THE REGULATION OF HAEMOLYMPH POTASSIUM ACTIVITY DURING INITIATION AND MAINTENANCE OF DIURESIS IN FED RHODNIUS PROLIXUS

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Accepted 17 December 1992

Summary
The blood-sucking insect Rhodnius prolixus rapidly eliminates a Na⁺-rich K⁺-poor urine after its large meals. K⁺-rich fluid is first secreted by the upper Malpighian tubules and passes to the lower tubules where most of the potassium is reabsorbed.

During the initial stimulation of the tubules, the lower tubules must be activated first to avoid loss of potassium. The major element in this is that they respond more rapidly than do the upper tubules to particular hormonal concentrations rather than that they react to lower hormonal concentrations than do the upper tubules.

During subsequent diuresis, regulation of the haemolymph potassium concentration depends on three cooperative homoeostatic mechanisms in the tubules. A fall in potassium concentration of the medium bathing the tubules causes (i) a decrease in the rate of fluid secretion by the upper tubules, (ii) a decrease in potassium concentration in the fluid secreted by the upper tubules and (iii) an increase in the rate of potassium absorption by the lower tubules. The tubules respond in the opposite direction to an increase in potassium concentration of the medium. As a result, the potassium concentration of the urine can be adjusted to match the potassium concentration of the fluids absorbed from the gut, so that the potassium concentration of the insect’s haemolymph remains unaltered.

Introduction
Haematophagous insects differ from herbivores in that the immediate excretory pressure on them is more to eliminate excess dietary sodium than to eliminate potassium. The blood-sucking bug Rhodnius prolixus ingests at each meal a volume of

Key words: potassium regulation, excretion, diuretic hormones, cooperative action, fluid secretion, Rhodnius prolixus, Malpighian tubules, epithelia.
blood equal to as much as ten times its unfed weight (Buxton, 1930). Immediately after the meal, it produces a sodium-rich urine at high rates in response to two synergistic diuretic hormones, 5-HT and a peptide (DH), whose release is stimulated by feeding (Maddrell et al. 1993). During steady diuresis, fluid is transported from the midgut into the haemolymph, whence it is removed at an equal rate by the activity of the four Malpighian tubules. Potassium levels in the ingested plasma are low, but almost regardless of the level of K⁺ in the midgut, the fluid transported into the haemolymph from the midgut contains only about 3 mmol l⁻¹ potassium (Farmer et al. 1981). Unlike most other blood-feeding insects, the iso-osmotic fluid secreted by the upper lengths of the Malpighian tubules contains as much potassium as sodium (Ramsay, 1952; Maddrell, 1969), so that, by itself, this process would rapidly deplete the haemolymph of potassium. Potassium (chloride), but not sodium (chloride) or water, is reabsorbed from the fluid leaving the upper tubules by the activity of the lowermost lengths of the lower Malpighian tubules, resulting in the elimination of hypo-osmotic urine (Maddrell and Phillips, 1975). The blood ingested is hypo-osmotic to that of the insect, so the secretion and subsequent reabsorption of potassium (chloride) serves to maintain both ionic and osmotic balance.

There are two obvious ways in which the regulation of haemolymph potassium concentration may be compromised by the activity of the epithelia involved in the removal of surplus fluid from the blood meal.

The first is that during initial stimulation of the Malpighian tubules, were the upper tubules to become active before the lower tubules, they would rapidly lower the potassium concentration in the haemolymph ([K⁺]ₕ). [K⁺]ₕ is about 4 mmol l⁻¹ (Ramsay, 1952; Maddrell and Phillips, 1975) and, in saline containing 5 mmol l⁻¹ potassium, stimulated upper tubules secrete a fluid containing about 80 mmol l⁻¹ potassium (Maddrell, 1969). It follows that, if this happened in vivo and could be maintained, it would remove all potassium from the haemolymph within a minute. There is an obvious need to activate the lower tubules rapidly, so as to prevent any such loss. This might be achieved in one of two ways. The lower tubules might respond to lower concentrations of the hormones appearing in the haemolymph or they may be no more sensitive but respond more rapidly. In the present paper, we describe both the dose–response relationships and the speeds of response for the activation of upper and lower Malpighian tubules resulting from stimulation by 5-hydroxytryptamine (5-HT) and by the natural stimulant, haemolymph from recently fed Rhodnius. Such haemolymph is known to contain both 5-HT and the peptide diuretic hormone, which collaborate synergistically to stimulate activity by the upper Malpighian tubules (Maddrell et al. 1993).

The second potential source of changes in [K⁺]ₕ is any discrepancy between the input of potassium ions to the haemolymph by the midgut epithelium and the removal of potassium ions from the haemolymph by the combined activities of the upper and lower Malpighian tubules. In other words, the activities of all three epithelia during sustained diuresis must be coordinated. We describe the ways in which the transport activities of both the upper and lower tubules are affected by changes in potassium activity in the bathing medium. We can then estimate how successfully the combined effect of the two segments of the tubules contributes to the stability of [K⁺]ₕ.
Materials and methods

The insects used were *Rhodnius prolixus* Stål (Hemiptera) kept at 27°C in laboratory culture at the Department of Zoology, Cambridge, UK. Experiments were performed with third- and fifth-stage (juvenile) insects.

Malpighian tubules were dissected from insects under saline using a binocular microscope and isolated in drops of saline under liquid paraffin as described earlier (Maddrell et al. 1988). The standard saline had the following composition (mmol l\(^{-1}\)):

- NaCl, 129;
- KCl, 8.6;
- CaCl\(_2\), 2.0;
- MgCl\(_2\), 8.5;
- NaHCO\(_3\), 10.2;
- NaH\(_2\)PO\(_4\), 4.3;
- Heps, 8.6;
- glucose, 20; pH adjusted to 7.0 with NaOH. The saline contains bicarbonate to avoid the difficulty, emphasised by Thomas (1989), that cells without available bicarbonate are not able to regulate their internal pH.

The secretory activity of the upper Malpighian tubules was determined as described in detail in Maddrell et al. (1988). In this technique, isolated tubules, usually from fifth-instar insects, are placed in drops of saline under liquid paraffin. Drops of saline larger than 100 \(\mu\)l are required to ensure sufficient supply of oxygen for rapidly secreting tubules from fifth-instar *Rhodnius* (B. J. Holloway, unpublished results). The cut end of the tubule is then pulled out of the saline and wrapped around a fine glass rod pushed into the wax base of a small Petri dish. To stimulate rapid fluid secretion by the tubules, 5-hydroxytryptamine (5-HT; obtained from Sigma) was included in the saline at the appropriate concentration (Maddrell et al. 1971). A second method of stimulation was to add haemolymph taken from fifth-instar *Rhodnius* that had been fed within the preceding 2 h. When a range of concentrations was required, the haemolymph was appropriately diluted with saline. Not more than 5–10 \(\mu\)l of haemolymph can conveniently be collected from fed fifth-instar insects. So, for experiments that involved stimulation by active haemolymph, we used the smaller tubules from third-instar *Rhodnius* and put them in 30 \(\mu\)l bathing drops. The dose–response curves for tubules from third-instar *Rhodnius* to stimulants are quantitatively similar to those of tubules from fifth instars (Maddrell et al. 1991, 1993). The transport activity of the lower Malpighian tubules was followed using the techniques developed by Maddrell and Phillips (1975), in which the upper and lower tubule segments are bathed in separate drops of saline or haemolymph, so that they can each receive different stimulating regimes.

Potassium concentrations in drops of haemolymph or tubule fluid were measured with potassium-selective microelectrodes. Unfilamented borosilicate glass capillary tubing (GC100-15, Clark Electromedical Instruments, Reading, UK) was washed in nitric acid for 5 min, rinsed 3–5 times in deionized water, and dried for a minimum of 20 min at 200°C on a hot plate. Micropipettes were pulled from the tubing using a vertical micropipette puller (Narishige, Tokyo, Japan), and replaced on the hot plate for 10 min before silanization. The latter process makes the glass surface hydrophobic and facilitates filling with, and retention of, the hydrophobic neutral carrier cocktail. A drop of dimethyl dichlorosilane was pipetted onto the inside of a 150 mm diameter Pyrex Petri dish, which was then inverted over the micropipettes which had been placed on the hot plate. Micropipettes were removed after a minimum of 20 min exposure to the silane vapour, and could be stored over silica gel for up to 2 weeks before filling.
Micropipette tips were filled with a short length of a potassium-selective neutral carrier ionophore cocktail based on valinomycin (Potassium ionophore 1, cocktail B, Fluka, Ronkonkoma, NY). The cocktail was taken up in the tip of a plastic tuberculin syringe pulled out over a low flame to a fine tip (Thomas, 1978), then injected into the shank of the micropipette. The cocktail ran to the tip of the micropipette by capillarity, and any trapped air bubbles were removed with a fine glass fibre. The micropipette was then backfilled with a 0.5mol l$^{-1}$ KCl solution injected through a second tuberculin syringe pulled to a fine tip. Reference electrodes were fabricated from 1mm o.d. filamented glass tubing. The tip and shank were filled with 1mol l$^{-1}$ sodium acetate, and the rest of the barrel was then backfilled with 1mol l$^{-1}$ KCl.

Electrodes were connected through chlorided silver wires to an electrometer of high input impedance ($>10^{14}$Ω; Keithley Instruments 600B) and were calibrated in solutions containing the following concentrations (in mmol l$^{-1}$) of KCl and NaCl respectively; 2 and 178, 8 and 142, 20 and 130, and 80 and 70. Although ion-selective electrodes measure ion activity and not concentration, data can be expressed in terms of concentrations if it is assumed that the ion activity coefficient is the same in calibration and experimental solutions. Expression of data in terms of concentrations simplifies comparisons with previous studies in which ion concentrations were measured by techniques such as flame photometry. Potassium concentrations in drops of haemolymph or secreted fluid were measured under paraffin oil by positioning potassium-selective and reference electrodes in the drop and measuring the potential change relative to that in drops of calibration solutions.

Potassium concentrations were calculated from the equation:

$$[K^+]_e = [K^+]_c \times 10^{(V/s)},$$

where $[K^+]_e$ is the potassium concentration of the experimental drop, $[K^+]_c$ is the concentration of the calibration drop, $V$ is the change in potential (mV) between the unknown and the calibration drop, and $s$ is the slope (mV) for a tenfold change in potassium concentration. All experiments were done at room temperature, 22–26°C. Values are given as mean ± S.E.

**Results**

**Potassium regulation in the initial stages of diuresis**

*Dose–response curves of upper and lower tubules*

Lower Malpighian tubules in a feeding *Rhodnius* might be activated earlier than the upper tubules after feeding if they were more sensitive to the hormones that appear in circulation. During feeding in *Rhodnius*, 5-HT increases rapidly in concentration from the low values found in the resting insect (Lange *et al.* 1989). It reaches a concentration of about $5 \times 10^{-8}$mol l$^{-1}$ after 5min (Maddrell *et al.* 1991) and then steadily declines to a lower level, $1 \times 10^{-8}$–$2 \times 10^{-8}$mol l$^{-1}$, by 1h after the start of feeding (Lange *et al.* 1989; Maddrell *et al.* 1991). This suggests that stimulation by 5-HT might be important in the early stages of diuresis after feeding begins. However, 5-HT is not the only hormone that
appears in circulation during the first few minutes after feeding begins, and its concentration reaches no more than $5 \times 10^{-8}$ mol l$^{-1}$. This level is insufficient to cause more than partial stimulation of the upper tubules (Maddrell et al. 1971, 1993) and both 5-HT and the peptide diuretic hormone (at least) are required for full stimulation (Maddrell et al. 1993). In addition, ketanserin, which causes very effective blockade of stimulation of upper Malpighian tubules by 5-HT alone (Maddrell et al. 1991), does not affect fluid secretion by upper tubules bathed in samples of haemolymph taken at any time during the period after feeding begins (S. H. P. Maddrell, unpublished observations). So, in the experiments described below we have investigated not only the dose–response relationship for stimulation of upper and lower tubules by 5-HT alone but also the relative sensitivities of the two segments of the tubule to samples of active haemolymph taken from fed *Rhodnius*.

The relationship between the potassium concentration of fluid after passage through the lower Malpighian tubule and the concentration of 5-HT in a bathing drop is shown in Fig. 1. A concentration of a little above $10^{-8}$ mol l$^{-1}$ is sufficient to produce about 50% of the maximum reabsorption. Previous studies suggest that an appreciably higher concentration, $3 \times 10^{-8} - 4 \times 10^{-8}$ mol l$^{-1}$ (Maddrell et al. 1993), is required to

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**Fig. 1.** Dose–response curve for 5-HT stimulation of K$^+$ reabsorption by lower Malpighian tubules of fifth-instar *Rhodnius*. For these experiments, whole tubules (upper plus lower segments) were arranged with the upper tubule in a drop of Na$^+$-free saline containing $2 \times 10^{-7}$ mol l$^{-1}$ 5-HT. This caused secretion of K$^+$-rich fluid that passed through the lumen of the lower tubule, which was bathed separately in a drop of standard saline containing the test concentration of 5-HT. The dotted line shows the sensitivity of upper Malpighian tubules of *Rhodnius* to 5-HT (data from Maddrell et al. 1993). The response of the lower tubules (continuous line, open circles) is plotted as the reduction in potassium concentration of the secreted fluid. This variable was calculated by subtracting the concentration of K$^+$ in drops from whole tubules from the concentration of K$^+$ in drops collected from the upper tubule. Droplets of upper tubule fluid were collected at the end of the experiment by cutting the tubule at the junction of the upper and lower segments of the tubule. The mean concentration of K$^+$ for drops collected from upper tubules bathed in Na$^+$-free saline was 147.4 ± 3.0 mmol l$^{-1}$ ($N=31$). Values are mean ± s.e.
produce 50% of the maximum fluid secretion rate by the upper tubule (Maddrell et al. 1993). The lower tubule is evidently more sensitive to 5-HT than is the upper tubule.

The effects of a relatively narrow range of different dilutions of haemolymph collected from recently fed fifth-instar Rhodnius on fluid secretion rates by upper tubules and potassium reabsorption by lower tubules are shown in Fig. 2A. It is important to point out that the results shown do not provide a full dose–response curve. The experimental method was to test a range of dilutions of active haemolymph on upper tubules and then treat lower tubules only with those dilutions that led to partial activation of the upper tubules. As Fig. 2A shows, in every case these dilutions also gave partial activation of the lower tubule. These results demonstrate that there were no dramatic differences in the sensitivity of upper compared to lower tubules in response to haemolymph containing diuretic hormones.

Fig. 2B shows the result of an experiment to test this finding directly. It records the responses of an upper and a lower tubule from a third-instar Rhodnius to two successively higher concentrations of active haemolymph and to a final saturating concentration of 5-HT. Both upper and lower tubules showed threshold stimulation by 10% haemolymph, partial stimulation by 14% haemolymph and maximal stimulation by \(2.3 \times 10^{-6} \text{ mol l}^{-1}\) 5-HT. Seven other similar experiments gave the same type of result.

**Speed of response of upper and lower tubules to stimulation**

If the lower tubules are not activated more quickly than the upper tubules by being *more sensitive* to the diuretic hormones in the haemolymph, perhaps they respond *more quickly* to particular doses of these hormones. Stimulation by 5-HT of K⁺ reabsorption by tubules from fifth-instar insects (Fig. 3A,B) was apparent within 1 min, and maximal stimulation was achieved in as little as 3 min. The mean time for 50% stimulation of the lower tubule was 2.7 ± 0.4 min \((N=3)\). In contrast, upper tubules did not begin to secrete fluid at significant rates until about 3 min after addition of 5-HT to the bathing drop, and the mean time for secretion to reach 50% of the maximum rate was 4.1 ± 0.2 min \((N=9)\). For third-instar Rhodnius, the mean times for 50% response of lower and upper tubules to 5-HT were 2.0 ± 0.1 min \((N=4)\) and 3.9 min \((N=2)\), respectively.

The initiation of fluid secretion, before drops of measurable size emerged from the open end of the tubule, could be detected by the movement of crystals of uric acid in the tubule lumen. In experiments with tubules from fifth-instar insects, uric acid movements were detected on average 2.2 ± 0.1 min \((N=8)\) after addition of 5-HT, about 1.9 min before fluid secretion rates had reached 50% of the maximum value.

Samples of active haemolymph (from recently fed fifth instars) also stimulated lower tubules more rapidly than upper tubules from third-instar insects (Fig. 4). The mean times for 50% stimulation of K⁺ reabsorption and fluid secretion were 1.6 ± 0.1 min \((N=5)\) and 4.2 ± 0.3 min \((N=5)\), respectively.

These results show that potassium reabsorption by the lower tubule was more rapidly stimulated by a single dose of either 5-HT or haemolymph than was fluid secretion by the upper tubule.
Potassium regulation in fed Rhodnius

Fig. 2. (A) Stimulation of K⁺ reabsorption and fluid secretion for third-instar Malpighian tubules bathed in drops containing haemolymph collected from fifth-instar insects in the diuretic phase. Data for secretion rates (filled circles) are expressed as a percentage of the rate in response to 35% haemolymph, which produces maximal stimulation (Maddrell et al. 1993). The percentage of K⁺ reabsorption (open circles) was calculated using the formula \((80-[K^+]_{sl}/80)\times100\), where 80 represents the mean potassium concentration (in mmol l\(^{-1}\)) of fluid secreted by upper tubules bathed in saline containing 8.6 mmol l\(^{-1}\) K⁺. (B) The effects of haemolymph on fluid secretion rate of an upper tubule (Bi) and potassium concentration of fluid secreted by a whole tubule (Bii) from the same third-instar Rhodnius. The inset at the top shows the arrangement of the tubules and bathing drops. The concentration of haemolymph was increased from 10% to 14% at 38 min after the tubules had first been placed in a drop of saline which initially contained no haemolymph. The dashed line in Bii is the potassium concentration in fluid secreted by the upper segment of the whole tubule. This concentration was determined by cutting the whole tubule at the upper–lower junction at the end of the experiment and collecting fluid from the upper tubule bathed in saline containing 0.2 μmol l\(^{-1}\) 5-HT. The potassium concentration in this fluid was 70 mmol l\(^{-1}\), somewhat lower than the usual value of 80 mmol l\(^{-1}\).
**Potassium regulation during steady-state diuresis**

**Regulation of haemolymph potassium concentration**

During post-prandial diuresis, the potassium concentration of the insect’s haemolymph depends on the rate of potassium absorption into the haemolymph from the midgut and its rate of removal from the haemolymph by the Malpighian tubules. To find out how well potassium is regulated, we measured the potassium concentration in the urine ([K⁺]₀) and

![Graph A](image1.png)  ![Graph B](image2.png)

**Fig. 3.** (A) The time course of stimulation of fluid secretion by an upper tubule (filled circles) and potassium reabsorption by a lower tubule (open circles) after addition of \(2 \times 10^{-7}\) mol l\(^{-1}\) 5-HT to the bathing saline. Potassium reabsorption is indicated by the decline in K⁺ concentration in drops collected from a whole tubule. Both tubules were obtained from the same fifth-instar *Rhodnius*. (B) The data in A are replotted as a percentage of the maximum for fluid secretion rate (filled circles) and the decline in potassium concentration (open circles). The lower tubule was fully stimulated before movements of uric acid crystals in the tubule lumen provided the first indication of fluid secretion by the upper tubule.

![Graph A](image3.png)  ![Graph B](image4.png)

**Fig. 4.** (A) The time course of stimulation of fluid secretion by an upper tubule (filled circles) and potassium reabsorption by a lower tubule (open circles) both in haemolymph collected from fed fifth-instar insects and diluted to 50% with standard saline. Both tubules were isolated from the same third-instar *Rhodnius*. (B) The data in A replotted as a percentage of the maximum for fluid secretion rate (filled circles) and the decline in potassium concentration (open circles). The lower tubule was more than 80% stimulated before movements of uric acid crystals in the tubule lumen provided the first indication of fluid secretion by the upper tubule.
haemolymph ([K⁺]₀) in insects during diuresis after the meal. We found no systematic changes with time after the meal in either of these variables. The mean values of [K⁺]₀ and [K⁺]ₜ were 2.5±0.3mmol l⁻¹ (N=8) and 3.6±0.2mmol l⁻¹ (N=19), respectively. These figures are similar to those determined by Ramsay (1952).

Effects of bathing saline [K⁺] on secretion rate and [K⁺] of fluid secreted by upper tubules

Previous studies (Maddrell, 1969) have shown that fluid secretion rates are relatively unaffected by a range of bathing saline potassium concentrations ([K⁺]₀). These early studies, however, mostly used unphysiologically high potassium concentrations to examine the stability of fluid secretion rates over a wide range of conditions. Experiments with potassium concentrations closer to those found in vivo show that both the rate of fluid secretion (Fig. 5A) and the potassium concentration of the secreted fluid ([K⁺]sf, Fig. 5B) decline considerably with decreases in potassium concentration of the bath. The decline in secretion rate was especially noticeable about 20min after the reduction in [K⁺]₀ (Fig. 6). These changes in fluid secretion rate and [K⁺]sf are additive in that the net rate of potassium movement from the upper tubule to the lower is the product of [K⁺]sf and the rate of fluid secretion.

Effects of bathing saline [K⁺] on potassium reabsorption by the lower tubules

It was known that the bathing saline potassium levels also affect the potassium concentration of the fluid emerging from the end of the lower tubules (Maddrell and

Fig. 5. (A) The effects of bathing saline potassium concentration on fluid secretion rate of upper Malpighian tubules of third-instar Rhodnius. Rates were measured after 35min in 30μl drops of saline containing 3×10⁻⁷mol l⁻¹ 5-HT. Values are shown as mean ± S.E. The numbers of determinations are shown by each mean value. Inset: fluid secretion rates for a single tubule bathed in salines containing 2, 4, 6 or 8mmol l⁻¹ K⁺. (B) The effects of bathing saline potassium concentration on the potassium concentration of the fluid secreted by upper tubules. Values are shown as mean ± S.E. The numbers of determinations are shown by each mean value.
Phillips, 1976). We have now investigated this in a more systematic way. Fig. 7 shows a typical experiment and Fig. 8 shows the aggregated results of a set of experiments to assess the effects of reductions in $[K^+]_b$ on the reduction of potassium levels in the secreted fluid. Clearly, $K^+$ reabsorption is considerably more effective when the potassium level in the bathing medium is lower.

**Discussion**

The results demonstrate that the diuretic hormones and autonomous homeostatic control systems work in concert to regulate haemolymph potassium levels closely.

The dose–response characteristics and the time course of the responses of the upper and lower tubules to 5-HT solutions in saline and to haemolymph samples containing 5-HT and the peptide DH help to explain how it is that potassium reabsorption in the lower tubule is fully stimulated before significant rates of ion and fluid secretion by the upper Malpighian tubule are established during the initiation of diuresis. It is not clear what the effective stimulus for the tubules is in the initial stages (0–20min) because the concentrations of 5-HT are then relatively higher and rapidly changing relative to the steady state later on (20–240min; Lange et al. 1989). Since the lower tubules are sensitive to lower concentrations of 5-HT than are the upper tubules (Fig. 1), it could well be that the activation of the lower tubules before the upper tubules in diuresis owes something to their greater sensitivity to the 5-HT appearing in the haemolymph at that time. Even if this is not important – the lower tubules are no more sensitive than the upper tubules to the concentrations of 5-HT and peptide diuretic hormone that occur in the
haemolymph at a later stage (Fig. 2A) – then the swifter response of the lower tubule to particular concentrations of 5-HT or active haemolymph (Figs 3, 4) will ensure that the lower tubules will be activated before the upper ones at the onset of diuresis.

These initial, hormonally controlled, events cannot regulate the haemolymph levels of potassium once diuresis is fully under way, because the haemolymph then contains concentrations of diuretic hormones significantly higher than that required to activate fully both the upper and the lower Malpighian tubules (see Fig. 2A and Maddrell et al. 1993). Instead, autonomous mechanisms to minimise potassium loss now come into play. These include decreases in upper tubule potassium and fluid secretion rates and an accompanying increase in lower tubule potassium reabsorption when bathing fluid potassium concentrations are reduced. In short, potassium homeostasis now depends upon the direct responses of both upper and lower tubules to any changes in haemolymph potassium concentration.

When K⁺ levels in the haemolymph fall, the upper tubule secretes potassium more slowly because both the fluid secretion rate and the potassium concentration in the secreted fluid decline. This adjustment occurs in two stages. Once the bathing medium K⁺
concentration falls below 12 mmol l\(^{-1}\), the potassium levels in the secreted fluid decline steadily (Fig. 5B). But when the medium K\(^+\) concentration falls further, to below about 6 mmol l\(^{-1}\), the rate of fluid secretion is also reduced (Fig. 5A). So, at the normal haemolymph levels of K\(^+\) (3–4 mmol l\(^{-1}\), see above), both effects occur and the rate of change of potassium concentration in the secreted fluid will be a sensitive function of haemolymph potassium levels. Not only does the upper tubule secrete less potassium in response to a lower [K\(^+\)], but the lower tubule reabsorbs potassium more effectively (Figs 7, 8). The concerted actions of the upper and lower Malpighian tubules constitute an elegant negative feedback system that very effectively regulates the potassium concentration of the haemolymph.

**References**


