985), the only known example among insects. Elevated NaCl concentrations, however, affect only the lower tubules (Fig. 4A), where they cause fluid secretion to decrease. Since both portions are equally sensitive to media made hypertonic by addition of sucrose (Fig. 3), elevated Na^+ concentrations might enhance fluid secretion in upper tubules, which is then counteracted by the inevitable increase in osmolality.

Electrochemical gradients for K^+ and Na^+ were calculated by using the Nernst equation. To determine the transepithelial electrochemical gradients, the measured concentrations of Na^+ and K^+ in the bath and in the tubule fluid were used. The basolateral and apical electrochemical gradients were estimated from assumed intracellular concentrations. Owing to the negative potential of the tubule cells, the electrochemical gradient for Na^+ is directed into the cell and apical transport of Na^+ must be active in *L. forficatus*. Paracellular Na^+ transport as a major factor in urine formation – albeit possible in upper tubules (Fig. 2) – is unlikely, since furosemide blocks fluid secretion completely (Fig. 5, see also below). During diuresis, Na^+ transport is stimulated more than K^+ transport in the Malpighian tubules of blood-sucking insects (Gee, 1975; Maddrell, 1969; Williams *et al.* 1983; Williams and Beyenbach, 1983) and in the nephridia of the blood-sucking medicinal leech *Hirudo medicinalis* (Zerbst-Boroffka *et al.* 1982). Elevated rates of Na^+ transport ensure efficient clearance of the major cation of the ingested meal. The rate of Na^+ transport (without diminishing K^+ transport) is also increased in other insect species during diuresis: twofold in a desert beetle (Nicolson and Hanrahan, 1986) and fivefold in the house cricket (Spring and Hazelton, 1987), stimulated with corpora cardiaca, brain and/or prothoracic ganglion homogenates. In *L. forficatus*, the Na^+/K^+ ratio does not change significantly in the presence of locust DH (Table 1), indicating that both ions are transported at the same rate under these conditions. When fluid production is stimulated by lowering the external osmolality, K^+ secretion is enhanced more than Na^+ secretion in upper, but not in lower, tubules. Apical electrochemical gradients for K^+ were estimated by using the measured concentrations of the tubule fluid and assumed intracellular concentrations. Transcellular K^+ transport

is against the electrochemical gradient (Fig. 2), but – as calculated using the Nernst equation – apical K^+ transport would be downhill when intracellular $[K^+]$ exceeds 85 mmol l^{-1} in upper and 150 mmol l^{-1} in lower tubules. Intracellular K^+ concentrations between 95 and 140 mmol l^{-1} have been measured in insect tubule cells (Baldrick *et al.* 1988).

The urine flow rate in upper tubules is largely unaffected by changes in Na^+ concentration between 140 and 350 mmol l^{-1} and K^+ concentration between 3 and 21 mmol l^{-1}. Lower tubules secrete comparatively less fluid when the K^+ concentration is varied (between 3 and 21 mmol l^{-1}) and have a more negative transepithelial potential difference with respect to the lumen (Fig. 2). This makes them more 'insect-like' than upper tubules, which resemble those parts of the Malpighian tubules that have been studied in the pill millipede (lower portions, see Farquharson, 1974*a*).

Pharmacology and the effect of diuretic hormones on fluid production

Ouabain, at a concentration of 10^{-5} mol l^{-1}, inhibits fluid production in isolated Malpighian tubules of the pill millipede (Farquharson, 1974*b*). It has no effect on fluid secretion in most insect species (Bradley, 1985), with the noteworthy exception of *Drosophila hydei* (Wessing *et al.* 1987). At high concentrations, it even stimulates urine flow in *Rhodnius prolixus* (Maddrell and Overton, 1988). In *L. forficatus*, ouabain has been tested at four different concentrations. Upper and lower tubules differ in their sensitivity to ouabain. Compared to their respective control periods, upper tubules have consistently higher flow rates than lower tubules (Fig. 6; and unpublished data). At 10^{-3} mol l^{-1}, ouabain inhibits fluid production in lower tubules dramatically, although not completely. Its effect on upper tubules varies greatly.

An important mechanism for Na^+ and Cl^- entry into the cells of many transporting epithelia is $Na^+/K^+/2Cl^-$ cotransport, which is specifically blocked by furosemide and bumetanide. Furosemide inhibits fluid secretion in isolated Malpighian tubules of *L. forficatus* (Fig. 5), *Rhodnius prolixus* (O'Donnell and Maddrell, 1984), *Drosophila hydei* (Wessing *et al.* 1987) and *Locusta migratoria* (Baldrick *et al.* 1988). At high concentrations (10^{-3} mol l^{-1}), bumetanide (not tested in *L. forficatus*) inhibits fluid secretion by the Malpighian tubules of ants (Verhulst *et al.* 1988). In the centipede *L. forficatus*, the entry of Na^+ and Cl^- into the epithelial cells might be by Na^+/Cl^- cotransport, since fluid secretion continues in nominally 0 mmol l^{-1} K^+ but ceases in 50 mmol l^{-1} Na^+. Na^+/Cl^- cotransport was proposed by Nellans *et al.* (1973) and also suggested for *Rhodnius prolixus* (O'Donnell and Maddrell, 1984).

In insects, blood-borne diuretic and antidiuretic peptides modulate urine formation and urine flow of the Malpighian tubules (Maddrell, 1963; cf. Spring, 1990; Spring *et al.* 1988; Hayes *et al.* 1989). The arginine-vasopressin-like diuretic hormone from *L. migratoria*, which has been isolated and sequenced (Proux *et al.* 1987), doubles the initial rate of fluid secretion in isolated tubules of *L. forficatus*

(Fig. 8), but the effect is transient. Whether and where a diuretic factor is produced is currently being investigated in *L. forficatus*.

At high concentrations (10^{-3} mol l^{-1}), dbcAMP inhibits fluid secretion in *L. forficatus* (Fig. 7) and in isolated tubules of the pill millipede *G. marginata*, the other myriapod species studied (Farquharson, 1974*a*). At the same high concentrations, dbcAMP mimics the action of diuretic hormones on insect Malpighian tubules (Spring, 1990). However, the maximal effect of dbcAMP in mimicking the action of synthetic locust DH in locusts occurred at a concentration of 10^{-5} mol l^{-1} and that of IBMX at 2×10^{-4} mol l^{-1} (Proux and Hérault, 1988). High concentrations – as used in most studies (see above) – might lead to a 'run-away' of cyclic AMP metabolism. As a consequence, tubule stimulation might be slowed down owing to a down-regulation of intracellular cyclic AMP concentration or to a negative effect on other second-messenger systems. In tubule cells, where intracellular cyclic AMP levels were high, fluid secretion increased only slightly (Proux, 1991).

Water and ion regulation in intact animals

Fluid secretion by insect and myriapod Malpighian tubules is inversely correlated with medium osmolality (Phillips, 1981; Bradley, 1985; Nicholls, 1985; Farquharson, 1974*b*; Fig. 3). The underlying mechanism, however, is not clear (Bradley, 1985), especially the contribution of the transport systems involved in cell volume regulation (see, for example, Lang *et al.* 1990). In *L. forficatus*, the inverse correlation between flow rate and osmolality is important for water regulation in the intact animal. The hindgut of *L. forficatus* is, unlike that of many terrestrial insects (Bradley, 1985), incapable of reabsorbing fluid hypotonic with respect to the haemolymph (Wenning, 1978, 1979). Under these circumstances, drastically reduced urine production is essential for water conservation. Upon dehydration, haemolymph osmolality cannot be held at its normal value (350–400 mosmol kg^{-1} H_2O; Wenning, 1978; Riddle, 1985) and may increase to 800 mosmol kg^{-1} H_2O (Riddle, 1985). The limited ability to conserve water imposes ecological restrictions on activity (Rilling, 1968; Wenning, 1978; Riddle, 1985). In the intact animal, rehydration may be facilitated through uptake of water vapour by coxal glands located on the last four hindlegs (Rosenberg, 1983; Rosenberg and Bajorat, 1983).

Although it is unable to reabsorb hypotonic fluid, the rectum of *L. forficatus* selectively reabsorbs ions (Wenning, 1978). This capacity appears to be essential for ion homeostasis, since no net ion reabsorption occurs along the Malpighian tubules.

In conclusion, isolated Malpighian tubules of most insects produce a K^+-enriched, hypertonic fluid and the transepithelial potential difference is positive with respect to the haemolymph. The same is true for the non-insect tracheate *L. forficatus*, a chilopod. The ability to enrich K^+ in the urine appears to be a common feature of tracheate Malpighian tubules rather than being unique to 'higher' insects (discussed in Nicholls, 1985). Furthermore, the urine flow rate in

insect tubules is controlled by diuretic factors and locust DH enhances fluid secretion in *L. forficatus*. Unlike that in the majority of insects (Bradley, 1985; Baldrick *et al.* 1988; Van Kerkhove *et al.* 1989), however, fluid secretion in the Malpighian tubules of *L. forficatus* is strongly dependent on $[Na^+]$ and independent of $[K^+]$. Along with a haemolymph with Na^+ as the main cation, these are features shared with the 'primitive' dragonfly (Nicholls, 1985) and the pill millipede (Farquharson, 1974*b*). The 'common cation pump' (Maddrell, 1977) and the coupled entry of Na^+ and Cl^- through the basolateral membrane appear to be early evolutionary characteristics of tracheate Malpighian tubules, modified by diet or by the ionic concentrations in the haemolymph. While the rectum in *L. forficatus* is important for ion regulation, the Malpighian tubules contribute greatly to volume regulation.

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References

ANESHANSLEY, D. J., MARLER, C. E. AND BEYENBACH, K. W. (1989). Transepithelial voltage measurements in isolated Malpighian tubules of *Aëdes aegypti*. *J. Insect Physiol.* **35**, 41–52.

BALDRICK, O., HYDE, D. AND ANSTEE, J. H. (1988). Microelectrode studies on Malpighian tubule cells of *Locusta migratoria*: effects of external ions and inhibitors. *J. Insect Physiol.* **34**, 963–975.

BERRIDGE, M. J. (1968). Urine formation by the Malpighian tubules of *Calliphora*. I. Cations. *J. exp. Biol.* **48**, 159–174.

BRADLEY, T. J. (1985). The excretory system: structure and physiology. In *Comprehensive Insect Physiology, Biochemistry and Pharmacology*, vol. 4 (ed. G. A. Kerkut and L. I. Gilbert), pp. 421–465. Oxford, New York, Toronto, Sydney, Paris, Frankfurt: Pergamon Press.

COOPER, P. D., SCUDDER, G. G. E. AND QUAMME, G. A. (1989). Segmental differences in secretion by Malpighian tubules of the fresh water dwelling corixid, *Cenocorixa blaisdelli* (Hung.) (Corixidae, Hemiptera). *J. Insect Physiol.* **35**, 531–536.

FARQUHARSON, P. A. (1974*a*). A study of the Malpighian tubules of the pill millipede, *Glomeris marginata* (Villers). I. The isolation of the tubules in a Ringer solution. *J. exp. Biol.* **60**, 13–28.

FARQUHARSON, P. A. (1974*b*). A study of the Malpighian tubules of the pill millipede, *Glomeris marginata* (Villers). II. The effect of variations in osmotic pressure and sodium and potassium concentrations on fluid production. *J. exp. Biol.* **60**, 29–39.

FARQUHARSON, P. A. (1974*c*). A study of the Malpighian tubules of the pill millipede, *Glomeris marginata* (Villers). III. The permeability characteristics of the tubule. *J. exp. Biol.* **60**, 41–51.

FÜLLER, H. (1966). Elektronenmikroskopische Untersuchungen der Malpighischen Gefäße von *Lithobius forficatus* L. (Chilopoda). *Z. wiss. Zool.* **173**, 191–217.

GEE, J. D. (1975). Diuresis in the tsetse fly *Glossina austeni*. *J. exp. Biol.* **63**, 381–390.

GREGER, R. (1985). Ion transport mechanisms in thick ascending limb of Henle's loop of mammalian nephron. *Physiol. Rev.* **65**, 760–797.

HAYES, T. K., PANNABECKER, T. L., HINCKLEY, D. J., HOLMAN, G. M., NACHMAN, R. J., PETZEL, D. H. AND BEYENBACH, K. W. (1989). Leukokinins, a new family of ion transport stimulators and inhibitors in insect Malpighian tubules. *Life Sci.* **44**, 1259–1266.

LANG, F., VÖLKL, H. AND HÄUSSINGER, D. (1990). General principles in cell volume regulation. In *Cell Volume Regulation* (ed. K. W. Beyenbach), pp. 1–25. *Comparative Physiology*, vol. 4 (ed. R. K. H. Kinne, E. Kinne-Saffrau and K. W. Beyenbach). Basel: Karger.

MADDRELL, S. H. P. (1963). Excretion in the blood-sucking bug, *Rhodnius prolixus* Stål. I. The control of diuresis. *J. exp. Biol.* **40**, 247–256.

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. (1977). Insect Malpighian tubules. In *Transport of Ions and Water in Animals* (ed. B. L. Gupta, R. B. Moreton, J. L. Oschman and B. L. Wall), pp. 541–570. New York: Academic Press.

MADDRELL, S. H. P. (1980). Characteristics of epithelial transport in insect Malpighian tubules. *Curr. Topics Membr. Transport* **14**, 428–463.

MADDRELL, S. H. P. AND OVERTON, J. A. (1988). Stimulation of sodium transport and fluid secretion by ouabain in an insect Malpighian tubule. *J. exp. Biol.* **137**, 265–276.

MADDRELL, S. H. P. AND PHILLIPS, J. E. (1975). Secretion of hypo-osmotic fluid by the lower Malpighian tubules of *Rhodnius prolixus*. *J. exp. Biol.* **62**, 367–378.

MALNIC, G., KLOSE, R. M. AND GIEBISCH, G. (1964). Micropuncture studies of renal potassium excretion in the rat. *Am. J. Physiol.* **206**, 674–686.

MORGAN, P. J. AND MORDUE, W. (1981). Stimulated fluid secretion is sodium dependent in the Malpighian tubules of *Locusta migratoria*. *J. Insect Physiol.* **27**, 271–279.

MÜLLER, P. (1958). Experiments on current flow and ionic movements in single myelinated nerve fibres. *Expl Cell Res.* (Suppl). **5**, 118–152.

NELLANS, H. N., FRIZZELL, R. A. AND SCHULTZ, S. G. (1973). Coupled sodium–chloride influx across the brush border of rabbit ileum. *Am. J. Physiol.* **226**, 467–475.

NICHOLLS, S. P. (1985). Fluid secretion by the Malpighian tubules of the dragonfly *Libellula quadrimaculata*. *J. exp. Biol.* **116**, 53–67.

NICOLSON, S. W. AND HANRAHAN, S. A. (1986). Diuresis in a desert beetle? Hormonal control of the Malpighian tubules of *Onymacris plana* (Coleoptera: Tenebrionidae). *J. comp. Physiol.* **156**, 407–413.

O'DONNELL, M. J. AND MADDRELL, S. H. P. (1984). Secretion by the Malpighian tubules of *Rhodnius prolixus* Stål electrical events. *J. exp. Biol.* **110**, 275–290.

PHILLIPS, J. E. (1981). Comparative physiology of insect renal function. *Am. J. Physiol.* **241**, R241–R257.

PROUX, J. P. (1991). Lack of responsiveness of Malpighian tubules to the AVP-like insect diuretic hormone in migratory locusts infected with the protozoan *Malemeba locustae*. *J. Inv. Path.* (in press).

PROUX, J. P. AND HÉRAULT, J.-P. (1988). Cyclic AMP: a second messenger of the newly characterized AVP-like insect diuretic hormone, the migratory locust diuretic hormone. *Neuropeptides* **12**, 7–12.

PROUX, J. P., MILLER, C. A., LI, J. P., CARNEY, R. L., GIRARDIE, A., DELAAGE, M. AND SCHOOLEY, D. A. (1987). Identification of an arginine vasopressin-like diuretic hormone from *Locusta migratoria*. *Biochem. biophys. Res. Commun.* **149**, 180–186.

PROUX, J. P., PICQUOT, M., HÉRAULT, J.-P. AND FOURNIER, B. (1988). Diuretic activity of a newly identified neuropeptide – the arginine-vasopressin-like insect diuretic hormone: use of an improved bioassay. *J. Insect Physiol.* **34**, 919–927.

RAMSAY, J. A. (1954). Active transport of water by the Malpighian tubules of the stick insect, *Dixippus morosus* (Orthoptera, Phasmidae). *J. exp. Biol.* **31**, 104–113.

RIDDLE, W. A. (1985). Hemolymph osmoregulation in several myriapods and arachnids. *Comp. Biochem. Physiol.* **80** A, 313–323.

RILLING, G. (1968). Lithobius forficatus. Stuttgart: Gustav Fischer Verlag.

ROSENBERG, J. (1983). Coxal glands of *Lithobius forficatus* (Myriapoda, Chilopoda). *Cell Tissue Res.* **230**, 421–430.

ROSENBERG, J. AND BAJORAT, K. H. (1983). Feinstruktur der Coxalorgane bei *Lithobius forficatus* und ihre Beteiligung an der Aufnahme von Wasserdampf aus der Luft. *Verh. dt. zool. Ges.* **76**, 317.

SAWYER, D. B. AND BEYENBACH, K. W. (1985). Dibutyryl-cAMP increases basolateral sodium conductance of mosquito Malpighian tubules. *Am. J. Physiol.* **248**, R339–R345.

SPRING, J. H. (1990). Endocrine regulation of diuresis in insects. *J. Insect Physiol.* **36**, 13–22.

SPRING, J. H. AND HAZELTON, S. R. (1987). Excretion in the house cricket (*Acheta domesticus*): stimulation of diuresis by tissue homogenates. *J. exp. Biol.* **129**, 63–81.

SPRING, J. H., MORGAN, A. M. AND HAZELTON, S. R. (1988). A novel target for antidiuretic hormone in insects. *Science* **241**, 1096–1098.

VAN KERKHOVE, E., WELTENS, R., ROINEL, N. AND DE DECKER, N. (1989). Haemolymph composition in *Formica* (Hymenoptera) and urine formation by the short isolated Malpighian tubules: electrochemical gradients for ion transport. *J. Insect Physiol.* **35**, 991–1003.

VERHULST, H., VAN KERKHOVE, E. AND DE DECKER, N. (1988). KCl secretion by Malpighian tubules of *Formica*: evidence for the presence of a KCl cotransport. *Archs int. Physiol. Biochem.* **95**, 8–9.

WENNING, A. (1978). Struktur und Funktion des Exkretionssystems von *Lithobius forficatus* L. (Myriapoda, Chilopoda). *Zool. Jb. Physiol.* **82**, 419–433.

WENNING, A. (1979). Structure and function of the hind-gut of *Lithobius forficatus* L. (Myriapoda, Chilopoda). In *Myriapod Biology* (ed. M. Camatini), pp. 135–142. London, New York: Academic Press.

WENNING, A. (1989). Transporteigenschaften der Malpighischen Gefäße von *Lithobius forficatus* (Myriapoda, Chilopoda). *Verh. dt. zool. Ges.* **82**, 215.

WESSING, A., HEVERT, F. AND RÖNNAU, K. (1987). Ion transport and intracellular activity of ions in Malpighian tubules of *Drosophila hydei*. *Zool. Beitr. N.F.* **30**, 297–314.

WILLIAMS, J. C., JR AND BEYENBACH, K. W. (1983). Differential effects of secretagogues on Na^+ and K^+ secretion in the Malpighian tubules of *Aëdes aegypti* (L.). *J. comp. Physiol.* **149**, 511–517.

WILLIAMS, J. C., JR, HAGEDORN, H. H. AND BEYENBACH, K. W. (1983). Dynamic changes in flow rate and composition of urine during the post-bloodmeal diuresis in *Aëdes aegypti* (L.). *J. comp. Physiol.* **153**, 257–265.

ZERBST-BOROFFKA, I., WENNING, A. AND BAZIN, B. (1982). Primary urine formation during diuresis in the leech, *Hirudo medicinalis*. *J. comp. Physiol.* **146**, 75–79.