Blood feeders, such as leeches, mosquitoes, bugs and ticks, increase their salt and water output following a meal. Although these species occupy different habitats and have different mechanisms of urine formation, postprandial diuresis is always a marked natriuresis due to the high NaCl load. Most of the research on urine formation and its regulation has focused on the Malpighian tubules of terrestrial insects (for recent reviews, see Nicolson, 1993; Beyenbach, 1995). In the leech *Hirudo medicinalis*, a limnic species, the performance of the excretory system has also been extensively studied (Zerbst-Boroffka and Wenning, 1986). The present study was undertaken to elucidate further the mechanisms of urine formation and to examine the transition to natriuresis.

To compensate for the osmotic inflow of water, *H. medicinalis* produces an amount of final urine equal to its body mass within 24 h. Urine production can vary by two orders of magnitude. When leeches are exposed to brackish water, urine production decreases to 10% of the normal rate (Boroffka, 1968), but it increases eight-to tenfold after a meal (Zerbst-Boroffka, 1973).

The organs maintaining salt and water homeostasis are the metanephridia, which occur in midbody segments 2–18 as bilateral pairs. In jawed leeches, the nephridia function as secretory organs. They are innervated and highly vascularized (Boroffka and Hamp, 1969; Boroffka *et al.* 1970; Zerbst-Boroffka, 1975; Wenning, 1983). Canalicular cells secrete hyperosmotic, K+ -enriched primary urine into their interconnected lumina. The canaliculi of the main lobe and the inner lobe feed into the canaliculi of the apical lobe, where they give rise to the central canal (Fig. 1, thick arrow). The central canal courses through the nephridium in two loops (Fig. 1). For most of its length, the central canal is surrounded by and in close contact with canalicular cells. Final urine is formed in the central canal by salt reabsorption and flows through the final canal into the urinary bladder that is associated with each nephridium. Urine is voided through a nephridiopore on the ventral body surface.

Water reabsorption is negligible in adjusting final urine volume in leech nephridia (Zerbst-Boroffka, 1975). The volume diuresis observed after feeding is therefore entirely due to a higher primary urine secretion rate, while the strong natriuresis results from the combined efforts of the canalicular and central canal cells: the canalicular cells secrete more primary urine with a higher Na+ and lower K+ concentration; NaCl reabsorption by the central canal cells decreases, resulting in a considerable increase in the NaCl concentration.
and hence osmolality, of the final urine (Zerbst-Boroffka et al. 1982). As a result, NaCl output increases 80-fold.

We have combined data from in situ preparations and isolated nephridia and present a model which accounts for the formation of the normally K⁺-enriched primary urine as well as for the Na⁺-enriched primary urine after feeding. A key question addressed here is whether the volume and composition of the primary urine are regulated independently.

Some of the results have appeared in abstract form (Zerbst-Boroffka and Böhm, 1989; Zerbst-Boroffka et al. 1993).

Materials and methods
Leeches (Hirudo medicinalis L.) were obtained from commercial suppliers and kept in either tap water or artificial pond water (Muller et al. 1981) at 20±2°C without feeding.

Determination of urine flow of isolated nephridia
To measure urine flow from isolated nephridia, leeches were pinned ventral side up in a stretched position. A polyethylene catheter was inserted through the nephridiopore into the urinary bladder of a midbody segment. The corresponding nephridium was exposed by removing the surrounding tissue. The blood supply was cut. Nephridium and bladder were isolated along with a piece of the lateral vessel and transferred to a dish lined with Sylgard (Dow Corning, Belgium). The lateral vessel was pinned to hold the preparation in place. The bathing medium volume was 10ml. Preparation time was between 30 and 50 min. In one set of experiments, urine was collected directly from the final canal. A nephridium was isolated together with the dorsal bladder wall, the bladder was pinned inside out and a small polyethylene tube (inner diameter approximately 80 µm) was inserted into the final canal. Unless stated otherwise, nephridia were isolated in ‘artificial leech blood’ with a change of the bathing medium every hour (Table 1). Urine production was measured for 6h. After establishing a control rate for 2h, the bathing medium was exchanged for solutions of different ionic composition or solutions containing transport-active drugs (ouabain, bumetanide, 4-acetamido-4’-isothiocyanatostilbene-2,2’-...
disulfonic acid (SITS), 2,4,6-triaminopyrimidine (TAP); all from Sigma). Changes in the ionic composition were achieved by substituting choline chloride for NaCl, Na+ for K+, and succinate for Cl–.

**Determination of urine composition of isolated nephridia**

Nephridia were isolated in artificial leech blood (Table 1). Samples of primary urine from the canaliculi of the apical lobe or secondary urine from different areas of the central canal (Fig. 1) were obtained by micropuncture (Zerbst-Boroffka, 1975). Successful penetration of a canaliculus lumen was verified by the injection of a small coloured droplet from the oil-filled glass capillary (tip diameter 5–10 μm), which could be observed as it passed along the canalicular network. The Na+, K+ and Cl– concentrations and the osmolality of each sample (2–5 nl) were measured.

Cation (Na+, K+) concentrations were determined by ultramicro double-beam flame spectrophotometry (constructed by W. Hampel, Frankfurt; see Zerbst-Boroffka, 1975, for details), the Cl– concentration by electrometric titration (Ramsay et al. 1955), and the osmolality using a direct-reading Cryostat (nanolitre osmometer, Clifton Technical Physics, USA).

**Electrophysiology**

Cellular and transepithelial potentials were determined in nephridia isolated in malate-based artificial leech blood (Table 1) using an Axoclamp-2A amplifier (Axon Instruments Inc., Burlingame, USA). Glass microelectrodes were filled with 4 mol l–1 potassium acetate and had resistances of 20–30 MΩ. A Ag/AgCl electrode was used as reference. In successful impalements, potentials were stable for several minutes. For transepithelial potential measurements, the electrode was pushed through the cell into the canalicular lumen. Successful recordings were characterized by a sharp jump to more positive values and by the reappearance of the cellular potential when the electrode was withdrawn from the lumen. In each preparation, the cellular and transepithelial potentials of 2–3 cells were determined within 15–20 min after isolation.

<table>
<thead>
<tr>
<th>Table 1. Composition of salines</th>
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<tr>
<td></td>
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<tr>
<td>Artificial leech blooda</td>
</tr>
<tr>
<td>Malate-based artificial leech bloodb</td>
</tr>
<tr>
<td>Leech saline</td>
</tr>
</tbody>
</table>

aZerbst-Boroffka et al. (1970); samples were kept frozen until use.
bHoeger et al. (1989); used for measurements of the cellular and transep棘ular potentials.

Data are expressed as means ± S.E.M. (unless otherwise indicated) with the number of experiments indicated in parentheses. Student’s t-test was used to assess significant differences.

**Results**

The leech excretory system allows quantitative measurements of the volume and salt output of a single nephridium in situ and upon isolation. In artificial leech blood, urine production by isolated nephridia remains stable for at least 6 h and is approximately twice as high as in situ (in situ 4.5 μl cm–2 body surface h–1; isolated nephridia 8.4 μl cm–2 body surface h–1; Zerbst-Boroffka and Wenning, 1986).

With respect to the extracellular fluid, the canalicular cells secrete hyperosmotic and K+–enriched primary urine with Cl– as the only anion (Table 2), both in isolated nephridia and in situ. While the K+ concentration in isolated nephridia is similar to that in situ, the Na+ concentration is significantly higher in isolated nephridia (Table 2). The primary urine composition of isolated nephridia remains constant for at least 5 h after isolation.

Cellular and transepithelial potentials were determined using the large canalicular cells of the apical lobe (Fig. 1). The cellular and transepithelial potentials are negative, with respect to the bathing medium, in isolated nephridia (Table 3) and, as shown previously, in situ (Zerbst-Boroffka, 1987). As indicated by the electrochemical gradients, K+ and Cl– transport must be active and hence transepithelial, while Na+ may move paracellularly into the lumen. The ouabain-sensitive Na+/K+-ATPase, located basolaterally (Zerbst-Boroffka and Wenning, 1986), contributes to the cellular and transepithelial potential difference: poisoning with ouabain (10–4 mol l–1) leads to (1) significant depolarization, (2) a decrease in the transepithelial potential difference within 5–10 min (Table 3), (3) loss of intracellular K+ and (4) an increase in intracellular Na+ concentration (Zerbst-Boroffka and Böh, 1989; Böh, 1989).
In leech nephridia, final urine volume depends entirely on the primary urine secretion rate. As shown by stopped-flow experiments, the influence of water reabsorption as a mechanism for adjusting final urine volume is negligible (Zerbst-Boroffka, 1975). Water loss across the bladder wall of isolated nephridia is also negligible (I. Zerbst-Boroffka, unpublished observations). The strong correlation between primary and final urine volume allows the primary urine secretion rate to be determined from measurements of final urine flow. The drugs used here are not membrane-permeable and will therefore directly affect only transporters located on the basolateral side of the canalicular cells.

\[ \text{Na}^+ / \text{K}^+ / 2\text{Cl}^- \text{cotransport is essential for fluid formation (Zerbst-Boroffka and Wenning, 1986). In isolated nephridia, the location of this transporter on the basolateral side was confirmed by using the specific blocker bumetanide, which decreased urine production (Fig. 2). Bumetanide also induced a hyperpolarization of the canalicular cells and a decrease in the transepithelial potential difference within 5–10 min (Table 3).} \]

The disulphonstilbene SITS, known to inhibit anion permeability and anion/HCO_3^- exchangers (Greger and Kunzelmann, 1990), reduced urine production significantly. The effect was dose-dependent (Fig. 3). To further confirm paracellular Na^+ transfer, the organic cation 2,4,6-triaminopyrimidine (TAP) was added to the bathing medium. Urine flow was significantly reduced at a concentration of 5×10^{-3} \text{mol} \cdot \text{l}^{-1} and was completely blocked at a concentration of 2×10^{-2} \text{mol} \cdot \text{l}^{-1} (Fig. 4).

In their natural habitat, leeches produce a strongly hypoosmotic final urine through efficient ion reabsorption along the central canal (Boroffka, 1968; Zerbst-Boroffka, 1975). As shown by measurement of the ion composition along the central canal in situ and in vitro, reabsorption of KCl and NaCl takes place in different parts of the central canal. In situ, the K^+ concentration of urine samples taken at the end of the first loop of the central canal (Fig. 1, site B) is 6.3 \text{mmol} \cdot \text{l}^{-1}.

<table>
<thead>
<tr>
<th>In situ</th>
<th>Isolated nephridia</th>
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<tbody>
<tr>
<td>Leech blood^a</td>
<td>[Na^+] (mmol l^{-1})</td>
</tr>
<tr>
<td></td>
<td>126</td>
</tr>
<tr>
<td></td>
<td>±4.8 (23)</td>
</tr>
<tr>
<td>Primary urine from</td>
<td></td>
</tr>
<tr>
<td>canalar cells</td>
<td>[Na^+] (mmol l^{-1})</td>
</tr>
<tr>
<td>in the apical lobe</td>
<td>98</td>
</tr>
<tr>
<td></td>
<td>±4.6 (8)</td>
</tr>
<tr>
<td>Secondary urine from</td>
<td></td>
</tr>
<tr>
<td>the central canal</td>
<td></td>
</tr>
<tr>
<td>site A</td>
<td>76</td>
</tr>
<tr>
<td></td>
<td>±4 (4)</td>
</tr>
<tr>
<td>Site B</td>
<td>67</td>
</tr>
<tr>
<td></td>
<td>±9.5 (4)</td>
</tr>
<tr>
<td>Site C</td>
<td>25</td>
</tr>
<tr>
<td></td>
<td>±4 (5)</td>
</tr>
<tr>
<td>Final urine collected</td>
<td></td>
</tr>
<tr>
<td>from the urinary bladder^a</td>
<td>11</td>
</tr>
<tr>
<td></td>
<td>±1.9 (24)</td>
</tr>
</tbody>
</table>

The micropuncture sites (A, B, C) are indicated in Fig. 1.
^aData from Wenning et al. (1980).
^bData from Zerbst-Boroffka et al. (1982).
ND, not determined.
Values are means ± S.E.M. (N).

<table>
<thead>
<tr>
<th>Control</th>
<th>V_m (mV)</th>
<th>V_{TEP} (mV)</th>
</tr>
</thead>
<tbody>
<tr>
<td>~84±5.8 (47)</td>
<td>~16±7.1 (47)</td>
<td></td>
</tr>
<tr>
<td>10 min after incubation in bumetanide (10^{-4} \text{mol} \cdot \text{l}^{-1})</td>
<td>~91±8 (65)*</td>
<td>~9.4±4.8 (61)*</td>
</tr>
<tr>
<td>10 min after incubation in ouabain (10^{-4} \text{mol} \cdot \text{l}^{-1})</td>
<td>~57±10 (53)*</td>
<td>~7.3±4.6 (45)*</td>
</tr>
</tbody>
</table>

Data from Kaltenhäuser (1994).
*Significantly different from the control values, P<0.001.
Values are means ± s.d. (N).
compared with 52 mmol l\(^{-1}\) in the primary urine, while the Na\(^{+}\) concentration has not changed at this point (Table 2). NaCl is reabsorbed in the second, longer loop of the central canal, resulting in the low Na\(^{+}\) concentration of the final urine. In isolated nephridia, the K\(^{+}\) concentration of the fluid collected after the completion of the first loop of the central canal (Fig. 1, site B) is also low, indicating KCl reabsorption. The final urine, collected from the bladder, has a low Na\(^{+}\) concentration (Table 2).

These results indicate that the ion-transport mechanisms underlying primary and final urine formation are similar \textit{in situ} and \textit{in vitro}.

\textbf{Changes of the ionic environment affect nephridial performance}

Following a meal, leeches face prolonged changes in the ionic composition of their body fluids (Zerbst-Boroffka, 1973; Wenning \textit{et al.} 1980) compensated for by a strong natriuresis. Crucial questions are whether and how these changes in the ionic environment \textit{per se} affect nephridial performance. External Na\(^{+}\), K\(^{+}\) and Cl\(^{-}\) are required to sustain urine production in isolated nephridia, but their respective thresholds differ greatly (Fig. 5). To vary the Na\(^{+}\) concentration of the bathing medium, choline chloride was used as a substitute for NaCl, and control measurements were made in leech saline with Cl\(^{-}\) as the only anion (Table 1). At elevated Cl\(^{-}\) concentrations, urine flow decreases with time (Fig. 5A; Wenning and Janisch, 1996). However, urine flow is strongly correlated with the extracellular Na\(^{+}\) concentration and it ceases in a nominally Na\(^{+}\)-free medium (Fig. 5A). Nephridia isolated in a nominally Na\(^{+}\)-free medium for no more than 30 min resume urine production when Na\(^{+}\) is again added to the medium (Fig. 5B). Longer exposure to Na\(^{+}\)-free medium causes irreversible damage. The K\(^{+}\) concentration may drop to 1 mmol l\(^{-1}\) without affecting urine production (Fig. 5C), but urine production ceases within 2–3 h in nominally K\(^{+}\)-free medium. Urine flow is restored to its original rate when the bathing medium is exchanged for media containing either 1 or 5 mmol l\(^{-1}\) K\(^{+}\) (Fig. 5D). The Cl\(^{-}\)
Fig. 5. Urine flow in isolated nephridia in media of different ionic composition. After establishing a control rate, the medium was exchanged for either the same medium (‘control’) or the test medium (the time of exchange is indicated by the broken line). Varying concentrations of external Na⁺ were achieved by substituting choline chloride for NaCl. The control medium (leech saline; Table 1) and the media containing different Na⁺ concentrations have the same high Cl⁻ concentration of 107 mmol l⁻¹. The control medium for experiments with varying external K⁺ and Cl⁻ concentrations was artificial leech blood (Table 1). The ion concentrations of the bathing medium and the number of preparations are indicated for each experiment. Note that the removal of either Na⁺ (A,B), K⁺ (C,D) or Cl⁻ (E,F) inhibits urine production. Values are means ± S.E.M.
Urine formation in the leech 2223

Urine formation in the leech nephridium

The complex design of the leech nephridium (Fig. 1) prevents experimental access to the luminal surface (apical side) of the transporting cells. However, the data presented here strongly suggest that the canalicular cells function as a Cl⁻-secreting epithelium, as has been reported in such different epithelia as the shark rectal gland (Riordan et al. 1994), the avian salt gland (Friszell and Morris, 1994), the mammalian small intestine (Field and Semrad, 1993) and the branchial epithelium of brine shrimps Artemia salina (Holliday et al. 1990). In these epithelia, an apical Cl⁻ conductance creates a lumen-positive potential which provides the driving force for paracellular Na⁺ transport. Cl⁻ enters the cells via a Na⁺/K⁺/2Cl⁻ cotransporter, driven by a basolateral Na⁺/K⁺-ATPase. The fact that urine formation is not blocked completely by furosemide (in situ; Zerbst-Boroffka, 1987) or bumetanide (isolated nephridia; Fig. 2) indicates that an additional pathway might also exist. Inhibition of urine formation by SITS in isolated nephridia (Fig. 3) suggests the presence of an anion/Cl⁻ exchanger (A⁻/Cl⁻ in Fig. 7; Bougias, 1993).

As indicated by the negative transcellular potential of the canalicular cells, apical exit of Cl⁻ is downhill – presumably through an apical Cl⁻ channel (gCl in Fig. 7). A prerequisite for Cl⁻-driven transport is that the intracellular Cl⁻ concentration is raised above its electrochemical equilibrium to allow its apical exit (Reeves and Andreoli, 1992). In the leech canalicular cells, this means raising it above 9 mmol l⁻¹ Cl⁻. The Na⁺/K⁺/2Cl⁻ cotransporter may provide an intracellular concentration of 30 mmol l⁻¹ Cl⁻. Paracellular transport (of Na⁺ in the canalicular cells) is further supported by applying the organic cation 2,4,6-triaminopyrimidine (TAP), which inhibits urine formation (Fig. 4; Bougias, 1993).

The canalicular cells secrete substantial amounts of KCl (Fig. 7). K⁺ enters the cell via the Na⁺/K⁺/2Cl⁻ cotransporter and via the Na⁺/K⁺-ATPase. The high K⁺ conductance governs the basolateral membrane potential (gK in Fig. 7). Ba²⁺, known to block K⁺ channels, inhibits urine production, indicating the

Fig. 6. Urine flow of isolated nephridia (N=5) is inhibited in 3.6 mmol l⁻¹ Cl⁻, but does not recover when the bathing medium is exchanged for artificial leech blood to which bumetanide (10⁻⁴ mol l⁻¹) had been added. In these experiments, final urine flow was measured by catheterization of the final canal. Values are means ± S.E.M.
ATPase contributes substantially to the cellular and transcellular anion/Cl\(^-\) and Böhm (1989). Intracellular cation concentrations are taken from Zerbst-Boroffka (A. Zerbst-Boroffka, B. Bazin and A. Wenning, 1986). Unlike other K\(^+\)-secreting epithelia (e.g. the mammalian colon; Rechkemmer et al. 1996), there is no apical conductive pathway for K\(^+\) on the apical membrane. Apparently, apical Cl\(^-\) exit provides the driving force not only for paracellular Na\(^+\) transport but also for K\(^+\) secretion (indicated as a K\(^+\)/Cl\(^-\) cotransport in Fig. 7). Since Cl\(^-\) secretion is 2.3 times greater than K\(^+\) secretion, the stoichiometry of the K\(^+\)/Cl\(^-\) cotransport is either K\(^+\):Cl\(^-\) or K\(^+\):2Cl\(^-\). To account for the 10-fold increase in the K\(^+\) concentration in the primary urine over the bathing medium, a 1:1 stoichiometry would require an intracellular Cl\(^-\) concentration above 85 mmol l\(^{-1}\) to operate a K\(^+\)/Cl\(^-\) cotransport. A 1:2 stoichiometry would require an intracellular Cl\(^-\) concentration of approximately 30 mmol l\(^{-1}\). Thus, the coupled exit of K\(^+\) and Cl\(^-\) requires a higher intracellular Cl\(^-\) concentration (≥30 mmol l\(^{-1}\)) than is needed for paracellular Na\(^+\) transport alone (≥9 mmol l\(^{-1}\)).

Along the central canal, 97% of the secreted K\(^+\) and 85% of the secreted Na\(^+\) are reabsorbed to form the strongly hypotonic final urine typical for leeches not under osmotic stress (Zerbst-Boroffka, 1975; Zerbst-Boroffka et al. 1982). As shown here, Na\(^+\) and K\(^+\) reabsorption occur in different areas of the central canal (Fig. 1; Table 2). KCl reabsorption, confined to the first loop of the central canal, may follow its electrochemical gradient, since the transepithelial potential remains negative with respect to the external medium (A. Wenning, unpublished observations). Given a luminal K\(^+\) channel, uptake of K\(^+\) would then be determined by the K\(^+\) equilibrium potential (E\(_{K}\)) across the apical and basal membrane and would be independent of the amount of secondary urine. Indeed, absolute and relative KCl reabsorption is always high and is strongly correlated to its extracellular concentration. NaCl reabsorption, confined to the second loop of the central canal, changes with the physiological conditions and is correlated with the external NaCl concentration (Zerbst-Boroffka et al. 1982). The mechanisms of NaCl reabsorption have not been investigated in detail.

**Nephridial performance during postprandial diuresis**

The volume of the blood and its ionic composition both change after feeding or after loading of the crop with either hypotonic (72 mmol l\(^{-1}\) NaCl) or hypertonic (145 mmol l\(^{-1}\) NaCl, 5 mmol l\(^{-1}\) KCl) salt solutions (Zerbst-Boroffka, 1975; Wenning et al. 1980; Zerbst-Boroffka et al. 1982). The subsequent increase in urine flow is termed ‘hypotonic’ and ‘hypertonic’ diuresis, respectively.

An increase of blood volume is a prerequisite for an increase of urine flow. As in the Gauer–Henry reflex in mammals, a volume-mediated release of diuretic factors is assumed to trigger diuresis (Zerbst-Boroffka, 1978). Alternatively, since primary urine formation depends on the supply of ions and water, diuresis might result – at least in part – from an increased delivery. Indeed, isolated nephridia with a disrupted circulation but an unlimited supply of ions and water produce twice as much urine as those in situ (this study; A. Wenning, unpublished results).

Isolated nephridia respond differently to changes in the osmolality and composition of the extracellular fluid from those in situ. Changes in blood osmolality are inversely...
correlated with changes in urine volume in isolated nephridia (Zerbst-Boroffka and Wenning, 1986), but not in situ: blood osmolality increases after a blood meal, as does urine production (Zerbst-Boroffka, 1973). The Cl\textsuperscript{−} concentration of leech blood, for example, almost triples after a blood meal and during hypertonic diuresis (Zerbst-Boroffka et al. 1982), but exposing isolated nephridia to a similar Cl\textsuperscript{−} concentration (107 mmol l\textsuperscript{−1}; Fig. 5A) does not result in diuresis. Similarly, urine production decreases in isolated nephridia when the external Na\textsuperscript{+} concentration is lowered (Fig. 5A), but a similar decrease in the Na\textsuperscript{+} concentration (from 125 to 80 mmol l\textsuperscript{−1} after loading of the crop with hypotonic salt solution) results in diuresis in intact animals (Wenning et al. 1980). These results show that diuresis is not a consequence of a different ionic environment or blood osmolality. On the contrary, nephridial transport is stimulated despite changes in osmolality and ion concentrations.

To rid themselves of the high salt and volume load of a blood meal, the canalicular cells of leech nephridia secrete more primary urine of a higher Na\textsuperscript{+} concentration. Many bloodsucking insects employ a similar strategy in that their Malpighian tubules switch from secretion of K\textsuperscript{+}-enriched primary urine to secretion of Na\textsuperscript{+}-enriched primary urine after a meal. However, the underlying transport mechanisms are fundamentally different from those in the leech. In the Malpighian tubules, cations are transported transcellularly. The apical cation pump is a proton pump (V-ATPase) which creates a lumen-positive transepithelial potential. The V-ATPase works in parallel with a cation/H\textsuperscript{+} antiporter (reviewed by Maddrell and O’Donnell, 1992). Cl\textsuperscript{−} is assumed to move either paracellularly (Beyenbach, 1995; Wang et al. 1996) or through a different cell type (Dijkstra et al. 1995; O’Donnell et al. 1996).

Independent regulation of the paracellular shunt (responsible for urine volume) and the transcellular pathway (responsible for urine composition) has been described in the mosquito Aedes aegypti (Beyenbach, 1995). The natriuretic peptide selectively increases the basolateral Na\textsuperscript{+} conductance of the Malpighian tubules and hence promotes Na\textsuperscript{+} secretion, while peptides from the leucokinin family regulate the paracellular shunt and hence volume transfer (Pannabecker et al. 1993; Beyenbach, 1995; Wang et al. 1996). Interestingly, the freshwater larvae of A. aegypti are unable to switch to Na\textsuperscript{+} secretion. Here, secretagogues [diuretic peptide(s), 5-HT] increase fluid production without a change in the Na\textsuperscript{+}:K\textsuperscript{+} ratio (Clark and Bradley, 1996). In contrast, leeches lack the ability to control primary urine volume and composition independently since the Na\textsuperscript{+}:K\textsuperscript{+} ratio increases whenever urine production increases: in hypertonic and hypotonic diuresis (Zerbst-Boroffka et al. 1982), in isolated nephridia (Table 2) and in ‘post-depletion diuresis’ (Fig. 5F; Na\textsuperscript{+}:K\textsuperscript{+} ratio, 4.1±1; control, 1.3). Since at least two Cl\textsuperscript{−} are required to move one K\textsuperscript{+}, but only one Cl\textsuperscript{−} per Na\textsuperscript{+}, opening of the paracellular shunt inevitably leads to a higher concentration of Na\textsuperscript{+} at the expense of the K\textsuperscript{+} concentration in the primary urine.

In Cl\textsuperscript{−}-secreting epithelia, activation of transport results from (1) stimulation of the Na\textsuperscript{+}/K\textsuperscript{+}/2Cl\textsuperscript{−} cotransporter, (2) an increase in the apical Cl\textsuperscript{−} conductance and (3) upregulation of paracellular Na\textsuperscript{+} transfer (Riordan, 1994). In the leech, (paracellular) Na\textsuperscript{+} transport increases 15-fold, while (transcellular) KCl transport increases only fourfold in hypertonic and hypotonic diuresis (Zerbst-Boroffka et al. 1982). Since Cl\textsuperscript{−} secretion drives Na\textsuperscript{+} and K\textsuperscript{+} transport, basal Cl\textsuperscript{−} entry must also increase in diuresis, probably by activation of Na\textsuperscript{+}/K\textsuperscript{+}/2Cl\textsuperscript{−} cotransport. Manipulations that decrease the intracellular Cl\textsuperscript{−} concentration are known to activate Na\textsuperscript{+}/K\textsuperscript{+}/2Cl\textsuperscript{−} cotransport (Riordan, 1994). Indeed, Cl\textsuperscript{−} depletion, which itself inhibits urine formation (Fig. 5E) and will decrease intracellular Cl\textsuperscript{−} concentration, leads to a marked overshoot of urine production upon return to the normal Cl\textsuperscript{−} concentration (Fig. 5F). Bumetanide not only prevents this post-depletion diuresis but also blocks urine flow completely (Fig. 6), indicating that Na\textsuperscript{+}/K\textsuperscript{+}/2Cl\textsuperscript{−} cotransport is the main pathway for Cl\textsuperscript{−} entry.

The change from the K\textsuperscript{+}-enriched primary urine to a more Na\textsuperscript{+}-enriched primary urine is the first step towards the marked natriuresis observed after feeding. The second step is the downregulation of NaCl reabsorption. As demonstrated by loading of the crop with different salt solutions, NaCl reabsorption is inversely correlated with the actual NaCl concentration of the leech blood (Wenning et al. 1980; Wenning, 1995). NaCl reabsorption drops from 85% (approximately 7 μmol h\textsuperscript{−1}) of the secreted amount under normal conditions to 3% (approximately 1 μmol h\textsuperscript{−1}) during postprandial diuresis (Zerbst-Boroffka et al. 1982). The decrease in NaCl reabsorption after NaCl loading implies that, during natriuresis, virtually all NaCl must be supplied by the circulation. In leeches that are not in diuresis, in contrast, fluid secretion is thought to rely in part on ion recycling, facilitated by the close association between the secreting canalicular cells and the reabsorbing central canal cells (Fig. 1 and Zerbst-Boroffka, 1975).

The stimulus for switching to more copious, Na\textsuperscript{+}-enriched primary urine is still unknown. Secretagogues, which stimulate fluid secretion in many epithelia (for example, 5-HT, cyclic AMP, carbachol), fail to increase fluid production in leech nephridia (Zerbst-Boroffka and Wenning, 1986; A. Wenning, unpublished results). However, secretagogues might be released in situ. The nephridia are densely innervated by a single peripheral neurone, the nephridial nerve cell (NNC), which is still present and signalling in isolated nephridia (Wenning and Calabrese, 1991). The NNC is bifunctional: as a sensory neurone, it encodes changes in the extracellular Cl\textsuperscript{−} concentration (Wenning, 1989); as a neurosecretory neurone, it releases the tetrapeptide FMRF-NH\textsubscript{2} and a second, as yet unidentified, transmitter directly to the urine-forming cells (Wenning et al. 1993). The electrical activity of the NNC and, hence, peptide release depend on the external Cl\textsuperscript{−} concentration, suggesting that the NNC might regulate NaCl excretion (Wenning, 1995). The NNC’s endogenous peptide, FMRF-NH\textsubscript{2}, has a dual role. It modulates the activity of the NNC through autoreceptors (Wenning and Calabrese, 1995).
and it affects urine production by isolated nephridia (Wenning and Janisch, 1996; A. Wenning, unpublished observations).

Primary urine formation in the leech canalicular cells is driven by Cl\(^{-}\) secretion, and natriuresis requires an upregulation of Na\(^{+}\) transfer through the paracellular pathway. In insect Malpighian tubules, natriuresis requires more Na\(^{+}\) to enter the cells. Thus, in contrast to the water-dwelling medicinal leech, these terrestrial species may change the composition of their primary urine independently of its volume. Future studies will now focus on the role of nephridial innervation in regulating urine production in leeches.

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References


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