LOCALIZATION AND CHARACTERIZATION OF WATER UPTAKE FROM THE MIDGUT OF THE LOCUST, SCHISTOCERCA GREGARIA

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SUMMARY

1. Water, potassium and sodium fluxes were measured across various regions of the locust gut under in vitro internal perfusion.
2. The anterior caeca differed from the other gut regions, notably the posterior caeca, in being able to absorb water in the absence of an applied chemical or osmotic gradient.
3. Absorption of sodium from the caecal lumen was active, and inhibited by 2,4-DNP or ouabain.
4. Potassium was passively distributed across the caecal epithelium, and transmembrane fluxes were unaffected by toxins.
5. A model for caecal water uptake was advanced, in which passive fluxes of potassium and chloride from the KCl-rich luminal fluid, combined with active sodium uptake, drives water into the blood, thus concentrating solutes within the caecal lumen.

INTRODUCTION

The conventional description of nutrient uptake in insects is based largely on the model proposed by Treherne (1967), in which nutrients are taken up passively along a concentration gradient maintained by a rapid removal of water from the gut contents, and a removal of nutrients from the haemocoel into storage tissues, like the fat body. Berridge (1970) elaborated this into an elegant ‘counterflow’ model, in which a solid matrix of food, passing down the alimentary canal, meets a flow of enzymes and water, secreted by the posterior midgut. The digested nutrients are thus swept forward, against the flow of the food matrix, to a specialized absorption site in the anterior midgut, particularly the gastric caeca. This model has clear advantages to the insect; particularly because the ‘standing gradient’ of nutrients, which such a flow would generate, would ensure that nutrient levels were high at the site of uptake, and low at the posterior end of the midgut, thus reducing leakage of desirable substances into the hindgut. This model, although of considerable interest, has yet to gain any direct, experimental support. Treherne’s data implicated the caeca as the region of the midgut from which nutrients disappeared the fastest, and in which the dye amaranth was concentrated after injection into the gut lumen. This agrees with the subsequent work of Baines (1976), who observed the appearance of
concentrated dyes in the caecal lumen of *Locusta* nymphs, after they had been fed a meal of dyed grass. A previous paper (Dow, 1981a) has shown that the caeca are the only parts of the midgut or ileum to contain a hyperosmotic lumen *in vivo* and Bernays (1980) has shown that the posterior lobes of the caeca in *Schistocerca gregaria* contain specialized infoldings which appear to concentrate dyes. Thus there is some indirect evidence that a site of water uptake is located in the gastric caeca of the locust. However, there is no evidence yet for a secretion of water in the posterior midgut, an essential part of the counterflow model, which must, therefore, be considered speculative. By studying the ability of different regions of the gut to transport water and ions *in vitro*, it was hoped to gain sufficient evidence to test and counterflow model of nutrient uptake in the locust midgut.

There are two classical physiological techniques for the *in vitro* study of such transport phenomena. The simpler is the use of isolated sacs, in which a sac of tissue is filled with a known volume of fluid of known composition, and the change in composition, or efflux of radioactive label from the sac, is measured. This method has been used extensively on insect preparations (Phillips, 1964; Sauer, Schlenz-True & Mills, 1969; Weintraub & Teitz, 1973), but its value is limited by certain assumptions, notably that the lumen, before filling, contained no fluid or contaminating solutes to alter the initial composition of the injected fluid. Rinsing the sac before filling ensures that there are no contaminents, but precludes an accurate assessment of total fluid volume, once the sac has been filled.

A more sophisticated method is that of internal perfusion. In this method, the tissue is prepared as a tube, cannulated at either end, and immersed in saline. Fluid of known composition is pumped into the lumen of the tissue at a known rate, and all the fluid appearing at the other end of the tube is collected for analysis. If the input rate of a solute is known, and its output rate can be determined, it is a simple matter to calculate the rate of transmembrane flux. If the tissue is immersed in a bath which is also being perfused, it is possible to study the performance of the tissue under a wide range of chemical and osmotic gradients. This method has also been used extensively on insect preparations (O'Riordan, 1969; Harvey & Zerahn, 1969), and allows results of high reliability to be obtained.

The transmembrane water fluxes of different parts of the gut were measured *in vitro* with an isotope dilution technique by internally perfusing various isolated sections of gut with fluid identical to the bathing medium, containing $^{14}$C inulin as a volume marker. In this way, there were no chemical or osmotic gradients which might drive a passive water flux.

In further experiments, the transmembrane sodium, potassium and water fluxes across single anterior caeca were measured with normal bathing solution, and bathing solution poisoned with 1 mM-2,4-DNP or 1 mM-ouabain, and with luminal solutions containing potassium at 10 mM or 100 mM, the latter concentration more closely resembling the gut fluid observed *in vivo* (Dow, 1981a).

The animals used were freshly fed (‘fed-3 h’, as defined earlier (Dow, 1981a)). The gut was removed from the animal as described earlier, and immediately immersed...
in a small dish of saline. The composition of the saline was as described previously. The saline (Dow, 1981a) contained Na 60, K 10, Ca 5, Mg 5 and Cl 80 mM, with the addition of amino-acids, and 10 mM glucose. Sucrose was added to bring the osmotic pressure of the solution to 370 m-osmol, and the pH was buffered to 6·8–7·0 with PIPES (Good et al. 1966). Control experiments with whole midguts under internal perfusion confirmed that ion fluxes could be maintained for at least 2 h in this saline. The tissue under study was then dissected out, and transferred to the internal perfusion apparatus (Fig. 1). The bath volume was 2 ml, and the bath perfusion system, driven by a peristaltic pump, ran at around 1 ml/min. The interval perfusion was driven by a motorized 'Agla' micrometer syringe drive, fitted with a 'Hamilton' 100 μl gas-tight microsyringe, which produced a flow rate of 180–200 nl/min. The micrometer delivery system was calibrated manually, and the precise perfusion rate calculated for each experiment from the micrometer readings. The bathing solution was kept in a water bath at 32 °C; this produced a temperature of 30 °C in the perfusion bath, as measured with a sub-miniature thermistor thermometer. The tissue was tied on to the two cannulae, and flushed through with 0·2 ml of the filling solution, which produced a flow rate of 180–200 nl/min. The micrometer delivery system was calibrated manually, and the precise perfusion rate calculated for each experiment from the micrometer readings. The bathing solution was kept in a water bath at 32 °C; this produced a temperature of 30 °C in the perfusion bath, as measured with a sub-miniature thermistor thermometer. The tissue was tied on to the two cannulae, and flushed through with 0·2 ml of the filling solution, which produced a flow rate of 180–200 nl/min. 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No drop was left under oil for more than 10 min; Lubbock (1980, personal communication) has observed that the volumes of small droplets left overnight under paraffin changed drastically, unless the paraffin had previously equilibrated with a solution of the same osmotic pressure as the experimental samples. At the end of the experiment, the integrity of the preparation was tested by manually inflating the tissue, and visually inspecting for leaks. Any leaks too small to detect would result in a slight dilution of the radiolabel, producing an apparent secretion of water. Such a leak, therefore, could not artefactually produce an apparent uptake of water from the gut lumen.

For experiments in which the effect of metabolic toxins was studied, 2,4-DNP or ouabain was added to the external bathing solution at 1 mM, immediately before use. Preliminary experiments had shown that the transepithelial potential in DNP-saline took 10–20 min to decay to a steady value of around 3 mV (lumen negative) and so the isolated gut was pre-incubated in poisoned saline for 20 min before subsequent dissection, to allow time for the poison to take effect.

Transepithelial potentials were measured during later internal perfusion experiments, in which it was desired to calculate the transepithelial electrochemical potential differences (TEECPs) for sodium and potassium, and in experiments in which the potassium dependence of the TEP was determined. The potentials were measured with two low-resistance (1–10 MΩ) Ag/AgCl glass microelectrodes, filled with 1 M-KCl. At the start and end of each experiment, the null offset of the electrode pair was determined with both in the perfusion bath; during the determinations, one electrode was placed in the droplet forming at the tip of the second cannula, while the other was brought close to the outside of the tissue in the perfusion bath.

**Dependence of transepithelial potential on luminal potassium concentration**

The transepithelial potential across an anterior caecum was measured under internal perfusion with isosmotic mucosal solutions, containing potassium at 1, 2, 5, 10, 20, 50 or 100 mM. The serosal solution was normal saline, which was recycled through a 50 ml pool, and continuously bubbled with oxygen. The perfusion rates were high (1 ml/min external, 0.1 ml/min internal) to ensure that the TEP was not reduced by the presence of unstirred layers near the surface of the epithelium. Preliminary experiments showed that such effects first became noticeable (1 mV error) at an internal perfusion rate of 0.04 ml/min.

**Sample handling**

Photometric concentrations of sodium and potassium were measured on a Pye-Unicam SP90A flame photometer. The samples were added to 2 ml of distilled water on collection, producing a typical dilution of 1000x, and measured against 100 µM standard solutions of NaCl and KCl. The values measured were corrected for the interference of sodium on potassium, and multiplied by the precise dilution, to obtain the original concentration. Samples for scintillation counting were diluted in 10 ml of ‘Biofluor’ scintillation cocktail (New England Nuclear), and counted in a Packard scintillation counter. Samples were corrected for background activity and
quenching, and counts were converted to disintegrations per minute (d.p.m.). The activity of each sample, in d.p.m./μl, was calculated, using the measured sample volume, and divided by the activity of the original solution, which had been similarly measured. Thus a value greater than one implied a net movement of water from the lumen to the blood-side, and vice-versa. The transepithelial fluxes of sodium, potassium and water were calculated from the observed changes in luminal concentrations of sodium, potassium and inulin. The transepithelial electrochemical potentials for sodium and potassium were calculated from the mean transepithelial concentration differences of sodium and potassium, and the mean T.E.P. during the experiment. The parameters measured are shown schematically in Fig. 2.

RESULTS

The mean water fluxes across the various tissues under study with identical saline both sides (Na 60, K 10 mM, 370 m-osmol), are shown in Table 1. It can be seen that the anterior caeca were the only parts of the midgut or ileum capable of absorbing water significantly in the absence of a chemical or osmotic gradient. The posterior and anterior regions of the midgut differ significantly, but neither shows much ability to secrete water, as required by the counterflow model. The posterior caeca differ significantly from the anterior caeca in their inability to transport water.
Table 1. Water fluxes measured under internal perfusion, in the absence of a chemical or osmotic gradient, for various regions of the midgut and ileum

(Fluxes are expressed in nl/min from lumen to bath, and so a positive value implies an absorption of water from the lumen. The value n represents the number of animals used in a given experiment, and P represents the probability that the values shown do not differ significantly from zero (Student's t-test). As there are 6 gastric caeca, these data have been multiplied by 6, to give a balanced impression of their significance.)

<table>
<thead>
<tr>
<th>Gut region</th>
<th>Mean (nl/min)</th>
<th>S.E.</th>
<th>n</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anterior caeca</td>
<td>114.0</td>
<td>4.8</td>
<td>4</td>
<td>0.001</td>
</tr>
<tr>
<td>Posterior caeca</td>
<td>-6.0</td>
<td>3.6</td>
<td>6</td>
<td>N.S.</td>
</tr>
<tr>
<td>Anterior midgut</td>
<td>-13.4</td>
<td>1.4</td>
<td>5</td>
<td>0.001</td>
</tr>
<tr>
<td>Posterior midgut</td>
<td>1.6</td>
<td>2.4</td>
<td>4</td>
<td>N.S.</td>
</tr>
<tr>
<td>Ileum</td>
<td>-0.3</td>
<td>2.4</td>
<td>5</td>
<td>N.S.</td>
</tr>
</tbody>
</table>

Table 2. Potassium, sodium and water fluxes across an anterior caecum under internal perfusion, with normal saline on both sides

(P denotes the probability that the data do not differ significantly, from zero (Student's t-test). The final row indicates the significance of any reduction in the fluxes measured (Mann-Whitney U-test.).)

<table>
<thead>
<tr>
<th>Saline</th>
<th>Flux from lumen to bath</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$J_K$ (nmol min$^{-1}$)</td>
</tr>
<tr>
<td>Control</td>
<td>Mean</td>
</tr>
<tr>
<td></td>
<td>-2.2</td>
</tr>
<tr>
<td>DNP</td>
<td>Mean</td>
</tr>
<tr>
<td></td>
<td>-1.46</td>
</tr>
</tbody>
</table>

Studies on the anterior caecal lobes

In the presence of 1 mM-2,4-DNP, an oxidative phosphorylation uncoupler, water uptake from the anterior caecal lumen fell significantly to a level which did not differ significantly from zero (Table 2). It can be concluded that the water flux was totally abolished in DNP saline. The reduction in the potassium flux was not significant, while the reduction in sodium absorption was; it follows that the transepithelial flux of sodium requires an intact pathway of oxidative phosphorylation, while that of potassium does not, at least for the duration of the experiments. This suggests that the potassium flux is passive, but might also suggest that the potassium flux is being driven through the glycolytic pathway (Civan, Hall & Gupta, 1980). In the presence of ouabain at 1 mM on the bath side of the tissue, sodium absorption was significantly reduced, while the potassium and water fluxes were not, although some fall was seen.
Locust midgut water uptake

Table 3. Effect of ouabain at 1 mM on potassium, sodium and water fluxes across an anterior caecum under internal perfusion

(P denotes the probability that the data do not differ significantly from zero (Student's t-test). The final row indicates the significance of any reduction in the fluxes measured (Mann-Whitney U-test).)

<table>
<thead>
<tr>
<th>Flux from lumen to bath</th>
<th>$J_K$ nmol min$^{-1}$</th>
<th>$J_{Na}$ nmol min$^{-1}$</th>
<th>$J_{water}$ nl min$^{-1}$</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control Mean</td>
<td>-1.32</td>
<td>3.4</td>
<td>15.6</td>
<td></td>
</tr>
<tr>
<td>s.e.</td>
<td>0.2</td>
<td>1.0</td>
<td>2.4</td>
<td></td>
</tr>
<tr>
<td>$n$</td>
<td>4</td>
<td>4</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>$P$</td>
<td>0.01</td>
<td>0.05</td>
<td>0.01</td>
<td></td>
</tr>
<tr>
<td>Ouabain Mean</td>
<td>-0.09</td>
<td>-0.1</td>
<td>6.83</td>
<td></td>
</tr>
<tr>
<td>s.e.</td>
<td>0.3</td>
<td>1.2</td>
<td>7.1</td>
<td></td>
</tr>
<tr>
<td>$n$</td>
<td>8</td>
<td>8</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>$P$</td>
<td>N.S.</td>
<td>N.S.</td>
<td>N.S.</td>
<td></td>
</tr>
<tr>
<td>$P$</td>
<td>N.S.</td>
<td>0.05</td>
<td>N.S.</td>
<td></td>
</tr>
</tbody>
</table>

Table 4. Fluxes of potassium, sodium and water, and the transepithelial electrochemical potential differences for potassium and sodium, for an anterior caecum under internal perfusion with an isosmotic luminal solution, containing 100 mM-potassium, 50 mM-sodium, 80 mM-chloride and 50 mM-sulphate

(Data are shown for normal bathing solution (Na 50, K 10 and Cl 80 mM), and bathing solutions containing DNP or ouabain at 1 mM. The values for $P$ indicate the probability (Student's t-test) that the data do not differ significantly from zero.)

<table>
<thead>
<tr>
<th>Bathing solution</th>
<th>$J_K$ nmol min$^{-1}$</th>
<th>$J_{Na}$ nmol min$^{-1}$</th>
<th>$J_{water}$ nl min$^{-1}$</th>
<th>T.E.P. mV</th>
<th>$\Delta\mu_K$ KJ mol$^{-1}$</th>
<th>$\Delta\mu_{Na}$ KJ mol$^{-1}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control Mean</td>
<td>6.7</td>
<td>3.7</td>
<td>19.3</td>
<td>-21.6</td>
<td>-3.5</td>
<td>+1.9</td>
</tr>
<tr>
<td>s.e.</td>
<td>0.9</td>
<td>1.1</td>
<td>6.2</td>
<td>3.1</td>
<td>0.3</td>
<td>0.3</td>
</tr>
<tr>
<td>$n$</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>$P$</td>
<td>0.001</td>
<td>0.05</td>
<td>0.005</td>
<td>0.01</td>
<td>0.01</td>
<td>0.01</td>
</tr>
<tr>
<td>+ DNP Mean</td>
<td>2.4</td>
<td>-0.3</td>
<td>-0.6</td>
<td>-3.1</td>
<td>5.5</td>
<td>+0.4</td>
</tr>
<tr>
<td>s.e.</td>
<td>0.9</td>
<td>0.7</td>
<td>4.5</td>
<td>0.7</td>
<td>0.04</td>
<td>0.04</td>
</tr>
<tr>
<td>$n$</td>
<td>8</td>
<td>8</td>
<td>8</td>
<td>8</td>
<td>8</td>
<td>8</td>
</tr>
<tr>
<td>$P$</td>
<td>0.05</td>
<td>N.S.</td>
<td>N.S.</td>
<td>0.01</td>
<td>0.001</td>
<td>0.001</td>
</tr>
<tr>
<td>+ Ouabain Mean</td>
<td>5.8</td>
<td>2.0</td>
<td>18.3</td>
<td>-13</td>
<td>4.6</td>
<td>+1.0</td>
</tr>
<tr>
<td>s.e.</td>
<td>1.7</td>
<td>1.1</td>
<td>7.2</td>
<td>0.8</td>
<td>0.1</td>
<td>0.1</td>
</tr>
<tr>
<td>$n$</td>
<td>8</td>
<td>8</td>
<td>8</td>
<td>8</td>
<td>8</td>
<td>8</td>
</tr>
<tr>
<td>$P$</td>
<td>0.025</td>
<td>0.1</td>
<td>0.05</td>
<td>0.001</td>
<td>0.001</td>
<td>0.001</td>
</tr>
</tbody>
</table>

(Table 3). It follows that sodium absorption occurs via a ouabain-sensitive transport mechanism, while the water and potassium fluxes probably do not.

The data for caeca perfused with a luminal solution containing 100 mM-potassium and 50 mM-sodium are shown in Table 4. Potassium moves down an electrochemical gradient from lumen to bath, and this movement is substantially unaffected by DNP or ouabain, suggesting that this flux is not critically dependent on cellular metabolic energy. The flux of sodium, which also runs from lumen to bath in normal bathing solution, is against an electrochemical gradient, and is reduced, or abolished in DNP ouabain. This indicates that sodium absorption from the anterior caeca is an active
Fig. 3. Transepithelial potentials across an anterior caecum under internal perfusion with luminal solutions containing 1–100 mM-potassium, 50 mM-sodium, 80 mM-chloride and 0.5–50 mM-sulphate. The bathing solution contained Na 50, K 10 and Cl 80 mM. The date are shown as means in mV (lumen relative to blood), and the vertical bar denotes ± 1 standard error (n = 5). The dotted line indicates the 58 mV/decade slope of a perfect potassium electrode.

process, requiring an intact pathway of oxidative phosphorylation, and a ouabain-sensitive carrier.

The water flux, from lumen to bath in normal saline, is apparently abolished, along with the TEP, in DNP saline, despite a net absorption of cations. This seems to be because DNP has increased the permeability of the epithelium until inulin can pass freely into the bath, and the TEP is effectively shorted out. This is confirmed by the finding of a control experiment, in which the efflux of inulin from caecal sac preparations, immersed in saline, increased fivefold in the presence of DNP. This is consistent with the finding of Loewenstein (1970) that DNP affects intercellular junctional permeabilities. Bielawski, Thompson & Lehninger (1967) also observed that the electrical permeability of artificial bilayer membranes increased massively, upon application of 1 mM-DNP. It is thus likely that inulin volume-marking is not suitable for DNP-poisoned tissues. The water flux in ouabain-poisoned saline is not significantly reduced, and the TEP is only slightly reduced, thus suggesting that a water uptake can be driven by an absorption of either sodium or potassium.

The variation in TEPs for a range of luminal potassium concentrations are shown in Fig. 3. It can be seen that the epithelium behaves as if it were a leaky potassium electrode. In particular, the TEP is zero when the luminal potassium concentration equals that of the bathing saline, indicating that the TEP is largely determined by the potassium gradient, and not by electrogenic transports. This impression is strengthened by the observation that the TEP with 100 mM-luminal potassium (mM-luminal potassium...
Increased in the presence of luminal copper sulphate at 1 mM, from $-19 \pm 1.4$ mV ($n = 4$), to $-26 \pm 1.9$ mV ($n = 6$). This would be expected to increase the size of the TEP, as copper is believed to block passive chloride fluxes, which would contribute to the leakiness of the membrane (Berridge, 1969).

**DISCUSSION**

The only gut region capable of transporting water under experimental conditions *in vitro* was the anterior lobe of the caecum. It is interesting to note that the anterior lobes differ from the posterior lobes in this respect, as they do in general structure (Bernays, 1980), and in the *in vivo* TEP across them (Dow, 1981a); the TEP across the anterior lobes being $-14$ mV (lumen negative), and across the posterior lobes, 0 mV (difference significant at 0.1%). It is thus becoming increasingly clear that the anterior and posterior caeca of the locust must be considered separately in physiological studies.

Bernays (1980) suggested that the posterior lobes of the caeca might be involved in a rapid movement of water from the gut lumen, through specialized 'pockets' in the epithelium, into the blood, as dyes became concentrated in these pockets *in vivo*. Such a water uptake was not detected in these experiments. This could be for one of several reasons. The tissue might have been systematically damaged, perhaps by the removal of the peritrophic membrane from the pockets; the flux may be too small to be measured (i.e. below 0.1 µl/h); the tissue might require stimulation by some agent not yet isolated; or the dyes might have been concentrated through some preferential absorption on to the contents of the pockets. Out of 7 acridid species studied, pockets were only found in *Schistocerca gregaria*. This suggests that, whether their function is to absorb water, secrete enzymes or inactivate toxic molecules, such as tanins (the diet of *Schistocerca gregaria* is unusually rich in such compounds (Bernays, Chamberlain & McCarthy, 1980)), they are not the means by which the majority of acridids perform such functions. As any fluid entering the anterior lobes of the caeca is compelled to pass the bulbous portions of the posterior lobes, the pockets would be ideally suited for a role in detoxifying the midgut fluid, prior to concentration and absorption.

The distribution of potassium across the anterior caeca appears passive, while that of sodium is actively maintained by a process similar to that identified in cockroach midgut by O’Riordan (1969). This is consistent with predictions made from data obtained *in vivo* (Dow, 1981a). It may transpire that this transport is a widespread property of the midguts of herbivorous insects. A caecal site for water absorption supports the models of Treherne (1967) and Berridge (1970) for nutrient uptake; however, the posterior midgut does not seem to secrete water, as Berridge had predicted. So, if the countercurrent model is to hold, the site of water secretion must be elsewhere. The Malpighian tubules provide such a site, and their likely role in a countercurrent system will be examined in a subsequent paper (Dow, 1981b).

**Ionic basis of water transport across the anterior caeca**

The energetics and stoichiometry of water movement across an epithelium have long been a matter of great interest and controversy (Gupta *et al*. 1971; Berridge &
Fig. 4. Model for the uptake of water and ions from the lumen of an anterior caecum. Fluid from the midgut and crop enters the caecal lumen, and replaces that removed from the extracellular infoldings. Potassium and chloride both move into the blood (the electrochemical potential gradients are favourable for both transcellular and paracellular routes). Sodium moves passively into the cell, and is then pumped actively into the blood by an ouabain and DNP sensitive mechanism. Water is assumed to move freely across the tissue, either trans- or paracellularly, to equilibrate the osmotic imbalance thus generated.

Oschman, 1972). There are many articles on the various modes of transport hypothesized, for Malpighian tubules (Maddrell, 1971), rectum (Phillips, 1964; Gupta et al. 1980), and vertebrate intestine (Schultz, 1977; Gupta & Hall, 1979). O’Riordan (1969) proposed a model for the ionic movements she observed across the isolated midgut of the cockroach, which corresponds closely with ideas on water and ion movements across the vertebrate intestine. However, neither she nor Sauer et al. (1969) found a significant flux of water across the midgut to investigate further. It is now clear that this may well be because, as suggested by Berridge (1970), the midgut is not a significant site of water uptake. The model here proposed (Fig. 4) is based on the data presented in the earlier results, and in the preceding paper. The following assumptions were made:

1. It is clear, both from in vivo sampling of the fluids on either side of the epithelium (Dow, 1981a), and from results of in vitro internal perfusion experiments, that potassium is passively distributed across the caecal membrane, and that the fluxes which result from an imposed electrochemical potential difference are largely insensitive to metabolic toxins. It is thus reasonable to assume that potassium moves passively through a mainly paracellular route, although a transcellular route is also energetically feasible.

2. Similarly, it is clear that a low luminal sodium level must be actively maintained, both in vivo (Dow, 1981a), and in vitro. This flux is abolished by both DNP and ouabain, suggesting that a transcellular route, requiring an intact pathway of oxidative phosphorylation, is the most important path for sodium fluxes.
3. The distribution of chloride does not differ significantly from a passive distribution in vivo (Dow, 1981a). Although chloride is a necessary counter-ion for trans-epithelial movements (as indicated by the effect of copper ions on the TEP), there is no evidence at present that chloride is actively transported across the caecal epithelium, as it is in rectum (Spring et al. 1978). It will thus be assumed that chloride moves paracellularly, according to the electrochemical gradient across the whole epithelium, although a transcellular route is also energetically feasible if water absorption raises the luminal chloride concentration to 120 mM.

4. The fluid entering the caecum is assumed to be a mixture of crop fluid and midgut fluid, and thus contain roughly 5 mM-Na, 100 mM-K and 100 mM-Cl. The fluid is assumed to be isosmotic with, or less than 50 m-osmol hypertonic to, the blood. (These data follow from a 50-50 mixing of the crop and midgut fluids observed in vivo (Dow, 1981a); the possibility that this mix might change with time from feeding will be considered later (Dow, 1981a).)

5. The blood is assumed to contain 60 mM-Na, 10 mM-K and 80 mM-Cl; the blood osmotic pressure is assumed to be 370 m-osmol (Dow, 1981a). The composition of the blood is, for the sake of simplicity, assumed to be stabilized by the relatively large size of the blood pool.

6. The epithelial cells are assumed to contain 10 mM-Na, 150 mM-K and 20 mM-Cl. These data have been obtained by electron microprobe X-ray analysis in frozen, hydrated section (J. A. T. Dow, B. L. Gupta, T. A. Hall, in preparation).

7. The concentrations of solutes which are not readily absorbed within the caecal lumen, are assumed to be limited by back-diffusion from the site of uptake. There is some evidence that this might be the case for calcium and magnesium (Dow, 1981a).

It can be seen that a water absorption is driven by fluxes of either potassium or sodium, depending upon availability. This has a clear adaptive significance to a polyphagous organism. It can thus be seen that the potassium flux is likely to be much the more important in vivo, whereas the sodium flux, by a different mechanism, was the more important in the initial internal perfusion experiments.

The uptake of sodium, potassium and chloride from the lumen, and the corresponding water absorption, serve to increase the luminal concentrations of nutrient molecules. If the volume deficiency is ‘topped up’ with further midgut fluid, and further ionic fluxes occur, it can be seen that nutrients will rapidly build up to levels at which they can move passively into the blood. Thus a steady-state system will be established, the required energy being supplied either by the sodium-pump in the cellular epithelium, or, remotely from the site of uptake, by an active excretion of potassium into the gut lumen. This occurs in vivo (Dow, 1981a); the site of such transport is likely to be the Malpighian tubules (Dow, 1981b).

The possibility that the fluid entering the caeca is initially hyperosmotic does not affect the validity of the model, if it is assumed that water moves rapidly to equilibrate any imbalance. (This, indeed, is an essential aspect of such an epithelium, if a passive flux of an ion is to drive a water flux efficiently.) Provided that the potassium level, after an initial osmotic equilibration, is still well above the blood level of 10 mM, the process will still proceed. With a midgut potassium concentration of 150 mM, and a maximum hypertonicity in crop fluid of 200 m-osmol (or 50%), it can be seen that a
suitable fluid can always be composed of the appropriate mixture of crop and midgut fluids.

This model is necessarily simple, and so suffers from many limitations. In particular, the caecal epithelium is not circular in cross-section, but is thrown into deep folds, thus generating compartments with restricted access, both on the luminal and blood sides; these may well be of significance to the uptake process, resembling a 'backward facing' system (Diamond & Bossert, 1967). Furthermore, the luminal compartments appear to be packed with mucus (Baines, 1976), with a surplus of negative charges (J. A. T. Dow, B. L. Gupta & T. A. Hall, in preparation); this is likely to restrict further the access of charged molecules to the presumed site of absorption. In the current experiments, the mucus was washed off the lining of the tissue, in order to ensure a successful internal perfusion; the possibility that this might affect the results obtained should not be overlooked. In these respects, the tissue is very similar to rabbit ileum; in this tissue, the importance of functional specialization of different cells within the infoldings has become clear in recent years; and again, mucus is generally removed before in vitro studies on vertebrate intestine.

This tissue provides an interesting example of an active uptake process, a large part of whose energetic gradient seems to be supplied remotely from the tissue itself; this poses interesting problems of definition. The water flux is also of interest because it appears to be driven principally by a flux of potassium, not sodium, in vivo, and so bears only a superficial similarity to the system in vertebrate intestine. The fate of molecules concentrated within the caecal lumen requires further investigation, as this could be of great significance to an understanding of the effects of plant alkaloids and systemic insecticides. Finally, we can say that there is now some evidence to support the counterflow model of nutrient uptake, despite the apparent inability of the posterior midgut to secrete water; this will be considered further in a subsequent paper (Dow, 1981b).

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