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RECTAL COMPLEX ION ACTIVITIES AND ELECTROCHEMICAL GRADIENTS IN LARVAE OF THE DESERT BEETLE, *ONYMACRIS*: COMPARISONS WITH *TENEBRIO*

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Abstract—Double-barrelled, ion-selective microelectrodes were used to record simultaneous ion activities and potential differences in the tubule lumen and perinephric space of the rectal complex of larvae of the desert beetles *Onymacris plana* and *O. marginipennis*. Maximal tubule lumen activities of K^+, Na^+ and Cl^- were 3348, 114 and 3100 mM, respectively. Calculated net electrochemical potentials are consistent with passive movement of Na^+, K^+ and H^+ from the haemolymph to the perinephric space, and active accumulation of Na^+, K^+ and H^+ in the tubule lumen. Chloride activities are in equilibrium with the electrical potential in the tubule lumen and perinephric space.

Unlike *Tenebrio*, predicted maximal osmolalities in *Onymacris* tubules, calculated from the threshold for water vapour absorption, exceed KCl solubility. Although NaCl is more soluble and is readily available to *Onymacris* larvae, tubule lumen Na^+ was 9.9 times less abundant, on average, than K^+. The ratio of tubule lumen Na^+ to K^+ activities was lower in *Onymacris* than in *Tenebrio*.

We suggest that lower absorption thresholds and higher KCl activities in *Onymacris* tubules depend on KCl supersaturation. Morphological differences between the rectal complexes of the two genera are consistent with the superior ion-concentrating ability of *Onymacris*.

Key Word Index: *Onymacris*; *Tenebrio*; tenebrionid larvae; rectal complex; epithelia; ion transport; electrochemical gradients; KCl supersaturation

INTRODUCTION

The tenebrionid rectal complex resorbs water against large thermodynamic gradients during faecal dehydration (Ramsay, 1964) or water vapour absorption (Machin, 1979). The complex consists of the distal ends of the Malpighian tubules applied to the rectum and enveloped in a perinephric membrane (Saini, 1964). Analysis of fluids collected by micropuncture from the lumen of the Malpighian tubules in the complex of larval mealworms, *Tenebrio molitor*, indicated high concentrations of KCl (Ramsay, 1964). Recently, ion activities and electrochemical gradients in cellular and extracellular compartments of the mealworm rectal complex have been studied using ion-selective microelectrodes (O'Donnell and Machin, 1991). Potassium chloride activities exceeding 3 M were measured in the lumen of the perirectal tubules. Moreover, sodium and hydrogen ions are also actively accumulated in the tubule lumen, although the contribution of these ions to the osmotic "sink" created around the rectum to reabsorb water (Machin, 1979) is comparatively minor. Tubular K^+ activities in some preparations approached the high values necessary to drive osmotic reabsorption of water during faecal dehydration and vapour absorption (O'Donnell and Machin, 1991).

Analysis of *Onymacris*, another tenebrionid larva with a rectal complex (Coutchié and Machin, 1984), is of interest because the ionic basis of tubular concentration might well be different from *Tenebrio*. Water vapour is absorbed at relative humidities as low as 88.8% in *Tenebrio* (Coutchié and Machin, 1984) as compared with 81% in *Onymacris plana* (Coutchié and Crowe, 1979a) and 82% in *O. marginipennis*. The latter humidities are lower, in fact, than the humidity over a saturated solution of KCl in the same temperature range [84–85% (Winston and Bates, 1960)]. Crystal formation in saturated solutions might damage cell membranes of the apical brush border or occlude the spaces between the apical microvilli, impeding further transport.

Haemolymph electrolyte composition in the two genera differs slightly. At comparable hydration levels, *Tenebrio* haemolymph contains much higher levels of K^+ with correspondingly reduced Na^+ (Na/K is 1.39 and 1.08 in hydrated and dehydrated larvae, respectively (Ramsay, 1964) whereas in

Onymacris Na^+ is elevated at the expense of K^+. Na/K was 6.73 or 6.91 depending on hydration (Coutchié and Crowe, 1979b). We suggest earlier that *Onymacris*, in adapting to conditions in the Namib Desert of Southwestern Africa, may have evolved a rectal complex in which sodium makes a larger contribution to the osmolality of fluids in the lumen of the perirectal tubules (O'Donnell and Machin, 1988). The Namib Desert is subject to oceanic fogs (Seely, 1979) which might transport NaCl in aerosol form. Enhanced tubular transport of Na^+ in *Onymacris* might be expected, therefore, as a means of exploiting the greater abundance of environmental Na^+ and much higher haemolymph Na^+ levels. Importantly, NaCl is more soluble than KCl, and the relative humidity over a saturated solution is 76% (Winston and Bates, 1960), well below the minima for vapour absorption by *O. plana* and *O. marginipennis*.

This paper exploits recently developed ion-selective microelectrode techniques to study ion transport in the rectal complex of *Onymacris* and extends earlier morphometric and physiological comparisons with *Tenebrio*.

MATERIALS AND METHODS

Experimental animals

O. plana and *O. marginipennis* larvae were collected from the Desert Ecological Research Unit, Gobabeb, Namibia and placed individually in vials provisioned with food. They were shipped by air to Toronto along with a large sample of Namib Desert dune sand. On arrival, larvae were removed from the vials and transferred in groups of six or less to plastic 1 l tubs with perforated lids. The tubs has been prepared by half filling then with moistened desert sand which was then covered with fresh pieces of lettuce and a layer of dry sand. Larvae were recovered for experimentation or for replenishing of water and food by gentle sieving with a fibreglass screen.

In vitro preparations of the rectal complex

Dissection techniques for the rectal complex of *Onymacris* were identical to those used for *Tenebrio* (O'Donnell and Machin, 1991). Preparations were bathed in the saline described by Nicolson and Hanrahan (1986) during dissection, and were superfused with the same saline during electrophysiological recording. The composition of the saline (in mM) was: NaCl (110), KCl (25), $MgCl_2$ (5), $CaCl_2$ (2), $NaHCO_3$ (10), NaH_2Po_4 (5), glucose (100), glycine (10), proline (10), serine (10), histidine (10), glutamine (10). Saline pH was 7.

Electrophysiology

Electrical potentials and ion activities in the tubule lumen and perinephric space of the *Onymacris* rectal complex were measured by impalement with double-barrelled, ion-selective microelectrodes fabricated from thick-septum theta glass capillaries. Electrodes pulled from this type of glass effectively penetrate the perinephric membrane surrounding the complex, and the microelectrode tip can be positioned either in the perinephric space or the tubule lumen (O'Donnell and Machin, 1991). Potentials were measured with respect to a ground electrode placed in the bathing solution. Details of construction and calibration of the microelectrodes, impalement techniques and a method for identification of microelectrode tip location by dye injection, have been described in our earlier paper (O'Donnell and Machin, 1991).

Elemental analysis of Namib Desert sand

Interstitial electrolytes in our sample of Namib Desert sand (not previously used for animal culture) were analysed in an aqueous extract, prepared by shaking approx. 20 g sand with 6 ml glass-distilled water for 2 h. The water was filtered off and two aliquoits of 0.5 and 1.0 ml were analysed at the University of Toronto, SLOWPOKE neutron activation reactor facility. The samples were irradiated at 20 kW for 10 min and the resulting gamma activity counted for 10 min on a Canberra Industries 8180 Multichannel Analyser (Meridian, CT).

Measurement of saturation point in experimental solutions

Relative humidities in equilibrium with solutions of individual salts, up to and including the point of saturation, have been tabulated by, or can be derived from information in, Frazer *et al.* (1926), Wolf *et al.* (1982/83), and Winston and Bates (1960). However, there is little published information on the solubilities of mixtures of salts, particularly in the ratios found in the perirectal Malpighian tubules. Our interest in possible anomalous vapour pressure lowering by solutions of mixed electrolytes was stimulated by the earlier report (Winston and Bates, 1960) that the equilibrium humidity over a mixture of saturated NaCl and KCl was lower than the humidity over saturated NaCl alone.

Saturation point in our experiments was measured by visual detection of salt crystals in individual drops (50–150 nl) which had been placed by micropipette into an experimental chamber. Temperature and humidity within the chamber were controlled and the drops could be viewed through a microscope (Machin, 1979). Humidity was controlled and modified by changing the temperature of an external circulating water bath containing a condenser which regulated the vapour pressure of air entering the viewing chamber (Machin, 1976, 1984).

Chamber humidity was slowly lowered in progressively smaller steps, giving sufficient time between changes for the sample drop to reach a stable volume in equilibrium with the surrounding humidity. Equilibration of the samples was indicated by unchanging drop volume, calculated from drop height (a) and base diameter (b) measured with an

eyepiece micrometer in the microscope (Beament, 1958):

$$\text{drop volume} = \pi/6(a^2 + 3b^2).$$

Equilibration required 2–6 h, depending on original volume and the magnitude of the humidity change. The relative humidity in the chamber at the point of crystallization was estimated as the average of the two humidities immediately before and after salt crystals appeared.

Chamber humidities were calibrated by determining the equilibrium volume of standard NaCl drops whose initial volume and concentration were known. Values for vapour pressure lowering by NaCl solutions were calculated from depression of freezing point values obtained from tables (Wolf *et al.*, 1982/83). Initial concentrations of the drops were relatively high (about 4 M) so that the drops were still sufficiently large to permit accurate measurement of their dimensions as they became more concentrated in lower humidities.

Experimental solutions were made from analytical grade NaCl and KCl, either as pure solutions or mixed in ratios, including those found in *Tenebrio* rectal complex tubules. In some experiments salt solutions contained 1% bovine serum albumen, 20 mM trehalose or 20 mM glycerol to examine possible effects on supersaturation point. Fluid collected from the common duct of transporting *in vitro* rectal complex preparations of *Tenebrio* (Tupy and Machin, 1985) was also studied. The common duct of the *Onymacris* rectal complex was too short to permit similar collection of fluid. The time-dependence of KCl crystallization was also investigated. Samples were subjected to long-term exposure (7 days) to constant relative humidities between the equilibrium humidity over a saturated KCl solution (Winston and Bates, 1960) and the lower humidity at which crystals were observed after short-term exposure (2–16 h).

Morphometric analysis of the tenebrionid rectal complex

We were interested in relating rectal complex morphometry to solute concentrating ability in the two genera. Allometric equations were used to calculate transporting surface area and volumes of the tubules and boursouflures (i.e. tubule diverticulae) in the rectal complexes of *Tenebrio* and *Onymacris* larvae of similar mass. As a representative mass for comparison of *Onymacris* and *Tenebrio* we chose 100 mg, the most common size employed for physiological studies in this paper as well as in measurements by Coutchié and Machin (1984) and O'Donnell and Machin (1991). We made use of previously reported (Coutchié and Machin, 1984) and new allometric equations (Table 1). The new equations relating boursouflure diameter to live mass were derived from measurements made on freshly dissected complexes mounted in a depression slide in saline, using a calibrated eyepiece micrometer. *Tenebrio* larvae were available over a wide size range (10.9–125.9 mg). The size range of *Onymacris* available for morphometric analyses was more limited (150.3–445.3 mg). Mean boursouflure diameters were calculated from at least 10 measurements on each complex. Allometric equations were determined from the log-transformed data for mass or mean diameter.

RESULTS

Analysis of interstitial elements in Namib sand

Neutron activation analysis of sand washing indicated that NaCl was the primary electrolyte. Results from two aliquots were essentially the same and were pooled. The amount of Na^+ per kg sand exceeded the amount of K^+ by a factor of 82 (Table 2).

Table 1. Constants for allometric equations ($y = kx^{exp}$) used in morphometric comparison of larval *Tenebrio* (T) and *Onymacris* (O) rectal complexes

					Constant exponent		
Independent variable (x)	Dependent variable (y)	Genus	n	r^2	(k)	(exp)	$y, x = 100$ mg
	Data from Coutchié and Machin (1984)						
Head capsule width (mm)	Live mass (mg)*	T	65	0.900	7.980	2.958	—
		O	134	0.940	4.753	3.415	—
Live mass (mg)	Complex length (mm)	T	86	0.895	1.042	0.289	3.943
	(l_c)	O	23	0.874	1.795	0.248	5.624
Live mass (mg)	Radial diffusion distance (mm)	T	37	0.500	0.041	0.176	0.091
	(d_r)	O	11	0.770	0.035	0.211	0.092
Live mass (mg)	Rectal cuticle circumference (mm)	T	85	0.880	0.480	0.257	1.568
	(c_c)	O	21	0.939	0.368	0.342	1.778
	Unpublished data of Coutchié and Machin						
Live mass (mg)	Mean complex radius (mm)	T	48	0.940	0.070	0.256	0.228
		O	71	0.897	0.083	0.251	0.264
	Data Collected for this Study						
Live mass (mg)	Boursouflure diameter (mm)	T	6	0.249	0.052	0.108	0.086
		O	6	0.058	0.048	0.033	0.056

*Equation provided to facilitate use with alternative size reference, head capsule width.

Table 2. Soluble inorganic constituents of Namib desert sand detected by neutron activation

Element	mol. kg sand^{-1} × 10^5
Na	38.11
Cl	21.91
Ca	8.27
Al	1.47
K	0.47
Mn	0.01

Ion activities and electrical potentials in the rectal complex of Onymacris

Potential differences and activities of Na^+, K^+, H^+ and Cl^- in the rectal complex were proportionally similar to those of *T. molitor* (O'Donnell and Machin, 1991), but significant quantitative differences were apparent. Correlation of potential differences with the location and direction of spread of dyes injected through the same microelectrode indicated that the tubule lumen was invariably positive and the perinephric space invariably negative. When positive potentials were recorded ($n = 12$ injections), the injected dye clearly delineated the zig-zag pattern of the tubule, and gradually flowed anteriorly, eventually appearing in the common duct. When the potential difference was negative (nine injections), a more diffuse, roughly circular dye pattern in which the boursouflures were outlined as clear round dye-free areas indicated location of the microelectrode tip in the perinephric space, as found in earlier experiments with *Tenebrio* (O'Donnell and Machin, 1991). Ion activities and potential differences in the tubule lumen and perinephric space of the two species of *Onymacris* were not significantly different and the results were pooled. Mean potential differences (±SE) in the tubule lumen and perinephric space were 29.1 ± 1.2 mV ($n = 146$) and -29.6 ± 1.1 ($n = 165$) mV, respectively.

Figure 1 shows the relationship of perinephric and tubule lumen potassium activity to electrochemical equilibrium for *in vitro* complexes bathed in control saline. The abscissa is the potassium activity (a_K) recorded by the potassium-selective barrel of the double-barreled ion-sensitive microelectrode and the ordinate is the electrical potential measured simultaneously by the reference barrel. The solid line was calculated using the Nernst equation and represents electrical equilibrium with the bathing saline potassium activity, 15.2 mM. Points above the line have higher a_K values than would be predicted from the electrical potential at that site. All tubule lumen potassium activities were well above the value predicted from the Nernst equation. The maximal value was 3348 mM, and the mean tubule lumen value was 453 ± 64 mM ($n = 67$). Many of the values of a_K in the perinephric space were slightly above the line for electrochemical equilibrium, and the mean value was 102 ± 8 mM ($n = 68$). Potassium activity in the perinephric space of *T. molitor* was closer to equilibrium when bathed in a saline with a potassium activity of 60 mM than when bathed in a saline with a potassium activity of 42 (O'Donnell and Machin, 1991). Figure 1 suggests that the haemolymph potassium activity for many of the animals may have been slightly above the saline a_K of 15.2 mM.

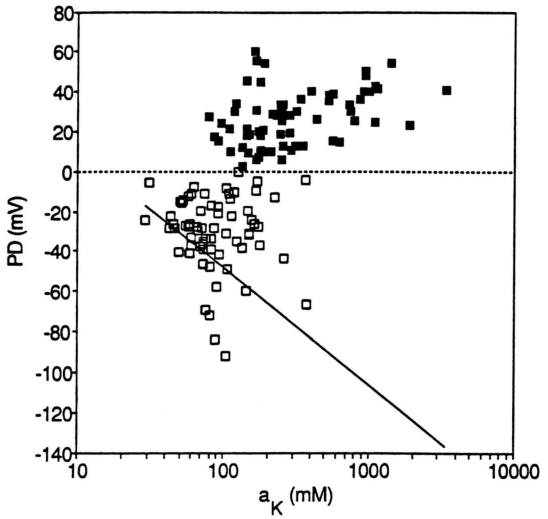

Fig. 1. Relationship between potassium activity and potential difference in the perirectal tubule lumen (■) and perinephric space (□). The solid line represents the electrochemical equilibrium calculated from the mean of measured potassium activities in the bathing saline, 15.2 ± 0.7 mM ($n = 15$).

Figure 2 is a plot of individual values of sodium activity and their relationship to electrochemical equilibrium for preparations bathed in Ca_2^+-free saline. The Na^+ sensor used is sensitive to interference by extracellular levels of Ca_2^+ (Steiner *et al.*, 1979), resulting in erroneously high apparent Na^+

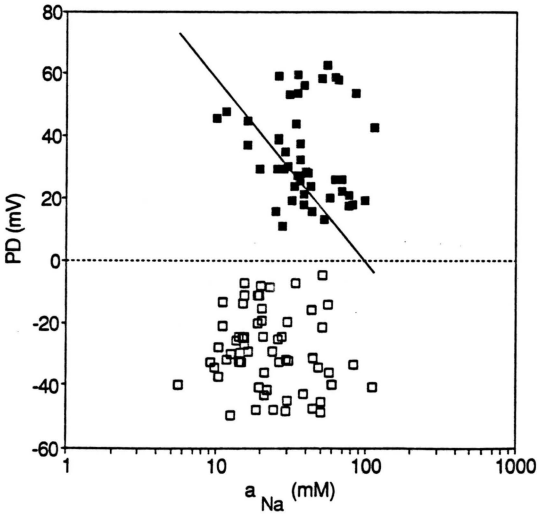

Fig. 2. Relationship between sodium activity and potential difference in the perirectal tubule lumen (■) and perinephric space (□). The solid line represents the electrochemical equilibrium calculated from the mean of measured sodium activities in the bathing saline, 99.2 ± 4.1 mM ($n = 12$).

activities on salines containing Ca_2^+. A point above the line representing electrochemical equilibrium has a value of a_{Na} that is higher than would be predicted from the simultaneously measured electrical potential. Measured values of a_{Na} in the tubule lumen were near or slightly above the equilibrium values. Maximal and mean values of a_{Na} in the tubulen lumen were 114 and 44 ± 3.4 mM ($n = 46$), respectively, indicating that sodium is much less concentrated than potassium in the tubule lumen. The mean a_{Na} in the perinephric space was 27 ± 2 mM ($n = 61$). Sodium activities were much lower than the values predicted from the simultaneously measured electrical potential. Similar results were found in *Tenebrio* (O'Donnell and Machin, 1991).

All but three of a_H in the perinephric space were also below those predicted from the simultaneously measured electrical potentials, and all but one of the tubule lumen values of H^+ activity exceeded those in equilibrium with the positive tubule lumen potential (Fig. 3).Mean proton activities in the tubule lumen and perinephric space were 97 ± 27 nM ($n = 9$) and 173 ± 50 nM ($n = 16$), respectively.

Measured values of a_{Cl} in both the perinephric space and tubule lumen were close to equilibrium (Fig. 4), as was found previously for *Tenebrio*. In this figure, data points below the equilibrium line indicated that a_{Cl} values are higher than would be predicted from the simultaneously measured electrical potential. Maximal and mean activities of Cl^- in the tubule lumen were 3100 and 555 ± 20 mM ($n = 20$), respectively. These values are similar to the corresponding activities for potassium, suggesting that Cl^- was the predominant anion, and that other anions were unlikely to be present in high contrast, mean a_{Cl} in the perinephric space was 63 ± 10 mM ($n = 20$), much less than the sum of a_K and a_{Na} in the perinephric space. This result suggests the presence of significant quantities of other anions in the perinephric space, such as the protein described in the space fluids of *Tenebrio* (Ramsay 1964).

Calculated net electrochemical potential ($\Delta\mu$) provides a useful means of quantifying the chemical and electrical driving forces on an ion. Values of $\Delta\mu$ are usually divided by F, the Faraday constant, so that the resultant quantity, $\Delta\mu/F$, expresses the net driving force in volts. A positive value indicates active transport of ions into a compartment, a zero value indicates that an ion is in passive equilibrium with the fluid bathing the compartment, and a negative value indicates that ions are actively transported out of the compartment.

Figure 5 summarizes net electrochemical potentials for K^+, Na^+, H^+ and Cl^- in the rectal complexes of *Onymacris*, and provides corresponding values for *T. molitor*, calculated from activity and potential difference measurements reported in an earlier study (O'Donnell and Machin, 1991). Chloride values are close to equilibrium in both tubule lumen and perinephric space in *Onymacris* and in *Tenebrio*. In both genera, potassium is actively accumulated in the tubule lumen. However, comparisons of net electrochemical potentials indicate important quantitative differences between *Onymacris* and *Tenebrio* with respect to sodium and hydrogen ion transport by the rectal complex. These cations are accumulated to a lesser degree in the tubule lumen in the *Onymacris* rectal complex as compared with *Tenebrio*. In addition, the gradient favouring movement of sodium from haemolymph into the perinephric space is greater in the rectal complex of *Onymacris* than in that of *Tenebrio*.

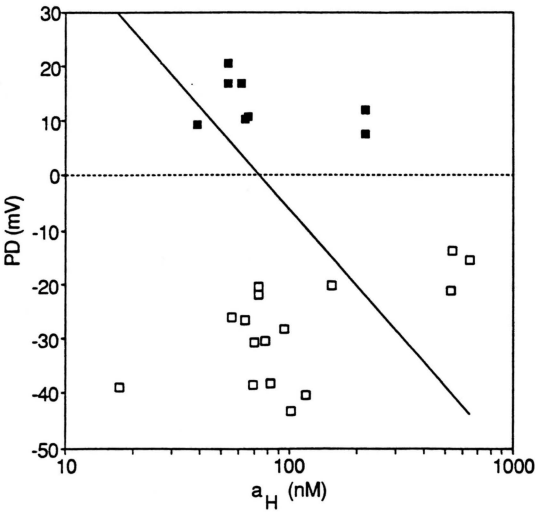

Fig. 3. Relationship between hydrogen ion activity and potential difference in the perirectal tubule lumen (■) and perinephric space (□). The solid line represents the electrochemical equilibrium calculated from the pH of the bathing saline converted to H^+ ion activity, 82 nM.

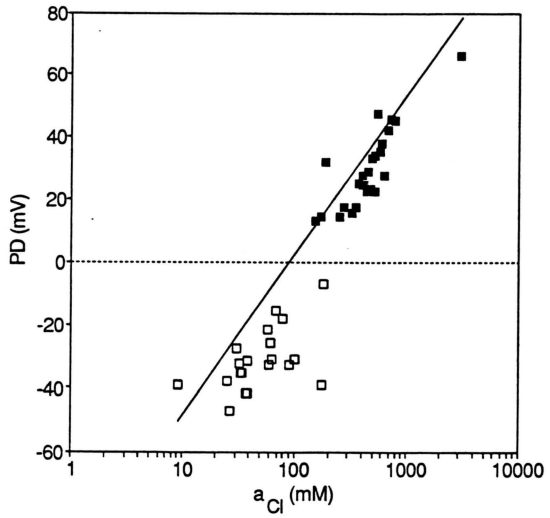

Fig. 4. Relationship between chloride activity and potential difference in the perirectal tubule lumen (■) and perinephric space (□). The solid line represents the electrochemical equilibrium calculated from the mean of measured chloride activities in the bathing saline, 137.2 ± 4.3 mM ($n = 6$).

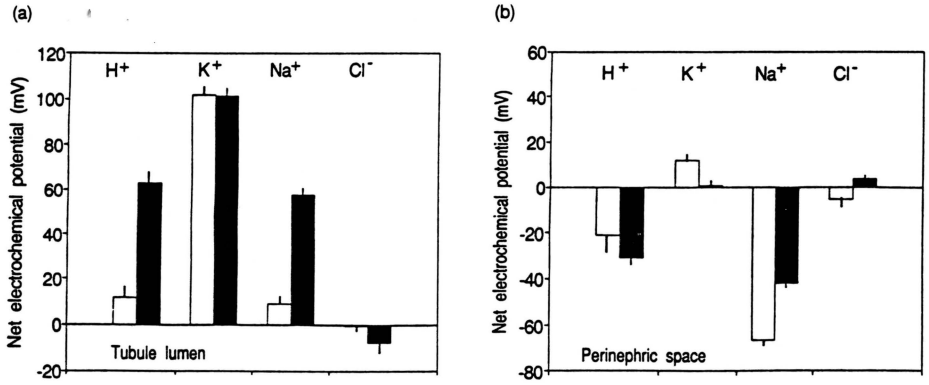

Fig. 5. Mean net electrochemical potentials, calculated with respect to the bathing saline, in (a) the perirectal tubule lumen, and (b) the perinephric space of *Onymacris* (open bars) and *Tenebrio* (solid bars); data taken from O'Donnell and Machin (1991)]. Error bars indicate 1 SE. $N = 8$–68 for *Onymacris* and 15–94 for *Tenebrio*.

Solubility of NaCl and KCl solutions

In all test solutions both NaCl and KCl were readily supersaturated. Table 3 shows that the relative humidities at which crystallization took place were all considerably less than the values quoted for KCl and NaCl in Winston and Bates (1960). Variability in our data was due to several factors. Some variability resulted from averaging of relative humidities bracketing the observed crystallization point. It is important to point out that KCl drops eventually crystallized at higher humidities (below the equilibrium a_w above saturated KCl) if given enough time, for example, 6 days at 82.9% r.h. This finding supports the view that the probability of crystallization increases as humidity is reduced further below the equilibrium humidity. Nonetheless, some of the variability was independent of these factors. For example, identical drops placed side by side in the chamber sometimes crystallized at different humidities. Table 3 shows that mean humidities at which crystallization occurred were significantly lower for solutions of NaCl or equimolar solutions of NaCl and KCl ($P < 0.001$). Mean crystallization points of solutions with K/Na ratios mimicking those of tenebrionid tubule fluid were not significantly different from the values for KCl alone ($P > 0.04$).

In equimolar solutions of NaCl and KCl, some crystals first appeared inside the drop at humidities where crystals formed in samples of pure KCl, and these initial crystals were presumably of KCl. The humidity at which all fluid was gone and only crystals remained in these samples was not significantly different from the humidity at which crystals appeared in solutions of NaCl alone. After the initial crystallization in drops where KCl was the predominant solute, it is presumed that the small quantity of water necessary to maintain NaCl in solution was invisible among the large mass of KCl crystals.

There was no evidence that fluid produced by the *Tenebrio* rectal complex contained any organic molecules which promoted supersaturation ($P > 0.1$). Furthermore, we found that neither protein (bovine serum albumen), trehalose nor glycerol, frequent molecular species employed in freezing-point depression or lowering of the supercooling point in cold-hardy insects (Zachariassen and Hammel, 1988), exerted any effects on crystallization points ($P > 0.4$, $P > 0.8$, $P > 0.4$, respectively).

Morphometry of the rectal complex: Onymacris vs Tenebrio

There are notable differences in the anatomy of the rectal complexes of *Tenebrio* and the two species of *Onymacris*. In both species of *Onymacris* the complex reveals a more extensive tracheal supply, with a large trunk entering the complex at the point where the

Table 3. Summary of crystallization points based on 2–16 h exposure

Sample	*n*	Crystallization points mean water activity ± SE
KCl	6	0.796 ± 0.011
KCl + 1% bovine albumen serum	8	0.758 ± 0.011
KCl + 20 mmol trehalose	6	0.786 ± 0.006
KCl + 20 mmol glycerol	8	0.798 ± 0.005
KCl:NaCl (1:0.073)	6	0.810 ± 0.012
NaCl	5	0.684 ± 0.006
KCl:NaCl (1:1)	8	0.694 ± 0.003
Internal crystallization	8	0.807 ± 0.007
Tenebrio fluid from common duct	3	0.760 ± 0.023

Sample column includes molar ratios given for salt mixtures.

perirectal tubules combine to form a short common duct. This trunk breaks into six prominent longitudinal trachaea which run between the tubules, each with perpendicular branches which pass between the lateral rows of boursouflures. Alternate branches supply each of the boursouflures on either side. By comparison, tracheal branches enter the *Tenebrio* rectal complex at irregular intervals along its length. The boursouflures of *Onymacris* are smaller and therefore more densely packed than in *Tenebrio*. In addition, the boursouflures occupy the entire surface of the complex in *Onymacris*, whereas they are found only in the posterior two-thirds of the complex of *Tenebrio*. One μm, expoxide sections of

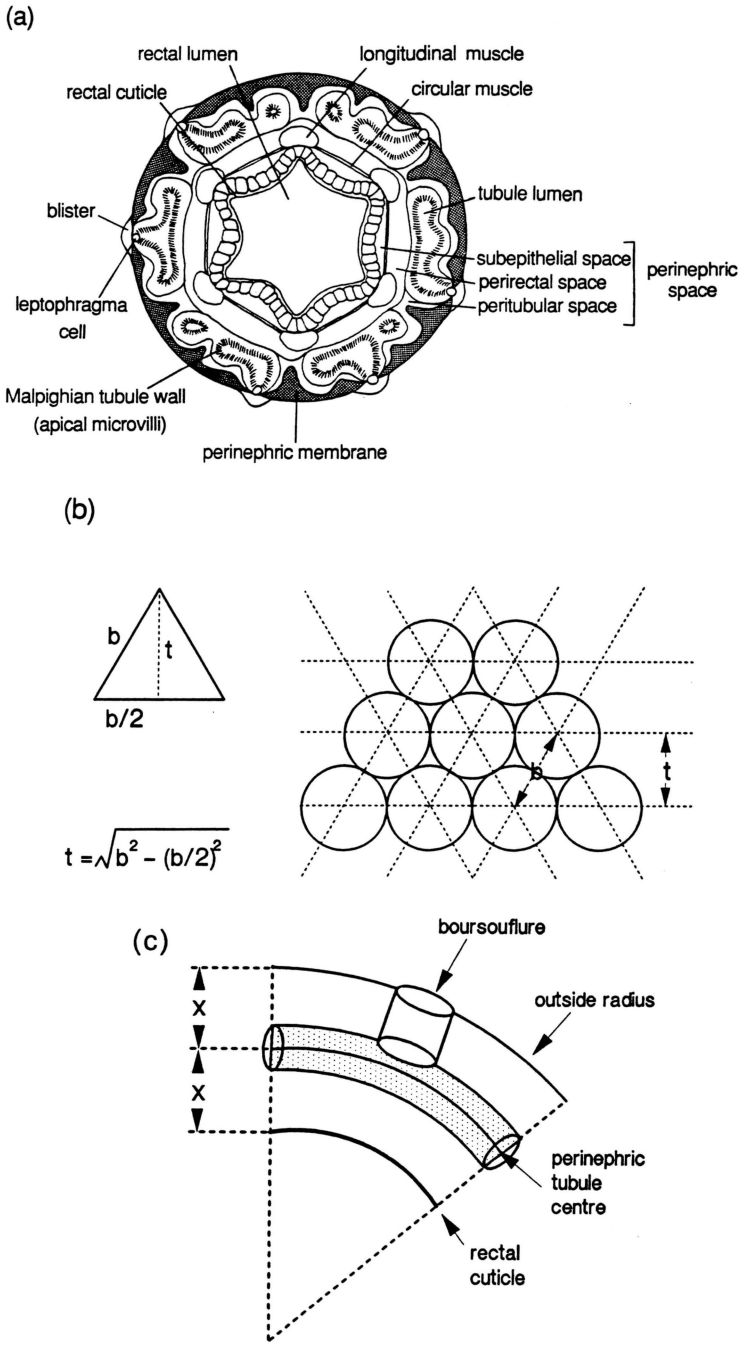

Fig. 6. Diagram illustrating various structural features of the rectal complex of tenebrionid beetle larvae. (a) Schematic transverse section of the rectal complex showing the distribution of perinephrine spaces (after O'Donnell and Machin, 1991). (b) Schematic diagram illustrating the hexagonal packing of bousouflures as seen on the rectal complex surface, showing calculation of perirectal tubule diameter (t) from bousouflure diameter (b). (c) Schematic diagram illustrating calculation of the radius at the perinephrine tubule centre and bousouflure height from reference points based on mean complex radius and rectal cuticle circumference.

Onymacris rectal complex, fixed in glutaraldehyde and counterstained with toluidine blue, revealed clear leptophragma cells under high-power observation (×40 to ×100). The leptophragmata are similar to those observed in *Tenebrio* (Grimstone *et al.*, 1968) although the prominent overlying "blisters" are absent.

Rectal complexes of the two genera were compared by estimating various morphometric parameters for 100 mg animals (Table 1). For estimates of tubule (plus boursourflure) area and volumes we first calculated the distance between the centre of the rectal lumen and the centre of the perirectal tubules (Fig. 6). This dimension could not be measured without sectioning, and was calculated by adding the radial diffusion distance (d_r; the distance from the centre of the perirectal tubules to the rectal cuticle) to the rectal lumen radius (calculated from rectal cuticle circumference, c_c). Both distances were calculated for 100 mg larvae using allometric equations in Table 1). Rectal complex tubules and boursouflures were treated as simple cylinders for calculation of their surface areas and volumes. The product of the circumference at the level of the perirectal tubules (Fig. 6) and the length of the rectal complex containing boursouflures (i.e. 2/3 × total complex length in *Tenebrio*) is the functional area for ion transport by the perirectal tubules. Total tubule length, i.e. the straightened length of all six tubules, was calculated by dividing functional area by the corresponding tubule diameter. Details of tubule diameter calculation are given in Fig. 6. Boursouflures form a closely packed hexagonal array over the surface of the rectal complex. Tubule diameter was assumed to be the perpendicular distance separating the centres of the boursouflures, calculated from the equations in Table 1. The radial height of each boursouflure was calculated by first subtracting the radius of the complex to the centre of the perirectal tubules from mean complex radius (Table 1), and then subtracting the radius of the perirectal tubule (Fig. 6).

Table 4 shows in essence that the rectal complex is proportionately larger in *Onymacris* than in *Tenebrio*. In conjunction with the more extensive distribution of boursouflures, this results in functional area for ion transport 2.4 times larger than in *Tenebrio*. Boursouflure diameters are smaller in *Onymacris* (Table 1) and cover all of the complex, not just 2/3 of its length, so they are 6.3 times more numerous than in *Tenebrio*. Tubule and boursouflure surface area in *Onymacris* is 3.5 times that in *Tenebrio* while the volume difference is 2.3-fold. Thus, in *Onymacris* there is 75.7 mm^2 of surface area per mm^3 of rectal complex volume, whereas in *Tenebrio* the corresponding ratio is 50.7 mm^2/mm^3. The ratio of the latter two values is 1.5, consistent with production of more concentrated fluids by the *Onymacris* complex.

DISCUSSION

The results show that in spite of the greater availability of NaCl in the environment and the haemolymph of *Onymacris* as compared to *Tenebrio*, NaCl does not account for a higher proportion of total osmolality of fluid in the perirectal tubules in *Onymacris*. The lower threshold for water vapour absorption in *Onymacris* seems more likely to depend upon supersaturation of KCl than to any effects of NaCl. In addition, morphometric analyses suggest that the rectal complex should be capable of producing more concentrated fluids in *Onymacris* than in *Tenebrio*.

Rectal complex electrophysiology: comparisions of Onymacris and Tenebrio

Tubule lumen electrical potentials were positive with respect to the haemolymph and perinephric space, though somewhat less possitive than in *Tenebrio*. In contrast, the perinephric space in *Onymacris* was more negative than in *Tenebrio*. As for *Tenebrio*, the ease and frequency with which the perinephric space could be impaled with theta-glass microelectrodes, and the extent and speed of spread of injected dye indicates that it is a functional compartment, as originally suggested by Ramsay (1964) and Koefeod (1975), and not an artefact of fixation for electron microscopy, as suggested by Grimstone *et al.* (1968) and Noble-Nesbitt (1990).

Our electrical measurements have shown as in *Tenebrio*, that rectal complex tubules in *Onymacris* actively accumulate K^+, Na^+ and H^+ while Cl^- enters primarily passively. Furthermore in the perinephric space surrounding the tubules, Na^+ and H^+ activities are depressed below electrochemical equilibria, while K^+ and Cl^- are much closer to equilibrium. These observations are consistent with the perinephric space acting as the source of ions for uptake across the basolateral membranes and accumulation in the tubule lumen, as with *Tenebrio*.

Table 4. Comparison of morphometric analyses of the rectal complexes of 100 mg *Onymacris* and *Tenebrio* larvae

Parameter	*Onymacris*	*Tenebrio*	*Onymacris*/*Tenebrio*
Functional area (mm^2)	12.6	5.3	2.4
Number of boursouflures	4225	673	6.3
Tubular* area (mm^2)	84.4	24.1	3.5
Tubular* volume (mm^3)	1.1	0.48	2.3
Area/volume (mm^2/mm^3)	75.7	50.7	1.5

*Includes both perirectal tubules and boursouflures.

Maximum activities of K^+ and Cl^- were about 3 M, sufficient to generate an osmolality of about 6 osmol kg^{-1}. Sodium activities were not high enough to raise this value more than about 11%. The absorption threshold is about 81–82% r.h., equivalent to 12–13 osmol kg^{-1}. Our measurements probably reflect considerable dilution of tubule lumen ion activities by osmotic water entry from saline which fills the rectal lumen in the *in vitro* preparation. This effect has been discussed in our earlier paper.

Sodium/potassium ratios in the tubule lumen were calculated from mean values of a_K and a_{Na} given in this paper and O'Donnell and Machin (1991). The ratios were 0.101 and 0.148 in *Onymacris* and *Tenebrio*, respectively. The Na/K ratio for fluid secreted by adult tubules is 0.118 (Nicolson and Hanrahan, 1986). In spite of the much greater availability of environmental and haemolymph sodium, the proportion of tubule fluid osmolality attributable to Na^+ in both larval and adult *Onymacris* is even less, surprisingly, than in *Tenebrio*.

Comparisons of rectal complex morphometry in Onymacris and Tenebrio

The significance of the morphometric differences described in the results is apparent in a comparison of rectal complex transport rates by the two genera. Rates of vapour absorption from humid air (96.3% r.h.) calculated for 25°C using allometric equations in Coutchié and Machin (1984) are 0.82 and 0.46 μl h^{-1} in 100 mg larvae of *Onymacris* and *Tenebrio*, respectively. By using the vapour absorption threshold to estimate the osmotic pressure of the fluid in the perirectal tubules, we estimate that the tubules would have to transport 7.91×10^{-6} and 3.03×10^{-6} osmol h^{-1} to sustain vapour uptake in *Onymacris* and *Tenebrio*, respectively. However, relative to tubule area, these values amount to 0.94×10^{-5} and 1.26×10^{-5} osmol h^{-1} cm^{-2} in *Onymacris* and *Tenebrio*, respectively. The superior concentrating power of *Onymacris* tubules may be related to their smaller volume relative to their surface area.

Significance of supersaturation for water vapour absorption

Supersaturation is similar in some respects to supercooling, where freezing can be delayed by the lack of nucleation (Franks, 1985). As for freezing, we found that the probability of crystallization increased in our supersaturated solutions with time. Differences between our measurements of humidity above saturated solutions and those of Winston and Bates (1960) are probably attributable to the intentional addition of excess undissolved salt to their solutions. Under these conditions crystal nucleating agents are always present. We found no evidence that agents such as trehalose and glycerol, familiar cryoprotectants in many insects (Somme, 1982), or proteins, which may serve either to promote or restrict ice nucleation (Franks, 1985) affected supersaturation. Nor was there any evidence that *Tenebrio* perirectal fluid crystallized in a different manner than solutions of pure salts.

The frequency distribution of threshold humidities for vapour absorption by different species of arthropods shows a large peak just below 84–85% r.h., corresponding to saturated KCl, and a smaller peak between 65 and 70% r.h. (O'Donnell and Machin, 1988). The discovery that KCl and NaCl supersaturation could account for these lower thresholds (Table 3) suggests that vapour absorption mechanisms based on these salts may be more widespread than previously thought.

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