

ION ACTIVITIES AND ELECTROCHEMICAL GRADIENTS IN THE MEALWORM RECTAL COMPLEX

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Summary

1. Ion activities and potential differences in cellular and extracellular compartments of the rectal complex of *Tenebrio molitor* L. larvae have been recorded simultaneously using double-barrelled ion-sensitive microelectrodes.

2. On average, the tubule lumen (TL) was 44 mV positive to the haemolymph. Values of a_K in the posterior rectal complex exceeding 2700 mmol l^{-1} were measured, sufficient to account for much of the osmolality of $6.8 \text{ osmol kg}^{-1}$ driving uptake of water from the rectal lumen. The mean value of 797 mmol l^{-1} exceeded the Nernst equilibrium activity more than 75-fold, indicating active transport of K^+ . Intracellular potassium activities in the tubules (153 mmol l^{-1}) were high relative to the values in other insect cells, but moderate relative to TL values.

3. Tubule lumen Na^+ activities as high as 400 mmol l^{-1} and pH values of 6.8 were well above the equilibrium values of 11 mmol l^{-1} and 7.9, respectively, indicating active transport of these cations as well.

4. The ease and frequency of impaling a perinephric space (PNS) surrounding the tubules established it as a functional compartment. On average, the PNS was 22 mV negative to the haemolymph. Potassium activities in the PNS were close to electrochemical equilibrium with the haemolymph, whereas mean a_{Na} and pH were reduced fivefold and 0.5 units, respectively, below the corresponding Nernst equilibrium values. The results suggest that cations move from haemolymph to PNS, and that the PNS is the immediate source for cation transport into the tubule lumen.

5. Cl^- was close to electrochemical equilibrium with the haemolymph in both compartments, and presumably enters the tubule lumen as a passive consequence of positive potential differences (PDs) in the tubule lumen.

Introduction

One of the pre-eminent physiological adaptations of insects to dry habitats is the

Key words: *Tenebrio molitor*, mealworm, epithelial ion transport, electrochemical gradients, rectal complex, transepithelial potential difference, ion activities, pH.

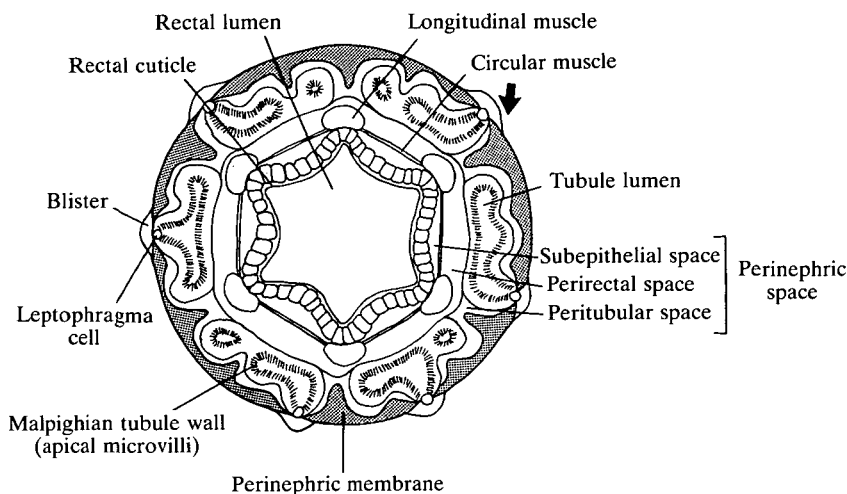


Fig. 1. Schematic transverse section of mealworm cryptonephridial (rectal) complex showing epithelia and fluid compartment interrelationships. Fig. 2 in Grimstone *et al.* (1968) has been redrawn to incorporate the distribution of perinephric spaces shown in Fig. 3 of Ramsay (1964). The downward arrow indicates the approximate position and angle for optimal impalement of the rectal complex by a double-barrelled theta glass microelectrode. Further details in text.

ability to recover water from the hindgut. In mealworms, the larvae of *Tenebrio molitor* L., the cryptonephridial or rectal complex is a particularly powerful water-absorbing organ consisting of the distal ends of the Malpighian tubules applied to the rectum and enveloped in a perinephric membrane (Saini, 1964). The tubules in combination with the perinephric membrane form a sheath enclosing the perinephric space (PNS; Fig. 1). The complex serves two functions. During faecal dehydration water is reabsorbed from faecal material, which enters the rectum anteriorly (Ramsay, 1964). Alternatively, mealworms can gain water vapour from the atmosphere through the open anal canal (Machin, 1976).

Both functions appear to depend upon osmotic gradients created by the Malpighian tubules of the rectal complex. Current understanding of the osmotic forces governing water transport in the rectal complex is based partly on determinations of melting points in frozen sections (Machin, 1979). Frozen section analysis is not compromised by the errors associated with micropuncture sampling of fluids from the rectal complex (Ramsay, 1964; Grimstone *et al.* 1968) and has demonstrated the existence of osmotic gradients necessary for the passive transfer of water from the rectal lumen to the Malpighian tubules during vapour absorption. Furthermore, the anomalous behaviour of perirectal fluid, thought to be related to its role as a water pump (Grimstone *et al.* 1968), has been shown to be a freezing artefact (Machin, 1979). Analysis of the volume of unfrozen perirectal

fluid samples over a range of ambient humidities indicated that the fluid is ideally suited to couple the rectum osmotically to the Malpighian tubules (Machin, 1979).

Unlike recta of insects such as the blowfly (Berridge and Gupta, 1967), locust (Wall and Oschman, 1975) and cockroach (Oschman and Wall, 1969), which lack cryptonephridia, the rectal wall of mealworms is devoid of the ultrastructural features of active ion transport and appears to act passively. The tough outer perinephric membrane also lacks such features. It is composed of many compressed cellular layers and has a low permeability to water, at least in the posterior complex. The perinephric membrane probably protects the complex from dilution by the haemolymph (Grimstone *et al.* 1968). Water activities of the faecal pellets and analyses of ion concentrations and osmolalities in the functioning complex suggest that ion accumulation in the tubules creates osmotic gradients as high as $6.8 \text{ osmol kg}^{-1}$ (Grimstone *et al.* 1968; Machin, 1979), sufficient for either faecal dehydration or vapour uptake. The minimum water activity from which water vapour can be absorbed (absorption threshold) is similar to that of the faecal pellets as they leave the rectum, about 0.9 (Ramsay, 1964; Coutchie and Machin, 1982).

Several types of evidence suggest that the complex actively transports potassium. Micropuncture studies suggest that high tubular osmolalities are almost entirely due to K^+ and Cl^- (Ramsay, 1964). Tubules in an *in vitro* rectal complex preparation transport a much higher proportion of the available K^+ in the bathing fluid relative to Na^+ (Tupy and Machin, 1985). Preliminary electrical measurements by Grimstone *et al.* (1968) on isolated rectal complexes showed that the tubule lumen (TL) is positive relative to the bathing fluid. Based on ion concentrations determined in other preparations, they concluded that K^+ was actively accumulated by the tubules and that chloride movements were passive.

Different kinds of rectal complex preparation, ranging from the partially dissected to the completely excised, have been used in previous physiological studies. Transport in all of them declines substantially over the course of a few hours. Full understanding of the ionic basis of water transport has been hampered, therefore, by the lack of an *in vitro* or *in situ* preparation which maintains ion fluxes sufficient to transport water at the rates observed in intact animals. An alternative approach is to make rapid surveys of the conditions within freshly excised rectal complexes using ion-sensitive microelectrodes. This approach requires the development of techniques for penetrating the rectal complex and determining the location of the microelectrode tip. In this paper we present simultaneous microelectrode measurements of ion activities and electrical potential differences (PDs) in the tubule cells and identified extracellular compartments. For the first time, ion activities in the extreme posterior part of the complex, where osmotic gradients are highest, have been obtained. Moreover, tubule lumen activities not only of K^+ but also of Na^+ and H^+ have been shown to be much greater than the corresponding Nernst equilibrium activities, indicating active accumulation of all three cations.

Materials and methods

Experimental animals

Mealworms were obtained from cultures maintained on wheat bran, either alone or with carrots and apples. Mealworms weighing 80–120 mg were transferred at least 1 month prior to experimentation to 'dry' culture, i.e. wheat bran only. Culture temperature was 22–24°C and humidity was normally 20–30 % RH during winter months (December to May). Humidities were higher, exceeding 50 % RH from June to September. In the summer months some small cultures were kept over silica gel in closed containers fitted with 4 cm long, 2 mm diameter air tubes to facilitate gas exchange for respiration.

Rectal complex preparations

Completely excised rectal complex preparations could be made in about 6 min. After decapitation and bisection of the abdomen, the dorsal integument of the hind end was rapidly cut to the posterior extremity with scissors. The abdominal integument was then pinned out under saline and the complex was examined with a dissecting microscope. Preparations in which the rectal complex was damaged by the longitudinal incision (<10 %) were discarded. The anal canal was severed as close to the anus as possible with iridectomy scissors. The complex was then removed by pulling with fine forceps gripping the anterior hindgut. The tracheal supply to the complex was broken by the procedure, generally without damaging the perinephric membrane. The anterior hindgut and common duct were then trimmed and any attached pieces of fat body were removed before the complex was transferred to the chamber used for electrophysiological measurements. For *in situ* preparations in which tracheal attachments and the anal canal remained intact, the complex was transferred for electrophysiological study after pinning out the abdominal integument. Such preparations could be made in about 3 min.

Solutions

Ramsay's saline

We used the saline solution of Ramsay (1964) to facilitate comparison of our data with that of earlier studies (Ramsay, 1964; Grimstone *et al.* 1968). Salts (in g l⁻¹) were dissolved in the following order to ensure that the 'earthy phosphate' precipitate which formed (Ramsay, 1964) was of the same composition in all experiments: NaCl (3.8), Na₂HPO₄·12H₂O (1.4), KCl (4.1), CaCl₂ (1.1), MgCl₂·6H₂O (2.0). The precipitate was removed by filtration and the filtrate was adjusted to pH 7.1. The osmolality of this saline measured by a vapour pressure osmometer (Wescor, Logan, Utah) was 253 mosmol kg⁻¹.

Concentrated saline

Because Ramsay's saline is hypo-osmotic to the haemolymph of mealworms, even those from moist cultures (Ramsay, 1964), salines that more closely resembled the Na⁺ and K⁺ activities and osmolality of the haemolymph of

mealworms taken from a 'dry' culture (Ramsay, 1964) were prepared by dissolving 3.8 g l^{-1} NaCl and 6.15 g l^{-1} KCl, the other electrolytes remaining the same. This gave 99 mmol l^{-1} Na^+ and 82 mmol l^{-1} K^+ , compared with 85 and 55 mmol l^{-1} , respectively, in Ramsay's saline. Some concentrated salines also contained 50 mmol l^{-1} glucose and 10 mmol l^{-1} each of the amino acids glycine, proline, serine, histidine and glutamine, after the saline designed for the desert tenebrionid *Onymacris plana* by Nicolson and Hanrahan (1986). Sucrose was used to adjust osmolality up to $668 \text{ mosmol kg}^{-1}$. Potassium activities in the saline solutions were varied by changing the proportions of NaCl and KCl. Calcium was omitted from the saline used to determine bathing saline Na^+ activity by means of Na^+ -sensitive microelectrodes (described below).

Electrophysiology

Ion activities and PDs in the tubule lumen or perinephric space were measured using double-barrelled pH- or ion-selective microelectrodes (ISMEs) pulled from thick septum theta glass (TST150, WPI, New Haven, CT, USA). Electrodes pulled from this type of glass effectively penetrated the perinephric membrane, which had previously been accessible only by microelectrodes made of quartz (Grimstone *et al.* 1968). During fabrication, one barrel was filled with a 2–3 cm column of water before pulling. The other barrel was then silanized by exposing the stem to dichlorodimethylsilane vapour (Sigma) for 10–30 s, followed by heating the pipette for 1–2 h on a hot plate at 200°C . A small volume of the appropriate neutral carrier or ion exchanger was introduced into the shank of the silanized barrel and ran to the tip by capillarity. Air bubbles were removed with glass fibres and application of heat from a soldering pencil.

Tips were broken back to about $1 \mu\text{m}$ diameter if the electrode was noisy, the 90 % response time to a step change in activity exceeded 5 s, or the slope was less than 50 mV/decade change in ion activity. A modification of the method of Tripathi *et al.* (1985) was used for tip breakage. The electrode shank and tip were held under saline by a micromanipulator and observed through a dissecting microscope. The surface of a $5 \text{ mm} \times 1 \text{ mm}$ piece of tissue paper held under saline in fine forceps was gently brushed across the tip at an angle of $10\text{--}20^\circ$. With electrodes pulled at comparable settings and filled with 3 mol l^{-1} KCl it was possible to reduce the tip resistance in a series of steps from $40\text{--}60 \text{ M}\Omega$ to $5\text{--}10 \text{ M}\Omega$. Usually more than one pass with the tissue paper was necessary to produce a noticeable change in resistance.

Calibration of ion-selective microelectrodes: ion activity versus concentration

The activity of an ion corresponds to the 'free concentration' (Thomas, 1978), and is equal to the product of the concentration and an activity coefficient. The advantages of calibration of an ion-selective microelectrode in terms of activity have been pointed out by a number of authors (Thomas, 1978; Ammann, 1986; Djamgoz and Dawson, 1989). (1) The potential of an ISME varies with the logarithm of ion activity, not concentration. (2) Activity is the most physiologically

relevant property of an ion because it refers to the mobile ions that contribute to phenomena such as the generation of membrane potentials and enzyme catalysis. (3) The results are less affected by the composition of a sample. As noted by Thomas (1978), most users prefer to express ISME measurements in terms of activity. This convention is also recommended in other general reference works (Ammann, 1986), and data are expressed as activities in most recent studies of insect cells with ISMEs (e.g. Hanrahan and Phillips, 1984; Moffett and Koch, 1988; Chao *et al.* 1989; Djamgoz and Dawson, 1989). Importantly, expressing data in terms of activity permits the calculation of electrochemical equilibrium potentials (e.g. Djamgoz and Dawson, 1989) and electrochemical equilibrium activities (e.g. Chao *et al.* 1989) using the Nernst equation.

Calibration procedures were similar to those in other studies of insect epithelia (Hanrahan and Phillips, 1984). Experimentally determined activity coefficients for single electrolyte solutions were obtained from tables (Robinson and Stokes, 1970). To correct for the imperfect selectivity of ISMEs, mixed electrolyte calibration solutions containing the ion of interest (e.g. Na^+) and the main interfering ion (e.g. K^+) were also used. Where both single electrolyte (e.g. NaCl) and mixed electrolyte (e.g. NaCl/KCl) solutions were used, the ionic strength was maintained constant, thereby permitting calculation of mixed solution activity coefficients with the extended Debye–Huckel equation and Harned's rule (Lee, 1981) using parameters given by Whitfield (1979). Single-electrolyte activity coefficients are considered to be quite precise, and the values for mixed electrolytes can be taken to be accurate to within $\pm 5\%$ (Ammann, 1986).

Na^+ electrodes contained the neutral carrier ETH 227 (Fluka, Ronkonkoma, NY, USA) and were backfilled with 0.5 mol l^{-1} NaCl . The reference barrel was filled with 0.5 mol l^{-1} KCl . Na^+ electrodes were calibrated using the 'mixed solution' method with K^+ as the interfering cation (Armstrong and Garcia-Diaz, 1980; Lee, 1981). Concentrations of the calibration solutions were 500 mmol l^{-1} NaCl and 50 mmol l^{-1} NaCl plus 450 mmol l^{-1} KCl ; corresponding activities were obtained as described above. Bathing saline Na^+ activities in the rectal complex were measured with respect to Ca^{2+} -free concentrated saline, because Ca^{2+} is known to interfere with the Na^+ carrier ETH 227 (Steiner *et al.* 1979). The mean slope of the response of the Na^+ microelectrodes used was $59.9 \pm 1.1 \text{ mV/decade}$ change in a_{Na} ($N=7$).

K^+ electrodes contained the ion exchanger Corning 477317 (IE-190, WPI) and were backfilled with 0.5 mol l^{-1} KCl . The reference barrel was filled with 1 mol l^{-1} sodium acetate. The choice of calibrating solutions for K^+ (and Cl^-) electrodes represented a compromise between the activities which would bracket most or all of the anticipated tubular or perinephric K^+ activities and the possibility of damage to the preparation if very high KCl/NaCl activities ($2500\text{--}3000 \text{ mmol l}^{-1}$) were used. Electrode re-calibrations during an experiment lasting 15–20 min were sometimes necessary while the preparation was still in place in the experimental chamber. Electrodes were calibrated against solutions in which the concentrations (in mmol l^{-1}) were 50 KCl/450 NaCl and 500 KCl , or 100 KCl/900 NaCl and 1000

KCl; corresponding activities were obtained as described above. Slopes did not change significantly in higher potassium activities in solutions whose concentration of KCl was as high as 3000 mmol l^{-1} . The mean slope of the response of the theta glass K^+ microelectrodes used in this study was $51.6 \pm 0.5 \text{ mV}$ ($N=38$) per decade change in a_{K} .

Single-barrelled and double-barrelled K^+ electrodes used for intracellular impalements of tubule cells were pulled from 1 mm unfilamented and 'piggy-back' (PB-150, WPI) glass, respectively. Single-barrelled pipettes were silanized by adding a drop of silane to a Petri dish, which was then inverted over the pipettes on the hot plate. The large-diameter barrel of the piggy-back pipette was silanized by the vapour method and the smaller one was used as the reference barrel. Filling and calibration were as described for thick septum theta glass K^+ microelectrodes. Intracellular K^+ electrodes were calibrated with NaCl/KCl solutions of constant ionic strength (150 mmol l^{-1}). The mean slopes of the responses of single-barrelled and piggy-back K^+ electrodes were $51.5 \pm 0.4 \text{ mV}$ ($N=5$) and $53.1 \pm 0.8 \text{ mV}$ ($N=5$), respectively.

Chloride electrodes contained the Corning ion exchanger 477913 (IE-173, WPI) and the reference barrel was filled with 3 mol l^{-1} sodium acetate. Electrodes were calibrated against single solutions of $100\text{--}1000 \text{ mmol l}^{-1}$ KCl. The mean slope of the response of the Cl^- microelectrodes used in this study was $59.7 \pm 0.9 \text{ mV}$ per decade change in a_{Cl} ($N=6$).

A pH-sensitive neutral carrier based on tridodecylamine (Hydrogen ionophore I, cocktail B, Fluka) was used in pH-sensitive microelectrodes. The stem of the pH-sensitive barrel was backfilled with a solution of 0.1 mol l^{-1} citrate and 0.1 mol l^{-1} NaCl adjusted to pH 6 (Thomas, 1978). The reference barrel was backfilled with KCl. Electrodes were calibrated frequently during experiments using saline adjusted to pH values above and below the range of interest. Saline pH was adjusted by titration with HCl or NaOH while pH was measured with a glass macroelectrode (Metrohm 605, Herisau, Switzerland). The mean slope of the response of the microelectrodes used was $55.1 \pm 2.3 \text{ mV}$ per pH unit ($N=7$).

PDs from the reference (V_{ref}) and pH- or ion-sensitive barrels (V_i) were measured by a high input impedance differential electrometer (FD 223, WPI). V_{ref} was measured with respect to a Ag/AgCl electrode connected to the bath through a 3 mol l^{-1} KCl agar bridge. V_i was filtered through a low pass RC filter (1 s) to eliminate noise resulting from the high input impedance (approx. $10^{10} \Omega$) of the ion-sensitive barrel. V_{ref} and the difference ($V_i - V_{\text{ref}}$) were recorded using an AD converter and data-acquisition system sampling at 20 Hz (Axotape, Axon Instruments, Burlingame, CA, USA) and/or on a two-channel chart recorder (Linear Instruments, Reno, NV, USA).

Impalement procedures

Extracellular compartments

The microelectrode was mounted on a hydraulic micromanipulator (Narishige,

Tokyo, Japan) and slowly advanced towards the rectal complex at an oblique angle (approximately 20°) so as to contact the surface at a point 80–90 % of the distance between the midline of the complex and the periphery. A slight (<2 mV) change in PD and an increase in the noise of the ion-selective barrel indicated surface contact. The electrode was then rapidly advanced $50\text{--}60\text{ }\mu\text{m}$ and immediately retracted approximately $40\text{--}50\text{ }\mu\text{m}$ so as to penetrate the perinephric membrane. If the electrode tip was initially positioned so that the microelectrode was in line with the long axis of a tubule loop, the tip often passed through the basolateral and apical tubule cell membranes and into the tubule lumen as well. In effect, a tubule in the '1 o'clock' to '3 o'clock' position in the complex was cannulated by this impalement technique (Fig. 1). For perinephric space measurements, the tip was positioned along the length of the complex so that it would enter the complex between the loops of tubule.

Acceptable impalements were characterized by an abrupt monotonic potential change in the reference barrel, stable transmembrane PD (± 1 mV) for at least 30 s, and return to within 2 mV of the baseline potential after electrode withdrawal. Periodic waves of muscle contraction in all rectal complex preparations produced movements of up to 0.5 mm and limited the time for which impalements could be maintained. Stable transmembrane potentials were frequently recorded for several minutes, and usually these periods ended abruptly, coincident with a wave of muscle contraction in the rectal complex. Acceptable impalements were achieved only rarely if the microelectrode was aligned at right angles to the surface of the rectal complex. Overall, less than half the impalements met the criteria outlined above, usually because muscle contractions dislodged the electrode before transmembrane PD stabilized.

Determination of microelectrode tip location

For experiments requiring correlation of measured PD with tip location (i.e. tubule lumen *versus* perinephric space), both barrels of a thick septum theta glass micropipette were filled with amaranth dye (50 mmol l^{-1}) dissolved in 0.5 mol l^{-1} KCl. After impalement and PD measurement, the position of the microelectrode tip was determined by dye injection. Pressure was applied from a hand-held 50 ml syringe (Gillette *et al.* 1982) connected by PE tubing to the pressure-injection port of a commercially available microelectrode holder (WPI model MEH-2SW).

Tubule cell impalements

As demonstrated in the Results, tubule cells could not be impaled in intact rectal complexes using double-barrelled microelectrodes. However, successful tubule cell impalements could be achieved by approaching the tubules from the unprotected rectal side. The complete sheath of Malpighian tubules plus perinephric membrane was severed from its posterior attachment to the complex by a single transverse cut with iridectomy scissors. The rectum was then pulled free so as to remove the rectal cuticle, epidermis and muscles. A longitudinal cut permitted the tubules and perinephric membrane to be pinned out as a flat sheet, rectal side up,

so that the tubules were freely accessible. Preliminary measurements were made using single-barrelled K^+ - and voltage-sensitive electrodes in a bathing solution whose K^+ activity (95 mmol l^{-1}) approached that of the perinephric fluid in the intact complex. Alternatively, measurements were made in a range of K^+ concentrations so that intracellular potentials with typical intact perinephric K^+ activities could be interpolated. Measurements of intracellular K^+ activity were made using double-barrelled piggy-back electrodes or by alternating groups of PD measurements with those using a single-barrelled K^+ -sensitive electrode in the same preparation.

Calculations and statistics

The activity of an ion in Ramsay's or concentrated saline, a_i^{Bath} , was obtained by inserting the potential measured by the ion-selective barrel into the calibration curve. The activity of an ion (i) in the intra- or extracellular compartments of the rectal complex was calculated from the equation:

$$a_i = a_i^{\text{Bath}} \times 10^{(V_i - V_{\text{ref}})/S},$$

where V_i is the potential measured by the ion-sensitive barrel, V_{ref} is the potential measured by the reference barrel, a_i^{Bath} is the ion activity of the bathing saline determined during electrode calibration, and S is the slope of the electrode for a 10-fold change in ion activity. As pointed out by Ammann (1986), the use of this equation makes the determination independent of the ionic strength of the sample, an important consideration in the present study because of large differences in ionic strength between the bathing saline and the tubule lumen.

All values are reported as means \pm standard errors. Statistical significance was determined using paired or unpaired Student's t -tests. Differences were considered statistically significant at $P < 0.05$.

Results

PDs in the rectal complex showed a clear bimodal distribution with one peak at -21 mV and a second broad peak at $+40 \text{ mV}$ (Fig. 2). These two peaks correspond to two separate compartments. Based on observed tip location determined by dye injection in 25 preparations, the perinephric space was invariably negative (30 injections) and the tubule lumen was invariably positive ($N=37$ injections). Tubule lumen impalements were easily identified because the dye clearly delineated the meandering zig-zag pattern of the tubule (Fig. 2). The dye could be observed to flow anteriorly during the injection, and with time after injection, gradually appearing in the common duct (cf. Fig. 1 of Ramsay, 1964). When the PD was negative, the pattern of dye-spread varied, presumably with the depth of impalement. Dye sometimes filled a portion of the perinephric space so that the boursoufflures were delineated as clear round areas (Fig. 2). In other negative impalements, the dye readily spread longitudinally so that the colourless tubule could be seen above the dye. These injections corresponded to tip location

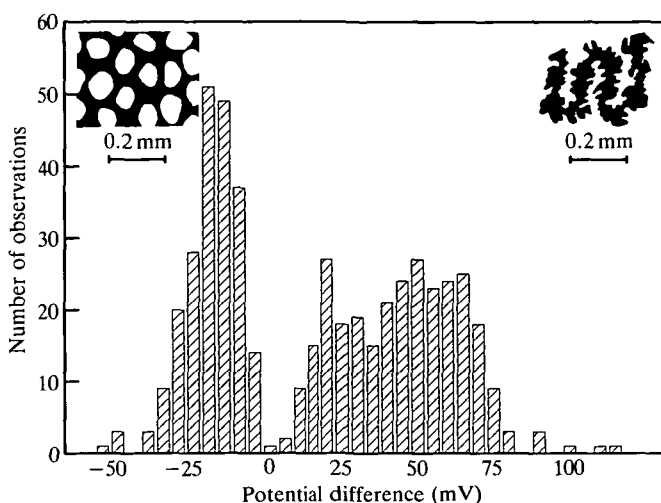


Fig. 2. Frequency distribution of rectal complex potential differences (PDs) measured with theta glass double-barrelled microelectrodes ($N=501$). Examples of patterns produced by dye injection at a negative PD site and a positive PD site are shown in the top left- and right-hand corners, respectively. The patterns were traced from photographs of the rectal complex after dye injection was complete.

in the perirectal or subepithelial space (Figs 1, 2). There was no evidence that the three divisions of the perinephric space were at different PDs. As discussed below, it appears that, as far as ion transport is concerned, the peritubular, perirectal and subepithelial regions of the perinephric space constitute a single compartment.

Once we became confident that the location of double-barrelled impalements could be identified by the polarity of the PD, the second barrel was used for ion activity measurement in place of dye injection. An example of a sequence of simultaneous measurements of a_K and PD is given in Fig. 3A. In addition to the different polarity and magnitude of PDs observed in perinephric *versus* tubular lumen compartments, correspondingly large differences in ion activity were also registered. Paired impalements where the PDs of tubular and perinephric compartments were registered in sequence, without passing through the zero value of bathing fluid, were a common occurrence, and resulted from micromanipulator advancement or retraction of the microelectrode or contraction of the preparation (Fig. 3B).

Although we expected the basolateral membrane potential of a tubule cell to be negative as well, we are confident that no negative PDs corresponded to cellular impalements. The electrode tips of theta glass electrodes used in the dye injection and ion-selective measurements were too coarse to impale single tubule cells exposed within the complex. Finer-tipped electrodes and exposure of the tubule surface, as described in Materials and methods, were required for intracellular impalements. Moreover, injected dye always spread over distances many times that of a single cell diameter. On no occasions were three discrete PDs recorded on

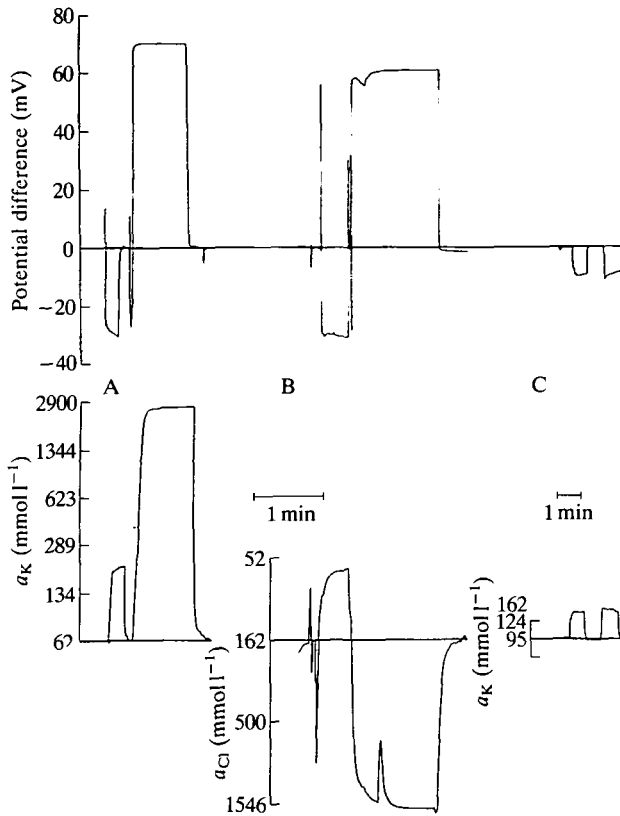


Fig. 3. Chart recordings of rectal complex impalements. (A) PD and potassium activity (a_K) measurements from separate perinephric (negative PD) and tubule lumen (positive PD) impalements. (B) PD and a_{Cl} measurements showing sequential perinephric and tubular impalements. Transient peaks in both recordings result from muscular contractions of the preparation. (C) Basolateral PD and intracellular a_K in an exposed tubule bathed in $120 \text{ mmol l}^{-1} \text{ K}^+$ saline ($a_K = 93 \text{ mmol l}^{-1}$). Two impalements with a piggy-back double-barrelled microelectrode are shown.

withdrawal of the microelectrode, as would be expected if the tip had passed from lumen to cell and then into the perinephric space.

Fig. 4A,B shows plots of individual measurements of perinephric and tubule lumen potassium activity in relation to electrochemical equilibrium. The abscissa of Fig. 4A is the estimated a_K in the rectal complex bathed in Ramsay's saline (mean $a_K^{\text{Bath}} = 42.0 \pm 0.7 \text{ mmol l}^{-1}$, $N=23$) and the ordinate is the electrical potential measured simultaneously by the reference barrel. The solid line represents electrochemical equilibrium with the bathing saline potassium activity. Points above the line have higher a_K values than would be predicted from the electrical potential at that site. For preparations bathed in Ramsay's saline, all tubule lumen potassium activities were well above the Nernst equilibrium value. Similarly, for preparations bathed in concentrated saline (Fig. 4B; mean $a_K^{\text{Bath}} = 60.3 \pm 0.5$

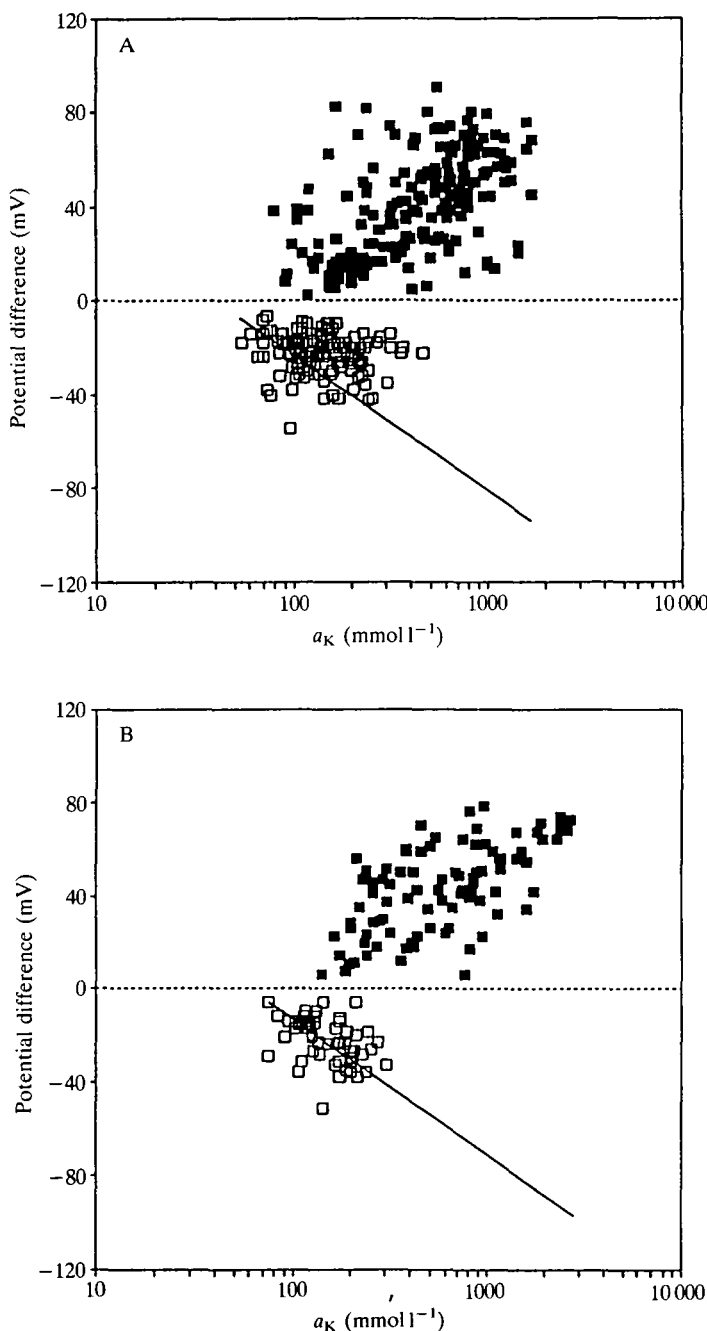


Fig. 4. Relationship between PD and a_K in the rectal complex tubule lumen (■) and perinephric space (□). Preparations were bathed in (A) Ramsay's saline or (B) concentrated saline. The solid lines are those of electrochemical equilibria calculated from the mean of the measured values of K^+ activity in Ramsay's bathing saline ($a_K=42$ mmol l⁻¹) or concentrated bathing saline ($a_K=60.3$ mmol l⁻¹).

mmol l^{-1} , $N=15$), tubule lumen potassium activities were as high as 2730 mmol l^{-1} , and all points were well above the value of 10.8 mmol l^{-1} that would be in equilibrium with $a_{\text{K}}^{\text{Bath}}$ and the mean tubule lumen PD. The average value of 797 mmol l^{-1} exceeded the Nernst equilibrium activity more than 75-fold (Table 1).

Values of a_{K} in the perinephric space were on average slightly above the line for electrochemical equilibrium in Ramsay's saline (Fig. 4A; Table 1) and were more tightly grouped about the line in concentrated saline (Fig. 4A; Table 1). This indicates that the PNS is normally in equilibrium with a saline approximating the ionic conditions in haemolymph obtained from dehydrated animals (Ramsay, 1964), on which the concentrated saline of the present study is based. The PNS values above the equilibrium value may indicate the presence of an impalement shunt which would result in an apparent PD lower than the true value. We suggest that activities on or below the equilibrium line indicate that the PNS is the immediate source for ion transport into the tubular lumen. As this source is drained by active K^+ accumulation in the TL, a_{K} falls below electrochemical equilibrium with the haemolymph, thereby providing a favourable gradient for movement of K^+ from haemolymph to PNS.

The impression given by previous vapour uptake studies is that maximum tubular osmotic pressures (indicated by the threshold for absorption, Coutchie and Machin, 1984) in intermoult mealworms is consistently in the region of $6.8 \text{ osmol kg}^{-1}$. Machin's (1979) frozen section study indicated that rectal complexes fell into two groups, those with high tubular osmotic pressure ($>4.8 \text{ osmol kg}^{-1}$), which absorbed water vapour, and moulting animals with nearly iso-osmotic tubule fluid ($0.0\text{--}0.9 \text{ osmol kg}^{-1}$), which did not. Since Ramsay's (1964) micropuncture study indicated that K^+ made up most of the cations in tubular fluid, we expected to observe tubular K^+ activities falling clearly into the two groups. In a survey of animals that had been in dry cultures for several months, we were surprised to find more continuous variation with very few values exceeding $800 \text{ mmol l}^{-1} \text{ K}^+$. After further experimentation, higher tubular K^+ activities in the region of $1800\text{--}2700 \text{ mmol l}^{-1}$ were obtained with mealworms that had been in dry cultures for only a few weeks. Our highest tubular K^+ activities were recorded in animals from a culture that had been dried over silica gel for about 3 weeks and where concentrated saline was used as the bathing medium (Fig. 4B). Reducing rectal water activity by filling the hindgut with concentrated sucrose, or the use of partially dissected *in situ* preparations instead of completely excised ones, did not result in substantially higher K^+ activities.

One factor contributing to the variability in Fig. 4 is the decline of tubular potassium activities with the duration of the experiment (Fig. 5A), as noted in previous studies of fluid transport (Tupy and Machin, 1985). In contrast, the transepithelial PD appears to be much less sensitive to the duration of the experiment, up to about 4 h (preparations 1–3 in Fig. 5B). Potassium activities and PDs do not decline as extensively in the PNS, presumably because the activities are close to equilibrium with the bathing saline. We suggest below that the decline

Table 1. Summary of ion activities, pH , electrical potentials and Nernst equilibrium activities in the tubule lumen and perinephric space of the mealworm rectal complex

Ion	Tubule lumen			Perinephric space		
	a^{Bath}	PD	a^{ISME}	a^{Eq}	N	a^{Eq}
Preparations bathed in Ramsay's saline; a^{Eq} calculated relative to bath						
K^+	42.0	39±2	525±25	8.9	198	104.3
Preparations bathed in concentrated saline; a^{Eq} , pH^{Eq} calculated relative to bath						
K^+	60.3	44±2	797±66	10.8	94	144.0
Na^+	86.1	52±2	118±8	11.2	47	175.5
Cl^-	156.8	54±3	1037±55	1306.8	38	63.1
H^+ activities expressed as pH :						
Preparations bathed in concentrated saline; pH^{Eq} calculated with respect to bath pH						
H^+	pH^{Bath}	PD	pH^{ME}	pH^{Eq}	N	pH^{Eq}
	7.28	36±3	6.81±0.07	7.91	53	6.94
Preparations bathed in concentrated saline; pH^{Eq} calculated with respect to rectal lumen pH (pH^{RL})						
	pH^{RL}	PD*	pH^{ME}	pH^{Eq}	N	pH^{Eq}
H^+	5.48	51±5	7.20±0.13	6.36	19	5.39

Abbreviations used in the table are as follows: a^{Bath} , ion activity in the bathing saline, mmol l^{-1} ; a^{Eq} , equilibrium activity in mmol l^{-1} , calculated from the Nernst equation using the mean PD and a^{Bath} ; a^{ISME} , ion activity determined by ion-selective microelectrode, mmol l^{-1} , mean \pm s.e.; PD, potential difference in mV relative to bathing saline, mean \pm s.e.; pH^{Bath} , pH of the bathing saline; pH^{Eq} , Nernst equilibrium pH ; pH^{ME} , pH determined by pH -selective microelectrode, mean \pm s.e.; pH^{RL} , pH of the rectal lumen.

* PDs measured with respect to the rectal lumen. The corresponding PDs measured with respect to the bathing saline were 43 ± 3 mV in the tubule lumen and -18 ± 2 mV in the perinephric space.

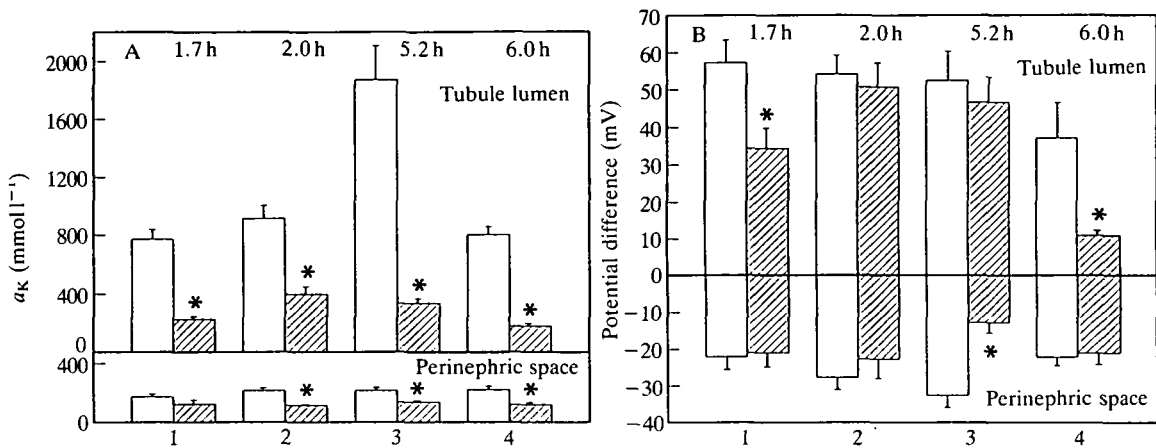


Fig. 5. Histograms showing changes in mean tubule lumen and perinephric (A) a_K and (B) PD with time in isolated rectal complex preparations numbered 1–4. Initial values in each preparation (unshaded columns) were measured no more than 30 min following the start of dissection. Corresponding minimum elapsed times before the start of a second 15 min survey (shaded columns) are indicated. Error bars are standard errors, and the asterisks indicate significant differences ($P < 0.05$).

in potassium activity in the TL is a consequence of osmotic water movement from the rectal lumen to the PNS and TL during dissection of the rectal complex in saline.

Intracellular impalements of rectal complex tubule cells were accomplished by first exposing the tubules through removal of the rectum. An example is shown in Fig. 3C. Basolateral membrane potentials were -52.5 ± 5.9 mV ($N=4$), -27 ± 2.5 mV ($N=22$) and -15 ± 1.9 mV ($N=26$) in bathing salines containing 5.5, 55 and 120 mmol l^{-1} K^+ , respectively. These data indicate that the basolateral membrane potential changed by about 27 mV/decade change in bathing saline potassium concentration over the range 5.5–55 mmol l^{-1} , and by about 33 mV/decade over the range 55–120 mmol l^{-1} .

For measurement of a_K , the basolateral membrane was impaled with a double-barrelled piggy-back K^+ microelectrode in a bathing saline with 120 mmol l^{-1} K^+ ($a_K=93 \text{ mmol l}^{-1}$). Potassium activity in this saline represented a compromise between mean perinephric levels (158 mmol l^{-1}), which would mimic *in vivo* conditions, and the lower activities required to get identifiably negative PDs when the basolateral membrane was impaled. Mean intracellular potassium activity of tubule cells was $143 \pm 10 \text{ mmol l}^{-1}$ ($N=8$) in 55 mmol l^{-1} K^+ saline and $153 \pm 7 \text{ mmol l}^{-1}$ in 120 mmol l^{-1} K^+ saline ($N=25$). Values obtained using single- and double-barrelled electrodes did not differ significantly, and were pooled. Mean values of a_K for preparations bathed in the two salines did not differ significantly, indicating that intracellular potassium activity is maintained within

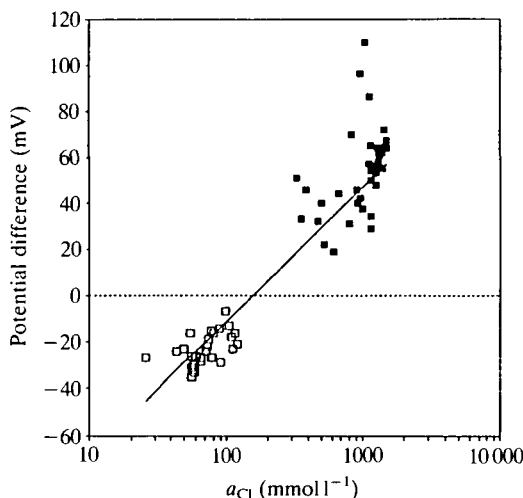


Fig. 6. Relationship between PD and a_{Cl} in the rectal complex tubule lumen (■) and perinephric space (□). The solid line is that of electrochemical equilibrium calculated from the mean of the measured values of Cl^- activity of concentrated saline, $156.8 \text{ mmol l}^{-1}$.

closely defined limits when the potassium activity of the saline bathing the exposed tubules is varied. Potassium activity in the tubule cells was slightly below electrochemical equilibrium with the bathing fluids (i.e. the PNS fluids). The Nernst equilibrium activity at the mean basolateral membrane potential of -15 mV for a cell bathed in saline with a potassium activity of 93 mmol l^{-1} ($120 \text{ mmol l}^{-1} \text{ K}^+$ saline) is 168 mmol l^{-1} .

Fig. 6 is a plot of individual values of a_{Cl} and their relationship to electrochemical equilibrium for preparations bathed in concentrated saline (mean $a_{\text{Cl}}^{\text{Bath}} = 156.8 \pm 4.7 \text{ mmol l}^{-1}$, $N=6$). In this figure, data points *below* the equilibrium line indicate a_{Cl} values that are higher than would be predicted from the corresponding electrical potential. In fact, measured values of a_{Cl} in both PNS and TL were close to the equilibrium line. Average TL chloride activity in concentrated saline was 1037 mmol l^{-1} (Table 1), which is broadly comparable to the sum of mean a_{K} (797 mmol l^{-1}) and mean a_{Na} (118 mmol l^{-1}). However, maximum recorded chloride activities in concentrated saline were 1510 mmol l^{-1} , considerably less than the highest measured a_{K} values. Our explanation for this difference is that relatively few preparations (6) were used for chloride compared with 27 for K^+ , and the probability of finding high salt concentrations was correspondingly reduced. All the animals were from cultures that had been dry for at least 2 months and that also yielded submaximal values of a_{K} . A chloride activity of 1307 mmol l^{-1} would be in equilibrium with the mean TL potential of these preparations, 53.6 mV (Table 1). The mean measured value of a_{Cl} is 21% less than the equilibrium value, and possible explanations for this discrepancy are considered in the Discussion. Nonetheless, the difference is small relative to the enormous differences between measured and equilibrium activities for cations, particular

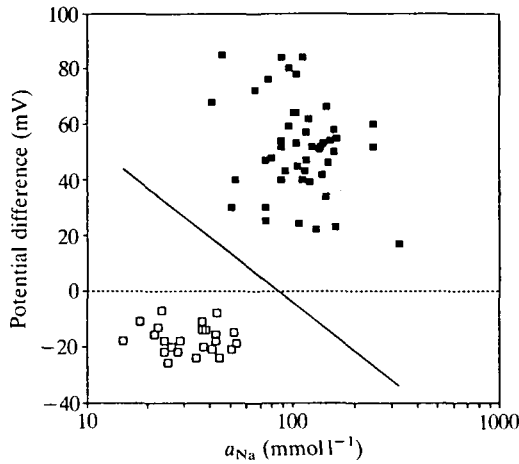


Fig. 7. Relationship between PD and a_{Na} in the rectal complex tubule lumen (■) and perinephric space (□). The solid line is that of electrochemical equilibrium calculated from the mean of the measured values of Na^+ activity of concentrated saline, 86.1 mmol l^{-1} .

K^+ , and the simplest explanation of the data is that TL chloride activities can be accounted for as a passive response to positive electrical potentials in the tubule lumen.

Fig. 7 is a plot of individual values of a_{Na} and their relationship to electrochemical equilibrium for preparations bathed in Ca^{2+} -free concentrated saline (mean $a_{\text{Na}}^{\text{Bath}} = 86.1 \pm 2.9 \text{ mmol l}^{-1}$; $N=6$). A point above the line representing electrochemical equilibrium has a value of a_{Na} that is higher than predicted from the simultaneously measured electrical potential. All measured TL values of a_{Na} were above Nernst equilibrium, whereas all values of a_{Na} in the PNS were below the equilibrium line (Fig. 7). On average, a_{Na} in the TL was more than 10-fold greater than the value in equilibrium with the bathing saline sodium activity and the mean TL electrical potential, whereas a_{Na} in the PNS was more than fivefold smaller than the Nernst equilibrium value (Table 1).

A similar pattern emerged from measurements of a_{H} (Fig. 8; Table 1). All tubule lumen values of a_{H} were above the values predicted from the electrical potential in the tubule lumen, and 37 of 40 values of a_{H} in the PNS were lower than the values that would be predicted from the simultaneously measured electrical potential. The line of electrochemical equilibrium is drawn for the mean a_{H} of concentrated saline, 52.5 nmol l^{-1} ($\text{pH} = 7.28 \pm 0.02$, $N=14$). In terms of pH units, the tubule lumen was acid by 1.1 units, on average, relative to the equilibrium value calculated from the mean TL electrical potential and the bathing saline pH (Table 1). The perinephric space was alkaline to the equilibrium value by 0.5 pH units, on average (Table 1). Figs 7 and 8 indicated that downhill electrochemical gradients favoured passive movement of Na^+ and H^+ from haemolymph

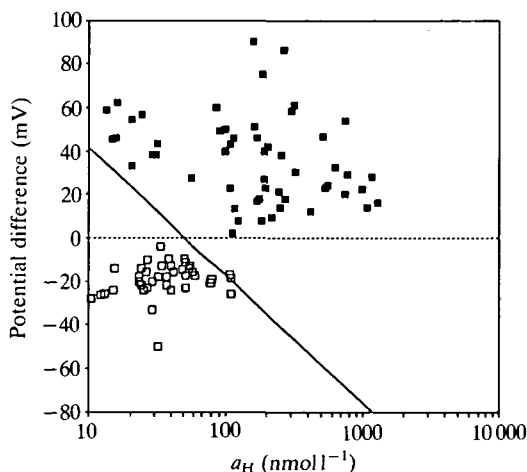


Fig. 8. Relationship between PD and a_{H} in the rectal complex tubule lumen (■) and perinephric space (□). The solid line is that of electrochemical equilibrium calculated from the mean of the measured values of H^+ activity of concentrated saline, 52.5 nmol l^{-1} (pH 7.28).

to PNS, and that these ions were actively accumulated in the tubule lumen against opposing electrochemical gradients.

An alternative possibility, that Na^+ and H^+ in the PNS might have been in equilibrium with the rectal lumen, was investigated by measuring PD and a_{H} in the rectal lumen. It was not possible to measure activities of sodium in the rectal lumen because of possible interference by Ca^{2+} in the rectal contents with the sodium microelectrodes. The rectal lumen pH was quite acid, in fact, with mean a_{H} of $3.31 \times 10^{-6} \text{ mol l}^{-1}$ (pH 5.48 ± 0.22 , $N=7$) and, in some preparations, apparent a_{H} values as high as $2 \times 10^{-5} \text{ mol l}^{-1}$ (pH 4.7) were observed. Mean rectal lumen PD, relative to the bathing saline was $-13 \pm 5.6 \text{ mV}$ ($N=7$). As a result, perinephric a_{H} was even further from electrochemical equilibrium relative to the rectal lumen (Fig. 9; Table 1) than it was relative to the bathing saline (Fig. 8; Table 1). Moreover, because of the acid conditions in the rectal lumen, H^+ in the tubule lumen was also below electrochemical equilibrium with the rectal lumen. If H^+ moved passively from the rectal lumen to the PNS and then to the TL, one would expect a_{H} values in the perinephric space to be intermediate relative to the other two compartments. In fact, values of a_{H} in the PNS are even further from the line of electrochemical equilibrium than tubule lumen values (Fig. 9).

The slopes of the responses of the pH microelectrodes based on tridodecylamine are above Nernstian values in the pH range 4–5.5 (Amman, 1986), and apparent values of rectal lumen pH below 5.5 are, therefore, underestimates. However, even if we assume these rectal pH values were as high as 5.5, perinephric pH was nonetheless nearly 2 units alkaline relative to the rectal lumen.

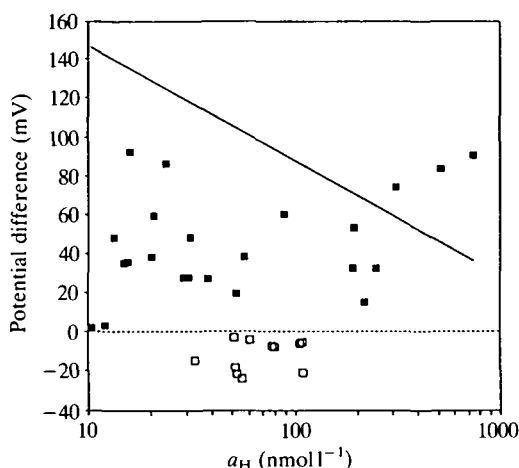


Fig. 9. Relationship between PD and a_H in the rectal complex tubule lumen (■) and perinephric space (□). The solid line is that of electrochemical equilibrium calculated from the mean of the measured values of H^+ activity in the anterior hindgut, $3.31 \mu\text{mol l}^{-1}$ (pH 5.48).

Effects of drugs

Bathing preparations in salines containing 1 mmol l^{-1} dinitrophenol (DNP) had little short-term (<15 min) effect on PDs in the rectal complex, presumably because relatively little DNP crossed the perinephric membrane. However, injection of DNP into the tubule lumen resulted in a dramatic and rapid decline in tubule PD, suggesting that the apical PD is not a diffusion potential. The latter view was supported by evidence that injection of dyes in 500 mmol l^{-1} KCl had little effect on tubular PD, whereas the basolateral membrane potential of the tubule cells was sensitive to changes in bathing saline a_K . The possible contribution of metabolically dependent electrogenic ion pumping to the apical PD is discussed below.

We attempted to reverse the time-dependent decline in tubule PD and ion activity by the addition of the phosphodiesterase inhibitor isobutyl methylxanthine (IBMX) or a non-hydrolysable derivative of cyclic adenosine monophosphate, chlorophenylthio cyclic AMP. Cyclic AMP is known to stimulate ion transport and fluid secretion by free Malpighian tubules (Maddrell *et al.* 1971). Neither drug reversed the time-dependent decline of the preparation.

Discussion

This paper presents simultaneous measurements of electrical potential difference and ion activity in the rectal complex for the first time. Potassium activities have been measured not only in the tubule lumen and perinephric space but also in the rectal tubule cells.

Based on the ease of impalement of the perinephric space, and results of dye injection, we consider the perinephric space to be a functional compartment. Thus, the PNS is probably not an artefact of fixation for electron microscopy, as suggested by Grimstone *et al.* (1968). Observation of the complex after dye injection at negative potential sites indicated good diffusional movements of dye within the complex, and these movements were aided by the muscular contractions of the complex. Because the dye continued to spread in the PNS after injection for as long as 1–2 h, we do not feel that the space was created artificially by the pressure associated with dye injection. Furthermore, the ease and frequency of double-barrelled impalements without applied pressure suggests that the PNS is an important permanent feature of the rectal complex, and our reinterpretation of its extent in Fig. 1 is justified. The unimodal distribution of negative PDs and the limited range of corresponding ion activities suggest that three structural divisions of the perinephric space, the peritubular, perirectal and subepithelial spaces, constitute a single functional physiological compartment.

In a few preparations, a_K in the tubule lumen approached 3 mol l^{-1} and a_{Na} was as high as 400 mmol l^{-1} , sufficient in the presence of an equivalent activity of an accompanying anion to account for much of the $6.8 \text{ osmol kg}^{-1}$ observed in previous studies (Machin, 1979). Our results, therefore, support the suggestion of Ramsay (1964) that tubule lumen osmolality can be explained on the basis of accumulation of inorganic salts, KCl and NaCl. However, the number of preparations with much lower a_K in the tubule lumen was much greater than expected. One component of this variability is the run-down with time of TL potassium ion activity. Since PD in the TL declines much more slowly than a_K and muscular contractions within the complex continue for more than 24 h *in vitro*, we think it unlikely that the run-down indicates a decline in the general health of the preparation. Significantly, a_K and PD decline more slowly in the PNS than in the TL, presumably because activities are closer to equilibrium. Tubular run-down probably does not reflect passive leakage into the surrounding PNS, because PNS ion activity would then increase. The most likely explanation for the run-down is that TL potassium activities are diluted by osmotic water entry. Rectal complex preparations in Ramsay's saline were probably exposed to lower osmotic pressures than those prevailing in the haemolymph. Alternatively, dissection in either Ramsay's or concentrated saline may expose the rectal lumen to fluids of far higher water activity than usual.

Variability of TL potassium activities is also related to culture conditions. There appears to be an optimum length of dehydration in culture to produce maximal TL potassium activities. In water-rich cultures, growth is rapid, with many moulting animals showing little rectal concentration (Machin, 1979). In contrast, in intermoult animals, high rectal osmolalities may be unnecessary or are diluted by moist faecal material. In response to an excessively long period of dehydration, a decline in rectal complex ion activity may be a pathological condition, resulting from reduced food intake. Alternatively, the decline in feeding rates and ion activity may be adaptive. If the gut is empty, the rectal complex need not maintain

gradients for faecal dehydration and, if ambient humidities are well below threshold (88 % RH), the gradients cannot drive water vapour uptake. In these conditions, allowing the ionic gradients within the rectal complex to run down may conserve energy.

In contrast to the active accumulation of cations in the tubule lumen, Cl^- appears to be close to electrochemical equilibrium in both PNS and TL. Mean values of apparent a_{Cl} in the PNS were 11 mmol l^{-1} above the equilibrium activity (Table 1). As discussed by Chao *et al.* (1989) and Hanrahan *et al.* (1984), the Cl^- ion exchanger is known to be sensitive to a number of organic and inorganic anions. The presence of any of these in the PNS would tend to increase the apparent a_{Cl} value, and the true value of a_{Cl} may be closer, therefore, to the equilibrium value. For example, the fluid in the PNS contains glycoproteins (Ramsay, 1964) which serve to couple the high osmolalities generated by ion accumulation in the TL to water in the rectal lumen (Machin, 1979). Any interference with the chloride electrode response caused by this material would result in an overestimate of a_{Cl} in the perinephric space.

In the tubule lumen, the mean value of a_{Cl} is some 21 % below the Nernst equilibrium activity, and a number of individual points deviated substantially from equilibrium. Values below the equilibrium line in Fig. 6 may result from impalement shunts, i.e. incomplete sealing of cell membrane to the microelectrode, resulting in an underestimate of tubule lumen PD. In such cases, the corresponding data point would fall below the line representing electrochemical equilibrium, and a_{Cl} would appear to be greater than the corresponding equilibrium activity. Data points above the equilibrium line may indicate that the chloride conductances of the pathways mediating passive chloride entry into the tubule lumen are imperfectly matched to the electrical driving force represented by the positive lumenal PD, and a_{Cl} is less, therefore, than the equilibrium activity. Higher chloride conductances would permit increased chloride entry, and a_{Cl} in the tubule lumen would then more closely approach the Nernst equilibrium value determined by the TL electrical potential. However, a lower chloride conductance may be necessary to maintain the selectivity of these pathways for chloride over other anions in the haemolymph.

Although previous studies indicated longitudinal osmotic pressure gradients in the rectal complex with maximal values at the posterior, or at least anteroposterior differences, we found no consistent longitudinal differences in ion activity. The primary reason for this discrepancy may be that most of our measurements were made in limited regions of the posterior half of the rectal complex. However, sequential measurements in preparations changing with time, along with sampling of different tubules and local impalement damage, undoubtedly contributed some variability to our data.

Values for rectal tubule intracellular a_{K} , basolateral membrane potential and the contribution of basolateral K^+ to basolateral potential have been measured for the first time. The mean value of a_{K} , 153 mmol l^{-1} , is higher than cytoplasmic potassium activity values measured by ISMEs in insect muscle (65 mmol l^{-1} ;

Djamgoz and Dawson, 1989), *Manduca sexta* midgut (95 mmol l^{-1} ; Moffett *et al.* 1982) and locust rectum (70 mmol l^{-1} ; Hanrahan and Phillips, 1984). In a study of locust Malpighian tubules by Morgan and Mordue (1983) ISME measurements of intracellular potassium are expressed as concentrations (95 mmol l^{-1}), which approximates to an activity of 67 mmol l^{-1} if an activity coefficient of 0.71 is assumed. The latter value was calculated by Dow *et al.* (1984) for *Manduca sexta* midgut cells, and is comparable to values for a variety of vertebrate and invertebrate cells (Lev and Armstrong, 1975). In *Rhodnius prolixus* Malpighian tubules potassium concentrations are 158 mmol kg^{-1} wet mass in the apical cytoplasm and 103 mmol kg^{-1} wet mass in the main cell cytoplasm (Gupta *et al.* 1976). The corresponding activity values, again using 0.71 as an estimate of the activity coefficient, are about 112 and 73 mmol l^{-1} . Similarly, in the rectal papillae of the blowfly *Calliphora erythrocephala*, K^+ concentrations in the apical and main cell cytoplasm are 187 and 167 mmol kg^{-1} wet mass, respectively (Gupta *et al.* 1980). These concentrations correspond to activities of about 133 and 119 mmol l^{-1} , respectively. These comparisons illustrate two points. First, a_{K} in rectal complex tubule cells is 15–60% above the values found in epithelia of other insects. Presumably this is related to the much higher gradient of a_{K} across the apical surface of the cell and also to the high bathing saline a_{K} , 93 mmol l^{-1} , which was chosen to mimic *in vivo* conditions. Second, the a_{K} values in this study are closer to the activity values calculated for the apical cytoplasm in the *Rhodnius* tubule cells (Gupta *et al.* 1976) and *Calliphora erythrocephala* rectal papillae (Gupta *et al.* 1980). This may indicate that the microelectrode tip was commonly positioned near the apical folds of rectal tubule cells, which is not unexpected in view of the extensive folding of the apical surface (Grimstone *et al.* 1968).

Values of intracellular a_{K} and basolateral membrane potential can be used in conjunction with the PDs and activities in the extracellular compartments of the complex to construct profiles for potential difference and potassium activity in the rectal complex (Fig. 10). It can be seen that potassium is near equilibrium across the basolateral membrane. The electrical and chemical gradients for K^+ between the PNS and the tubule cell cytoplasm are small. In contrast, movement of K^+ across the apical membrane from tubule cell to lumen is opposed by a large electrical gradient of 70 mV and by a fivefold increase in activity, on average. This suggests, as for other Malpighian tubules and insect transporting epithelia, that the active transport step resides at the apical membrane. This view is supported by the observation that the transepithelial potential was very sensitive to metabolic inhibition from the apical side, and less so from the basolateral side. Furthermore, the insensitivity of the TL potential difference to varying ion activities during dye injection into the tubule lumen is consistent with a substantial contribution of a metabolic electrogenic pump to the apical potential.

Relative to the bathing saline, activities of H^+ and Na^+ are below electrochemical equilibrium in the PNS and above equilibrium in the TL, indicating active accumulation of these ions as well. Because the neutral carrier used in the Na^+ microelectrodes was also sensitive to Ca^{2+} , the effects of the presence of Ca^{2+}

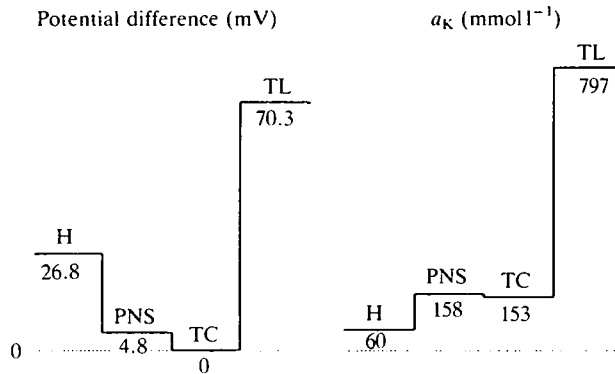


Fig. 10. Profiles of PD and a_K across from haemolymph (H) to tubule lumen (TL). Intervening compartments are perinephric space (PNS) and rectal Malpighian tubule cell (TC).

activities in excess of a few mmol l^{-1} in the PNS and/or TL would make our a_{Na} measurements inaccurate. However, any interference due to the presence of calcium in the PNS would elevate the apparent sodium activity, so true sodium activities in the PNS may, in fact, be even lower. In contrast, sodium activities in the TL would be lower, and therefore closer to equilibrium, if activities of Ca^{2+} exceed about 1 mmol l^{-1} . However, that would require active transport of Ca^{2+} into the tubule lumen, in view of the relatively high haemolymph activities of Ca^{2+} (approx. 1 mmol l^{-1} ; Ramsay, 1964) and the relatively large and positive TL potential differences. We consider it simpler, therefore, to assume that the values of a_{Na} are representative and that Na^+ is actively accumulated in the tubule lumen.

Tubular a_{H} is also far from equilibrium if the rectal lumen is chosen as the reference point. In fact, a_{H} in the PNS is even further from equilibrium with the rectal lumen than with the haemolymph. We think it likely that this result is a consequence of acidification of the gut anterior to the rectum, and is unrelated to H^+ transport into the TL.

Measured ion activities and electrical potentials in the compartments of the rectal complex are of relevance to a discussion of the possible route of ion movements leading to accumulation in the tubule lumen. There are four possible pathways by which ions could gain access to the tubules. Ions might pass through the rectal wall from gut fluid in contact with the anterior surfaces of the rectal lumen (route 1 in Fig. 11). Once in the perirectal space, ions could pass longitudinally along it to be taken up directly by the tubules. From the perirectal space, ions could gain access to outer tubule and boursouffure walls after passing through the membrane separating the perirectal and peritubular spaces. Ions might also enter the tubules directly from the haemolymph, through the specialized blister-leptophragma regions of the perinephric membrane (route 2 in Fig. 11). Alternatively, ions might pass from haemolymph to PNS through the thinner and more permeable anterior perinephric membrane (route 3), or they

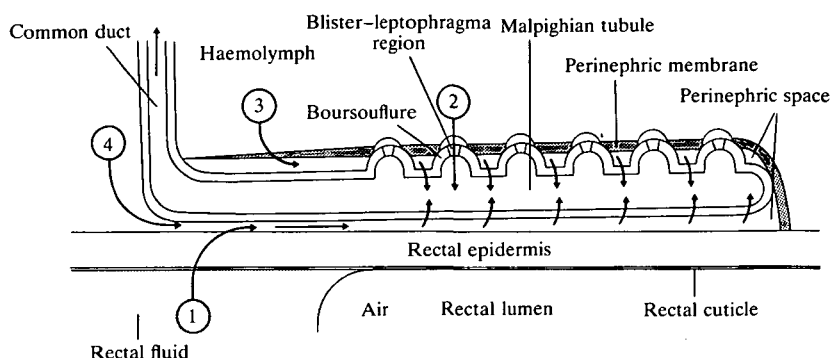


Fig. 11. Schematic longitudinal section of rectal complex showing possible routes of ion entry into the tubule lumen.

might enter the perinephric space if bulk movements of haemolymph occur between the gut and the collar formed at the anterior end of the complex by the tubules and perinephric membrane (route 4 in Fig. 11).

All the cations K^+ , Na^+ and H^+ are concentrated by tubules, yet Na^+ and H^+ are below equilibrium in the perinephric space whereas K^+ is at equilibrium. This suggests that the perinephric space acts as a conduit and as the immediate source of ions for transport in the tubules (route 1 or 3). Removal of ions by transport across the tubule cells and into the tubule lumen would result in ion activities near to, or lower than, the corresponding equilibrium activities. If ions were not transported from PNS to TL, we would expect some ion leakage from the elevated activities in the tubule lumen, resulting in higher than equilibrium cation activities in the PNS. We consider route 2 unlikely, therefore, at least for cation entry. We consider bulk entry of haemolymph (route 4) to be unlikely, since it would tend to maintain all ions in the PNS at equilibrium levels with the haemolymph, at odds with our findings for Na^+ and H^+ . A perinephric route (route 1 or 3) has the attraction of supplying ions to the basal side of the tubule cells whose total area and ultrastructure seem ideally suited for active ion transport.

The gut appears not to be an important source of ions, since filling the rectal lumen with concentrated sucrose neither abolished the fluid transporting properties of rectal complex preparations (Tupy and Machin, 1985) nor significantly altered PNS or TL K^+ activities or electrical potentials. Furthermore, if there were significant ion flow from the gut to the perinephric space, near equilibrium values would be expected. At least for H^+ , this was not the case, as the rectal lumen was 2 pH units acidic to the PNS. We favour route 3, therefore, for entry of cations into the rectal complex.

Chloride may enter the tubule lumen *via* the PNS and tubule cells (route 3), or access may be directly through the blisters and leptophragma cell (route 2). The potential profiles indicate that the main opposing electrical potential for Cl^-

accumulation is across the perinephric membrane, rather than the basolateral membrane of the tubule cells, as in preparations of Malpighian tubules that are free (i.e. not attached to the rectum; O'Donnell and Maddrell, 1984). If Cl^- follows route 2 then, although electroneutrality is preserved by equivalent cation and Cl^- influx into the tubule lumen, the sites of cation and anion entry into the lumen may be separate. Cations may enter *via* route 3, and cation transport across the apical membrane may generate the positive potential driving Cl^- across the blisters and into the lumen.

Alternatively, if Cl^- also follows route 3, then the blisters and leptophragmata may play indirect roles in rectal complex functioning, perhaps mediating entry of metabolites or excretory products. The results of a comparative study (Saini, 1964) indicated that the leptophragmata may not be essential for faecal dehydration because two important families of dry-feeding beetles (Ptinidae and Anobiidae) lack these structures. Saini (1964) suggested that the leptophragmata serve a structural role by attaching the tubules to the perinephric membrane.

Our suggestion for ion entry across the anterior part of the complex (route 3) requires that water movements should not be coupled to ion movements, or the perinephric space would tend to be iso-osmotic with the haemolymph. A precedent for this separation of ion and water movements is found in another insect epithelium, the proximal Malpighian tubule of *Rhodnius prolixus*. KCl reabsorption from tubule lumen to haemolymph occurs in the one-third of the proximal tubule closest to the junction of the tubule with the midgut (Maddrell, 1978). Water is not reabsorbed because it is in this same region where osmotic permeability is greatly reduced, more than eightfold relative to the value in the more distal regions of the tubule where KCl reabsorption does not occur (O'Donnell *et al.* 1982).

We have no specific information on hormonal or neural control of rectal complex ion transport. We attempted to elevate the concentration of cyclic AMP, a possible second messenger in Malpighian tubule ion transport, by application of the phosphodiesterase inhibitor IBMX or direct application of chlorophenylthio cyclic AMP. It is important to point out that sufficient concentrations of these drugs may not have reached the tubules across the perinephric membrane, or they may have been bound or inactivated within the PNS.

Evidence for active transport of H^+ into the lumen is of interest in view of the recent proposal of Wiczorek *et al.* (1989) for proton pumping by *Manduca sexta* midgut cells. Active ion transport by insect epithelia involves an ATPase, which probably resides in the repeating subunits found on the cytoplasmic surface of the apical plasma membranes of insect transporting epithelia, such as the rectal papillae of the blowfly *C. erythrocephala* (Gupta and Berridge, 1966). These subunits have been termed 'portasomes' by Harvey *et al.* (1983). Wiczorek *et al.* (1989) have suggested that electrogenic cation transport by insect epithelia involves a vacuolar-type proton-ATPase that is coupled to an electroneutral K^+/H^+ exchanger. K^+/H^+ exchange has been suggested earlier as the basis for acidification of the rectal contents in the *C. erythrocephala* (Berridge and Gupta,

1968). Our data would be consistent with the operation of a proton pump combined with K^+/H^+ and even Na^+/H^+ exchange.

Active accumulation of Na^+ in the tubule lumen has implications for other tenebrionids, especially those such as *Onymacris plana*, in which the threshold of vapour absorption is below the equilibrium humidity over saturated KCl (85 %) but above the value over a mixed solution of NaCl and KCl. Control over transport may be exerted either at the level of the entry step in the tubule cell or in mechanisms responsible for transport from cell to lumen. Sodium plays an important role in osmotic water uptake by the rectal pads of cockroaches *Periplaneta americana* (Wall and Oschman, 1970) and the rectal papillae of the *C. erythrocephala* (Gupta *et al.* 1980). Moreover, the ratio of Na^+ to K^+ in the extracellular fluids in the rectal papillae reflects the contents of the rectal lumen. With KCl in the lumen, the extracellular fluid contains 3.2 times more K^+ than Na^+ , but with NaCl in the rectal lumen the extracellular fluid contains equimolar amounts of Na^+ and K^+ (Gupta *et al.* 1980). In the case of tenebrionids, the haemolymph levels of Na^+ and K^+ and/or the relative conductance of the perinephric membrane to Na^+ versus K^+ may be the factor limiting the net transport of each species.

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References

- AMMANN, D. (1986). *Ion-Selective Microelectrodes. Principles, Design, and Applications*. Berlin: Springer-Verlag.
- ARMSTRONG, W. M. AND GARCIA-DIAZ, J. F. (1980). Ion-selective microelectrodes: theory and technique. *Fedn Proc. Fedn Am. Soc. exp. Biol.* **39**, 2851–2859.
- BERRIDGE, M. J. AND GUPTA, B. L. (1967). Fine-structural changes in relation to ion and water transport in the rectal papillae of the blowfly, *Calliphora*. *J. Cell Sci.* **2**, 89–112.
- BERRIDGE, M. J. AND GUPTA, B. L. (1968). Fine-structural localization of adenosine triphosphatase in the rectum of *Calliphora*. *J. Cell Sci.* **3**, 17–32.
- CHAO, A. C., KOCH, A. R. AND MOFFETT, D. F. (1989). Active chloride transport in isolated posterior midgut of tobacco hornworm (*Manduca sexta*). *Am. J. Physiol.* **257**, R752–R761.
- COUTCHIE, P. A. AND MACHIN, J. (1984). The allometry of water vapor absorption in two species of tenebrionid beetle larvae. *Am. J. Physiol.* **246**, R230–R236.
- DJAMGOZ, M. B. A. AND DAWSON, J. (1989). Ion-sensitive micro-electrode measurements of intracellular K^+ , Na^+ and Cl^- activities in lepidopteran skeletal muscle. *J. Insect Physiol.* **35**, 165–173.
- DOW, J. A. T., GUPTA, B. L., HALL, T. A. AND HARVEY, W. R. (1984). X-ray microanalysis of elements in frozen-hydrated sections of an electrogenic K^+ transport system: The posterior midgut of tobacco hornworm (*Manduca sexta*) *in vivo* and *in vitro*. *J. Membr. Biol.* **77**, 223–241.
- GILLETTE, R., GILLETTE, M. U. AND DAVIS, W. J. (1982). Substrates of command ability in a buccal neuron of *Pleurobranchia*. II. Potential role of cyclic AMP. *J. comp. Physiol.* **146**, 461–470.
- GRIMSTONE, A. V., MULLINGER, A. M. AND RAMSAY, J. A. (1968). Further studies on the rectal complex of the mealworm, *Tenebrio molitor* L. (Coleoptera, Tenebrionidae). *Phil. Trans. R. Soc. Ser. B* **253**, 343–382.
- GUPTA, B. L. AND BERRIDGE, M. J. (1966). A coat of repeating subunits on the cytoplasmic

- surface of the plasma membrane in the rectal papillae of the blowfly, *Calliphora erythrocephala* (Meig.) studied *in situ* by electron microscopy. *J. Cell Biol.* **29**, 376–382.
- GUPTA, B. L., HALL, T. A., MADDRELL, S. H. P. AND MORETON, R. B. (1976). Distribution of ions in a fluid-transporting epithelium determined by electron-probe X-ray microanalysis. *Nature* **264**, 284–287.
- GUPTA, B. L., WALL, B. J., OSCHMAN, J. L. AND HALL, T. A. (1980). Direct microprobe evidence of local concentration gradients and recycling of electrolytes during fluid absorption in the rectal papillae of *Calliphora*. *J. exp. Biol.* **88**, 21–47.
- HANRAHAN, J. H., MEREDITH, J., PHILLIPS, J. E. AND BRANDYS, D. (1984). Methods for the study of transport and control in the insect hindgut. In *Measurement of Ion Transport and Metabolic Rate in Insects*. (ed. T. J. Bradley and T. A. Miller), pp. 19–67. Berlin: Springer-Verlag.
- HANRAHAN, J. H. AND PHILLIPS, J. E. (1984). KCl transport across an insect epithelium: Electrochemical potentials and electrophysiology. *J. Membr. Biol.* **80**, 27–47.
- HARVEY, W. R., CIOFFI, M., DOW, J. A. T. AND WOLFERSBERGER, M. G. (1983). Potassium ion transport ATPase in insect epithelia. *J. exp. Biol.* **106**, 91–117.
- LEE, C. O. (1981). Ionic activities in cardiac muscle cells and application of ion-selective microelectrodes. *Am. J. Physiol.* **241**, H459–H478.
- LEV, A. A. AND ARMSTRONG, W. McD. (1975). Ionic activities in cells. *Curr. Top. Membr. Transport* **6**, 59–123.
- MACHIN, J. (1976). Passive exchanges during water vapour absorption in mealworms (*Tenebrio molitor*): a new approach to studying the phenomenon. *J. exp. Biol.* **65**, 603–615.
- MACHIN, J. (1979). Compartmental osmotic pressures in the rectal complex of *Tenebrio* larvae: evidence for a single tubular pumping site. *J. exp. Biol.* **82**, 123–137.
- MADDRELL, S. H. P. (1978). Physiological discontinuity in an epithelium with an apparently uniform structure. *J. exp. Biol.* **75**, 133–145.
- MADDRELL, S. H. P., PILCHER, D. E. M. AND GARDINER, B. O. C. (1971). Pharmacology of the Malpighian tubules of *Rhodnius* and *Carausius*: The structure–activity relationship of tryptamine analogues and the role of cyclic AMP. *J. exp. Biol.* **54**, 779–804.
- MOFFETT, D. F., HUDSON, R. L., MOFFETT, S. B. AND RIDGWAY, R. L. (1982). Intracellular K⁺ activities and cell membrane potentials in a K⁺-transporting epithelium, the midgut of tobacco hornworm *Manduca sexta*. *J. Membr. Biol.* **70**, 59–68.
- MOFFETT, D. F. AND KOCH, A. R. (1988). Electrophysiology of K⁺ transport by midgut epithelium of lepidopteran insect larvae. I. The transbasal electrochemical gradient. *J. exp. Biol.* **135**, 25–38.
- MORGAN, P. J. AND MORDUE, W. (1983). Electrochemical gradients across *Locusta* Malpighian tubules. *J. comp. Physiol.* **151**, 175–183.
- 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. B* **156**, 407–413.
- O'DONNELL, M. J., ALDIS, G. K. AND MADDRELL, S. H. P. (1982). Measurements of osmotic permeability in the Malpighian tubules of an insect, *Rhodnius prolixus* Stal. *Proc. R. Soc. Ser. B* **216**, 267–277.
- O'DONNELL, M. J. AND MADDRELL, S. H. P. (1984). Secretion by the Malpighian tubules of *Rhodnius prolixus* Stal: electrical events. *J. exp. Biol.* **110**, 275–290.
- OSCHMAN, J. L. AND WALL, B. J. (1969). The structure of the rectal pads of *Periplaneta americana* L. with regard to fluid transport. *J. Morph.* **127**, 475–509.
- RAMSAY, J. A. (1964). The rectal complex of the mealworm *Tenebrio molitor* L. (Coleoptera, Tenebrionidae). *Phil. Trans. R. Soc. Ser. B* **248**, 279–314.
- ROBINSON, R. A. AND STOKES, R. H. (1970). *Electrolyte Solutions*. (2nd edition, revised). London: Butterworths.
- SAINI, R. S. (1964). Histology and physiology of the cryptonephridial system of insects. *Trans. R. ent. Soc., Lond.* **116**, 347–392.
- STEINER, R. A., OEHME, M., AMMAN, D. AND SIMON, W. (1979). Neutral carrier sodium ion-selective microelectrode for intracellular studies. *Analyt. Chem.* **51**, 351–353.
- THOMAS, R. C. (1978). *Ion-Sensitive Intracellular Microelectrodes. How to Make and Use Them*. New York: Academic Press.

- TRIPATHI, S., MORGUNOV, N. AND BOULPAEP, E. L. (1985). Submicron tip breakage and silanization control improve ion-selective microelectrodes. *Am. J. Physiol.* **249**, C514–C521.
- TUPY, J. H. AND MACHIN, J. (1985). Transport characteristics of the isolated rectal complex of the mealworm *Tenebrio molitor*. *Can. J. Zool.* **63**, 1897–1903.
- WALL, B. J. AND OSCHMAN, J. L. (1970). Water and solute uptake by rectal pads of *Periplaneta americana*. *Am. J. Physiol.* **218**, 1208–1215.
- WALL, B. J. AND OSCHMAN, J. L. (1975). Structure and function of the rectum in insects. *Fortschr. Zool.* **23**, 193–222.
- WHITFIELD, M. (1979). Activity coefficients in natural waters. In *Activity Coefficients in Electrolyte Solutions*, vol. 2 (ed. R. M. Pytkowicz), pp. 153–300. Boca Raton: CRC Press.
- WIECZOREK, H., WEERTH, S., SCHINDLBECK, M. AND KLEIN, U. (1989). A vacuolar-type proton pump in a vesicle fraction enriched with potassium transporting plasma membranes from tobacco hornworm midgut. *J. biol. Chem.* **264**, 11 143–11 148.