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COMPARTMENTAL OSMOTIC PRESSURES IN THE RECTAL COMPLEX OF *TENEBRIO* LARVAE: EVIDENCE FOR A SINGLE TUBULAR PUMPING SITE

BY J. MACHIN

Department of Zoology, University of Toronto, Toronto, Ontario M5S 1A1, *Canada*

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SUMMARY

The distribution of osmotic pressures derived from melting points of frozen sections of mealworm rectal complex is described. Animals rapidly frozen during atmospheric absorption show radially increasing values from lumen to Malpighian tubules suggesting that water uptake is driven by a single pump located in the area of highest solute concentration. Volume–concentration relationships of isolated rectal cuticle and perirectal fluid are consistent with their transmitting water passively to an area of lower solvent activity.

The rectum of animals frozen with some delay after atmospheric absorption show high osmotic pressures with no radial gradients, a condition which is thought to exist when the rectum is in its faecal dehydration mode. Rectal osmotic pressures in all absorbing animals increased towards the posterior end. Several, presumably extreme posterior tubular, osmotic pressures agree well with the 6·7 osmol.kg^{-1} value expected for an 88% R.H. threshold for atmospheric absorption.

INTRODUCTION

The first comprehensive account of the distribution of osmotic pressure within the rectal complex of *Tenebrio* larvae was given by Ramsay in 1964. Freezing-point depression of compartmental fluids collected by micropuncture from Malpighian tubule and perirectal spaces showed that they were hyperosmotic to the haemolymph, with values increasing posteriorly. Ramsay advanced a model for faecal dehydration involving two energy-requiring processes, the first apparently located in the rectal epithelium because luminal water activity, equivalent to 90% R.H., appeared lower than that of the other rectal compartments. In the second phase of water transport, tubular concentration of electrolytes was thought to create the necessary osmotic gradients to convey water from the perirectal fluid to the tubules. More precise localization of micropuncture sampling (Grimstone, Mullinger & Ramsay, 1968) established higher osmotic pressures for posterior perirectal and tubular fluids than before, with the differences clearly favouring osmotic flow towards the tubules. Maximum tubular values were close to 10·3 °C, the freezing-point depression expected for reducing the faecal water content to 90% R.H. at ambient temperatures of

20 °C. Although rectal epidermal cells were found to lack many of the ultrastructural features associated with active water and electrolyte transport in the recta of other insects, an energy requiring step in the epithelium was still considered likely. Subsequently Maddrell (1971) accepted the double pump model, extending it without major change to incorporate Noble-Nesbitt's (1970) discovery that water-vapour absorption also took place in the rectum. This broadening of the model unfortunately introduces new problems because the well established threshold of 88% at 20 °C for atmospheric absorption would be consistent with a maximum tubular freezing point depression of 12·4 °C. In his determination of luminal humidities Ramsay (1964) used an inaccurate source for the equilibrium humidity of saturated KCl which is 85, not 87·5% R.H. (Winston & Bates, 1960). A reworking of the data brings conditions in the rectal lumen during faecal dehydration more into line with the 88% R.H. threshold for atmospheric absorption.

A technique (Machin, 1976), involving the continuous monitoring of weight changes in mealworms subjected to abrupt changes in humidities from which they are able to absorb water vapour, has recently identified a passively exchanging superficial fluid compartment apparently within the rectal complex. Water-vapour exchange with such a compartment temporarily disturbs uptake and is apparently located between the uptake mechanism itself and the source of water in the atmosphere. Further progress in the understanding of how the complex functions must therefore depend on better information on the transfer of water from compartment to compartment.

The present study is based on the analysis of melting of frozen sections of rectal tissues, used earlier by Machin (1974). While this technique yields less precise osmotic pressures than does micropuncture, it has certain advantages in studying large standing gradients. Osmotic pressures in adjacent compartments can be more reliably compared, and in addition intracellular values for epithelial layers can be obtained. In its use of experimental controls, the work exploits the fact that a proportion of a mealworm population is unable to absorb from the atmosphere apparently because they are involved in moulting (Machin, 1975).

MATERIALS AND METHODS

Mealworms were kept in dry meal cultures (19–23 °C and 20–50% R.H.) without added vegetables or fruit. Animals were separated for experimentation according to their ability to absorb water from the atmosphere on the basis of whether or not they gained weight during overnight exposure to 98% R.H. The combination of low environmental humidity and the lack of liquid water in the diet ensured they were all in a state of water deficit.

Analysis of frozen sections

The posterior abdomen containing the rectal complex was rapidly frozen by bisecting the animal and allowing the rear section to fall into isopentane cooled to its freezing point of − 140 °C by liquid nitrogen. The frozen tissue was then transferred to a Harris Cryostat (Harris Manufacturing, Cambridge, Mass.) at −40 °C and mounted on a brass specimen holder with a drop of water and then with Ames O.C.

compound (Division Miles Laboratories, Inc., Elkhart, Indiana, U.S.A.), which adhered more readily to the cuticle. In the cryostat chamber, frozen transverse sections, generally 10 μm thick, were cut and mounted in cold kerosene under glass and viewed with a specially lubricated Richert microscope fitted with a Mettler temperature controlled stage (temperature resolution 0·1 °C ≡ 54 m-osmol) (Machin, 1974). The eyepiece with automatic photographic attachment, its electrical controls and those of the stage were at room temperature outside the cryostat chamber. Sections were photographed at 1 or 2° intervals, usually from −16 to 0 °C. Marked differences were found in the cutting characteristics of the two experimental groups. Sections of absorbing animals were extremely fragile in contrast to non-absorbing individuals. The difficulty of obtaining intact sections suitable for analysis, particularly from the posterior zone of the rectal complex was therefore greatly increased.

Early analyses of sections from absorbing animals, although clearly showing longitudinal gradients, rarely indicated radial differences in melting point between the various layers of the complex. In these experiments freezing and weighing the animals involved a delay of up to 30 min from the first moment of removing the mealworms from the humidity chamber. Longer delays between uptake and freezing would be expected if the animals stopped absorbing during the night. To test the possibility that changes took place in the rectum during the delay before freezing, the weights of individual mealworms were continuously recorded in 98% R.H. (using a Mettler ME 22 electronic microbalance) until they established a steady weight gain. They were then quickly removed and frozen within 20 s of the last recorded water vapour uptake.

The volume–concentration characteristics of rectal cuticle and perirectal fluid were also studied in the isolated state.

Isolated rectal cuticle

The water content of freshly isolated rectal cuticle at different humidities was measured by weight using a special humidity controlling device which fitted into the weighing chamber of a Mettler ME 22 electronic microbalance (Fig. 1). The device consisted of a water-jacketed, rotatable aluminium block containing five humidity chambers maintained by different saturated salt solutions. Spring loading of this block permitted it to be depressed, rotated, then raised so that the sample hanging on the balance could be rapidly exposed to each humidity in turn. Preliminary testing using evaporation of a water drop showed that the humidity gradient within the chambers was almost, but not completely, eliminated by filter-paper wicks. Corrections for the remaining differences between equilibrium humidity over a given solution (Winston & Bates, 1960) and the actual humidity in each chamber were based on comparisons with a standard line drawn through two fixed points: saturated air where evaporation is zero and ambient humidity where there are no gradients in the chambers (Fig. 2). Preliminary experiments also established that it was impractical to maintain one chamber with dry air, as the working life of such a small amount of desiccant was too short. The dry weight of the samples was therefore obtained by extrapolation. The initial wet weight of the cuticle was converted for use in further analyses to volume using the density value for chitin of 1·4 given in Richards (1951).

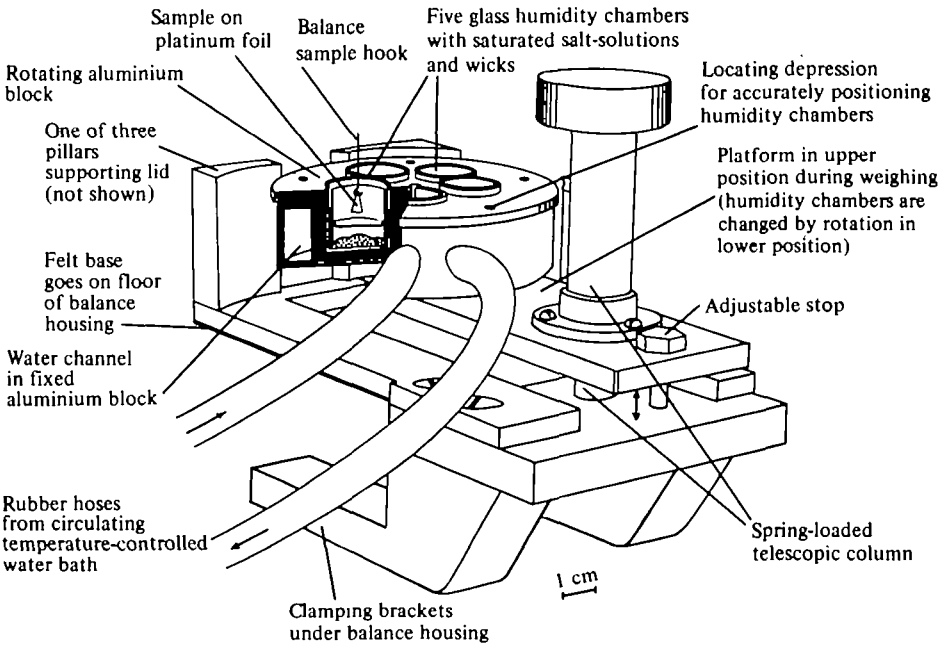

Fig. 1. Three-dimensional diagram of humidity controlling apparatus used with a Mettler ME22 electronic microbalance. The lid and attached locating pin, used to accurately position the chambers and seal them when not in use, has been removed. The temperature-controlled portion of the apparatus has been cut away (shaded area) to show details of one humidity chamber in use.

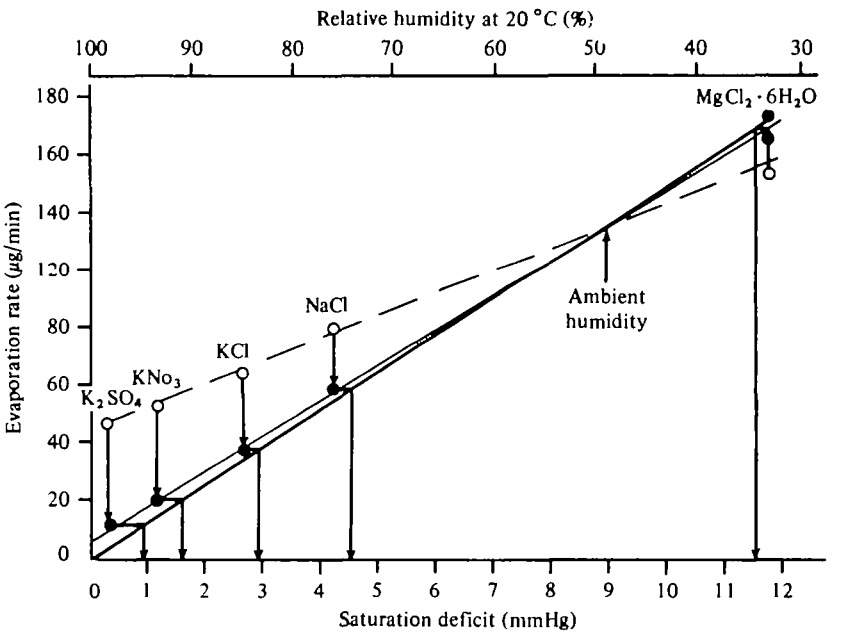

Fig. 2. Calibration graph of microbalance humidity chambers based on evaporation characteristics of a water drop, without wicks (open circles, interrupted line) and with wicks (closed circles). The two continuous lines indicate the discrepancy with wicks, between observed (fine line) and ideal evaporation rates (heavy line drawn between points at saturated and ambient humidities). The necessary corrections by interpolation to obtain the true humidity in each chamber are indicated by downward pointing arrows. The saturated salts used in the chambers are also shown.

To obtain cuticles the rectal complex was first removed from large mealworms (> 100 mg). With a pair of fine watchmaker's forceps the cuticle and attached epithelium was then pulled out of the rest of the complex. Drawing the cuticle and epidermis between the closed tips of the forceps stripped off the epidermal cells leaving a clean tube of cuticle after trimming the ends. The cuticles of ten animals provided enough material for analysis with the balance output amplified by a factor of 10 (full scale = 100 μg). These were adhered by superficial drying to a narrow ribbon of platinum foil. After recording the equilibrium weight in the five humidities the platinum was heated to red heat to burn off the sample, cooled and then reweighed to obtain the tare weight.

Isolated perirectal fluid

Fig. 3 shows the humidity chamber in which the volume–concentration relationships of perirectal fluid droplets could be studied. It consisted essentially of an observation well in a temperature-regulated (20·00 ± 0·01 °C) aluminium block into which air streams of differing regulated vapour pressure are passed. Two 45° prisms permitted the samples to be illuminated and viewed from the side with a compound microscope. Measurements of drop dimensions permitted determination of drop volume in known vapour pressures.

Humidity control was achieved by passing air through one of two temperature-regulated copper heat-exchangers after the method described by Machin (1976). Heat exchangers could be reliably maintained to 0·1 °C; the maximum dew-point obtainable in the chamber without risk of condensation was 19·9 °C (17·43 mmHg, 99·3% R.H.). Chamber vapour pressure was calculated from volume changes of a drop of KCl of known molal concentration and vapour pressure lowering and of similar size to the unknowns. For absorbing animals 1·5 M-KCl standard was used, whereas 0·2 M-KCl more closely corresponded to the concentrations of perirectal fluid from non-absorbing animals. The empirical relationships used in determining the vapour pressure of the chamber at 20 °C were

$$v_{p_0} - v_{p_1} = 0{\cdot}0153 + 0{\cdot}567M$$

(derived from data in Lovelace, Frazer & Sease, 1921), where $v_{p_0} - v_{p_1}$ is the vapour pressure lowering in mmHg and M is the molal concentration of the KCl, and

$$\text{relative volume of KCl drop} = -0{\cdot}524 + 21{\cdot}601/v_{p_0} - v_{p_1}$$

(derived from data in *The Handbook of Physics and Chemistry*, 49th ed., 1969. The Chemical Rubber Company, Cleveland, Ohio).

For fluid sampling, mealworms were decapitated, opened by dorsal incision with fine scissors and pinned out under oil. After the method of Ramsay (1964) a glass micropipette, held parallel to the rectum, was slipped under anterior valves formed by the ring of Malpighian tubules at the base of the common trunk. After insertion as deep as possible into the perirectal space the micropipette was withdrawn whereupon it was often observed to fill by capillarity. Since as much perirectal fluid as possible was essential for drop volume measurement no attempt was made to sample from different regions of perirectal space. After wiping the tip of the micropipette the

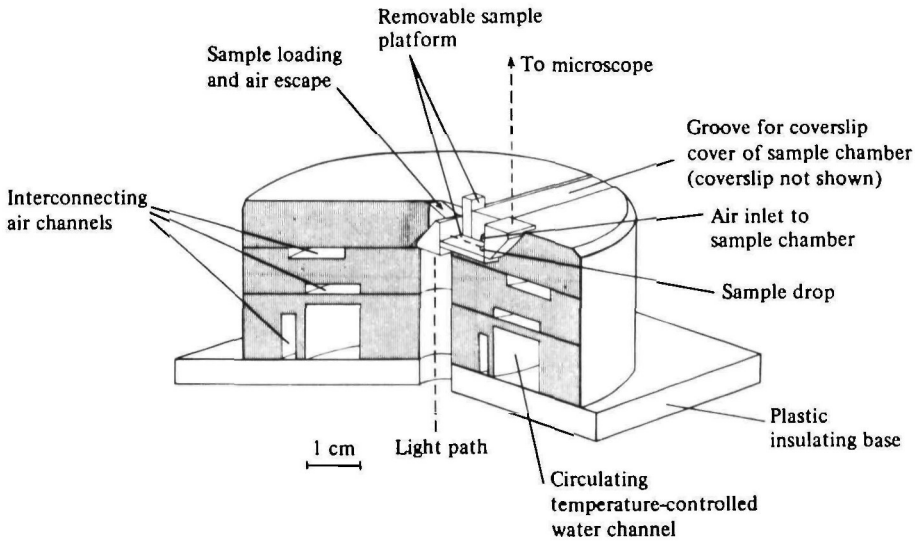

Fig. 3. Three-dimensional, cut away (shaded surfaces) diagram of humidity chamber used to measure volume-concentration relationship of perirectal fluid. The apparatus is contructed of aluminium blocks bolted together with milled temperature-controlled water and air channels. The length and narrowness of the air channels (interconnexion not shown) ensure that incoming air reaches the temperature of the blocks before passing over the sample drop. The prisms, which permit the sample to be viewed and illuminated from the side, together with the removable sample platform are drawn fully. The apparatus is mounted on a microscope stage with offset condenser and light source.

sample was gently blown out onto a silicone-coated Plexiglass platform in the humidity chamber. The entire sampling and loading procedure took less than a minute. As many as three unknowns and a single standard KCl drop were run simultaneously.

Sample volumes were calculated from eyepiece micrometer measurements of drop height and base radius using the following equation from Beament (1958):

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

where a is the drop's maximum height and b its radius at the plane of contact. Since the equation assumes that drops are portions of perfect spheres, errors in volume calculation probably occurred when the drop base diverged from circular. It was also found in some cases that a small ring of oil from the micropipette frequently formed round the base of the drops. A combination of visual estimation from the side and measurement from above where the oil ring could be more clearly distinguished, was used to estimate the true diameter of the drop base. Drops readily changed volume in response to randomly selected chamber humidities, coming to new volume equilibria within 30–60 min.

RESULTS

Distribution of osmotic pressures

Sections from non-absorbing animals were mechanically stable, uniformly crystalline and when partially melted, readily showed structures within the rectal complex. By contrast, even at −20 °C, ice crystals in the posterior regions of the rectal comp

Table 1. *Summary of rectal compartment osmotic pressures in moulting, non-absorbing and absorbing intermoult mealworms*

(Data for intermoult animals are separated into a rapidly frozen group showing radial gradients and those frozen after some delay where radial gradients were abolished. Values, given in osmol.kg^{-1}, are ranges based on the temperatures between which melting took place. Mean values were obtained by averaging the mid-value of each pair. Values in horizontal rows are from the same individuals. Values in brackets were calculated from relative humidity of the rectal lumen. Abbreviations: Epith., MT and Haem. refer to rectal epithelium, Malpighian tubules and haemolymph, respectively.)

Longitudinal zones of rectal complex												
Posterior third				Mid-third					Anterior third			
Lumen	Epith.	MT	Haem.	Lumen	Epith.	MT	Common trunk	Haem.	Lumen	Epith.	MT	Haem.
Absorbing mealworms – rapid freezing												
[1·1]	4·3–3·8	6·5–5·4	1·0–0·5									
[1·1]	4·3–3·8	4·8–4·3	1·0–0·5	3·2–2·2	3·2–2·2	5·4–4·8	2·2–1·6	1·6–1·0				
				3·2–2·7	3·2–2·7	4·3–3·8	— —	1·0–0·5	3·2–2·7	3·2–2·7	3·8–3·2	1·6–1·0
									2·7–2·2	2·7–2·2	2·2–1·6	1·0–0·5
[1·1]	4·3–3·2	7·0–6·5	2·2–1·0	3·8–2·2	3·2–2·2	5·4–4·3	2·7–1·6	2·2–1·0	2·7–1·6	2·7–1·6	4·8–3·8	1·0–0·5
Mean [1·1]	4·0	5·8	1·0	2·6	2·8	4·7	1·9	1·2	2·5	2·6	3·2	0·9
Absorbing mealworms – delayed freezing												
Rectal complex			Haem.	Rectal complex			Common trunk	Haem.	Rectal complex			Haem.
—	—	—	—	—	—	—	—	—	3·8–2·7	—	—	1·6–0·5
—	—	—	—	4·3–3·8	—	—	1·6–1·0	1·0–0·5	1·6–1·0	—	—	1·0–0·5
—	—	—	1·6–1·0	1·6–1·0	—	—	—	1·0–0·5	1·6–1·0	—	—	1·0–0·5
5·4–4·3	—	—	1·6–1·0	3·2–2·2	—	—	1·6–1·0	1·0–0·5				
5·4–4·3	—	—	1·6–1·0	4·3–3·2	—	—	2·2–1·6	1·6–1·0	3·2–2·2	—	—	1·0–0·5
4·3–3·8	—	—	1·0–0·5	3·2–2·2	—	—	3·2–2·2	1·0–0·5				
Mean 4·6			1·1	2·9			1·8	0·9	2·1			0·9
Non-absorbing mealworms – rapid freezing												
Whole animal				Whole animal					Whole animal			
1·0–0·5				1·0–0·5					1·0–0·5			
1·6–0·9				—					—			
1·0–0·5				—					—			
1·0–0·4				—					1·0–0·5			
—				—					1·3–0·8			
—				—					1·2–0·1			
—				—					1·0–0·1			
Mean 0·9				0·8					0·8			

were sparse, particularly in the tubules of absorbing animals, and tissues of the complex were shrunken and more difficult to discern.

For the purposes of comparison between individuals, transverse sections from any part of the posterior abdomen could be placed by means of anatomical clues into one of five longitudinal zones: extreme post-anal (no-gut); pre-anal (thick muscular wall, thick cuticle, narrow star-shaped lumen); posterior rectal complex (free pigmented Malpighian tubules also in section); mid-rectal complex (free Malpighian tubules and two other portions of gut loop in section); anterior rectal complex (including gut loop in section and common trunk). Analysed sections obtained within 20 s of water-vapour absorption showed radial osmotic gradients. In Table 1 it can be seen that the Malpighian tubules in the majority of cases melted at a lower temperature than the rectal epidermis, even in the most anterior sections of the rectum. In sections where rectal contents included sufficient water for melting to be observed, this process occurred at higher temperatures than in the surrounding tissues. Since the humidity in the more posterior, air-filled part of the rectal lumen is close to that of outside air (Machin, 1976) the value given in brackets (1·1 osmol.kg^{-1}) was directly calculated from ambient humidity. The above melting pattern is illustrated in the sequence of photographs, Fig. 4(*a–f*). It can be seen that ice crystals (black in the photographs) are much less densely distributed in the region of the Malpighian tubules. Note the sharp delineation between the early melting tubules and the haemolymph, which melts last. Such a steep osmotic gradient confirms the low water permeability in the perinephric membrane (Ramsay, 1964). In contrast the peritubular zone (*p*) melts progressively outwards. In the section illustrated in Fig. 4 longitudinal muscles (*m*) appear unfrozen at − 12 °C but this was not always the case.

Animals frozen following some delay after removal from high humidity showed low melting points as before, but no radial progression of melting (Table 1, Fig. 5*a*). The rectal complex of non-absorbing animals, on the other hand, melted uniformly at the same time and temperature as the haemolymph (Table 1, Fig. 5*b*).

Data presented in Table 1 for all absorbing animals also clearly demonstrate that there are marked longitudinal gradients; maximum osmotic pressures in the posterior region progressively decrease anteriorly. Melting points of the fluid in the common trunk seen in mid-rectal sections are always slightly higher than those of the haemolymph. This differential disappears in the free Malpighian tubules seen in posterior rectal sections.

After a great deal of difficulty a sufficient number of intact sections were obtained and successful melting analyses performed on the same individual for a more complete three-dimensional reconstruction to be attempted. Longitudinal measurements necessary to obtain the correct spacing between transverse sections was done by combining micrometer measurement with counting the intervening sections whose thickness was known. The radial dimensions of the rectal complex, which differ depending on the amount of cuticular folding round the lumen, were based on the means of six equally spaced radii fitted arbitrarily between maximum and minimum extents of the folding. The results of this analysis are presented in Fig. 6 as a longitudinal section of the rectal complex drawn to scale, showing the distribution of osmotic pressures in its major compartments. Since the temperatures chosen for the analysis were not identical, the means of values just above and below melting of the haemolymph, for example, show some variation.

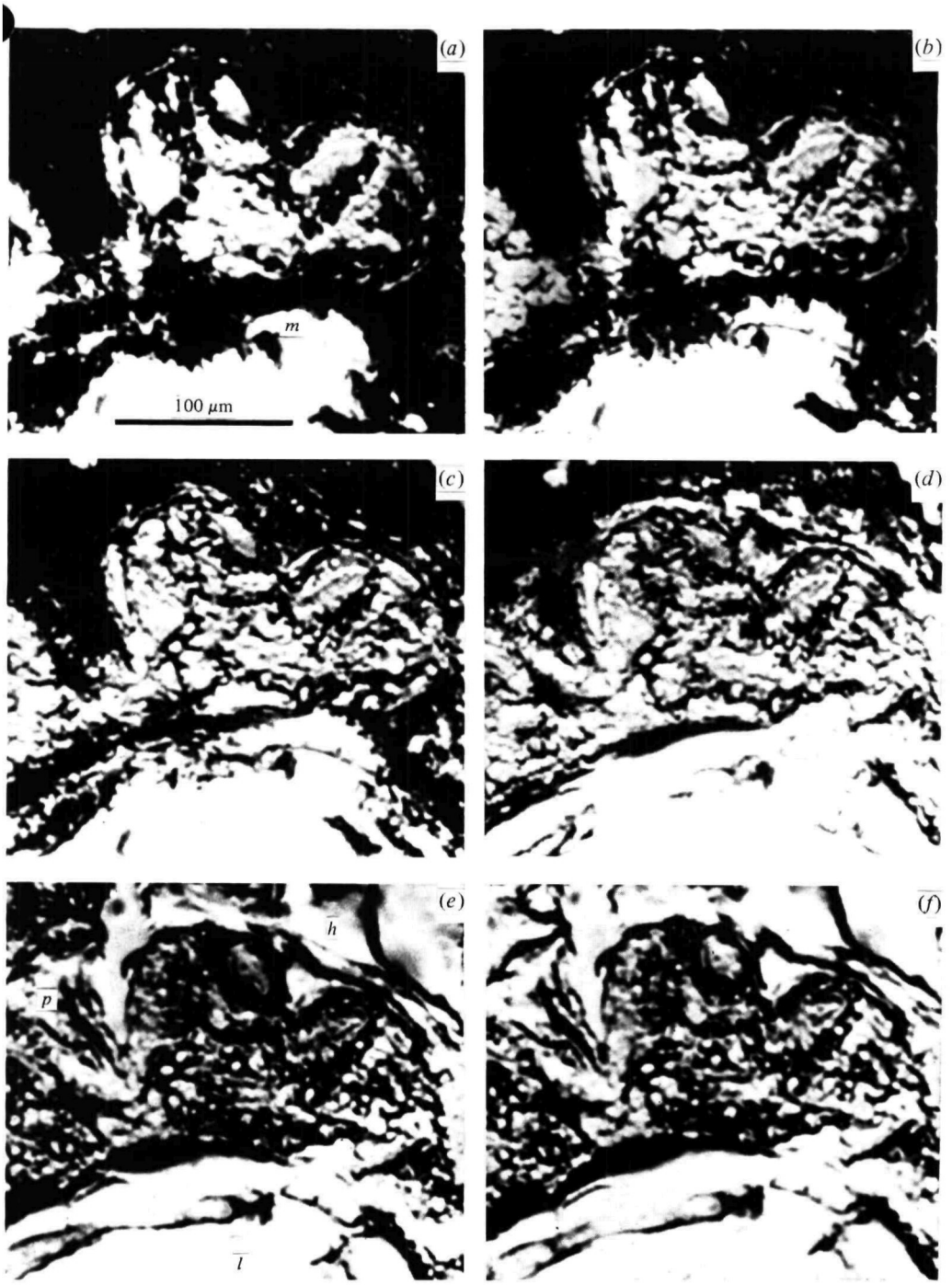

Fig. 4. Transverse section of a quadrant of quick-frozen posterior rectal complex of an absorbing mealworm photographed at (*a*) −12 °, (*b*) −10 °, (*c*) −8 °, (*d*) −6 °, (*e*) −4 °, and (*f*) −2 °C. *m*, Longitudinal muscle; *p*, peritubular space; *l*, rectal lumen; *h*, haemolymph.

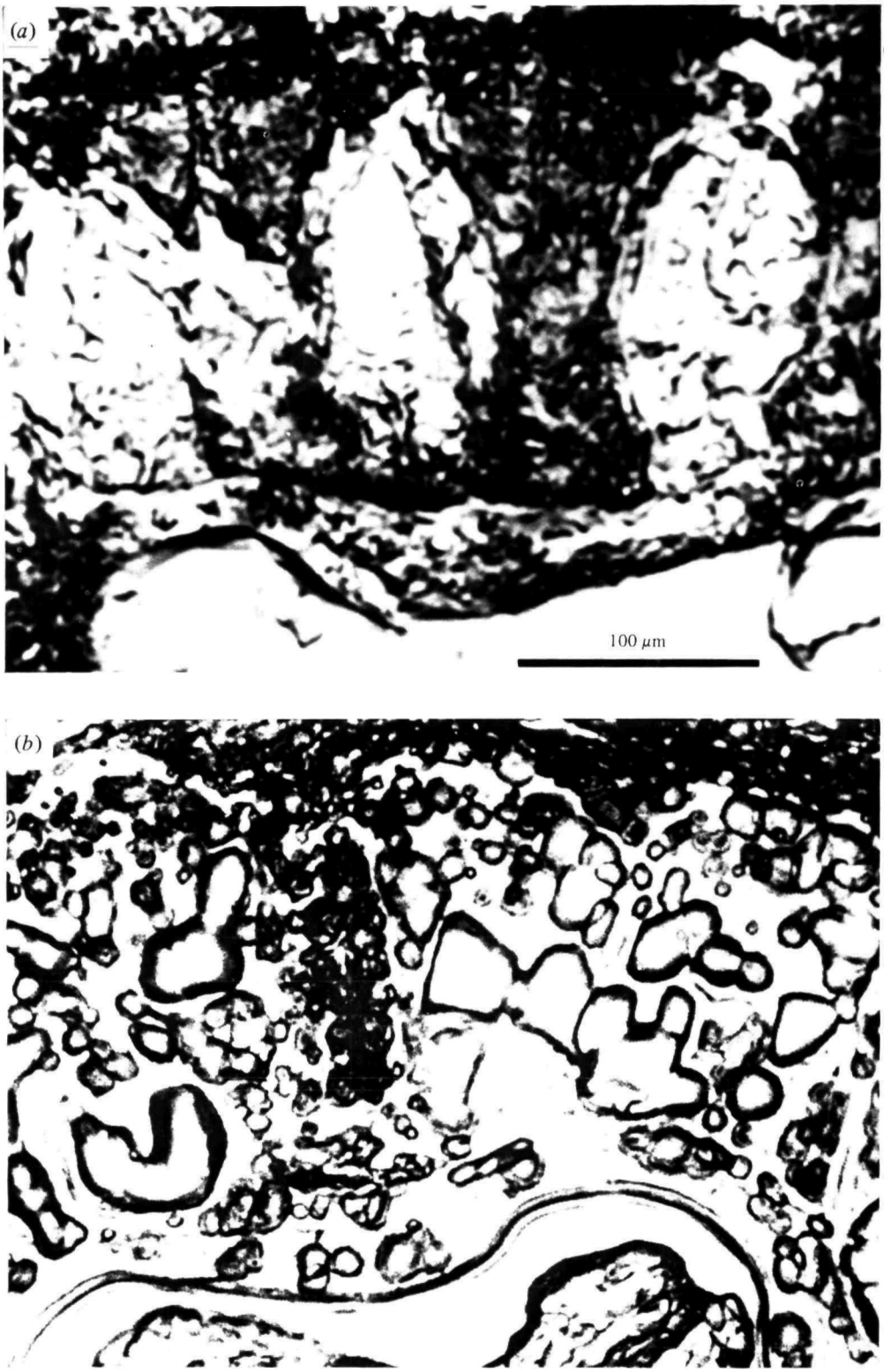

Fig. 5. Partially melted transverse sections showing the uniform melting in the complex of (*a*) absorbing mealworms with delayed freezing (taken at −7 °C) and (*b*) non-absorbing mealworms (taken at −5 °C). Differences in resolutions are partly due to the different optical systems used in obtaining the photographs.

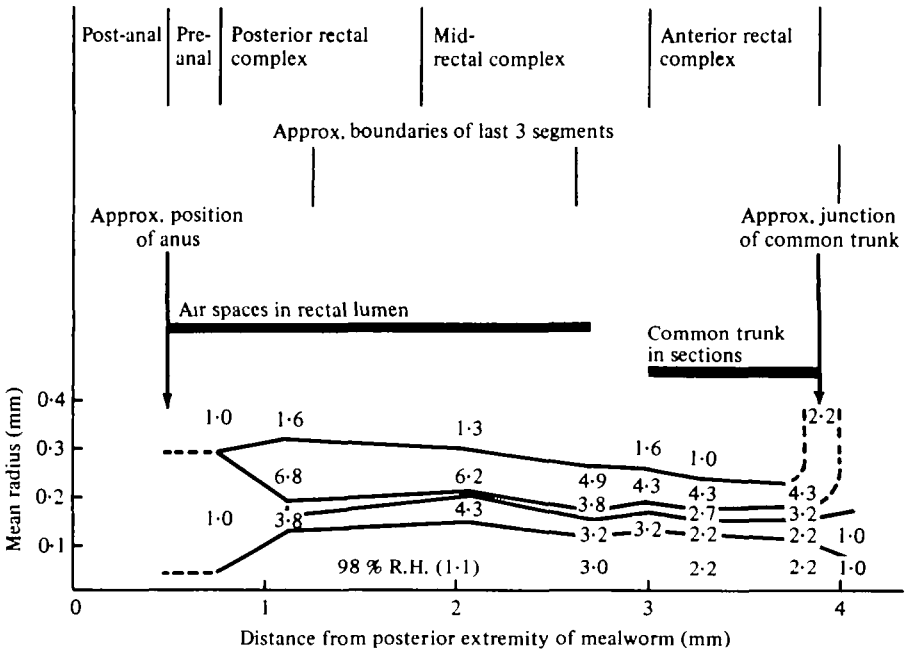

Fig. 6. Longitudinal distribution of rectal compartment osmotic pressures (osmol.kg^{-1}) reconstructed from serial transverse sections. Sections were taken at measured longitudinal intervals from a single mealworm, rapidly frozen after it has been absorbing in 98% R.H. at 20 °C. The base-line is drawn through the longitudinal axis of the rectum. Radial dimensions of each rectal compartment and corresponding osmotic pressures are means of six values taken from each transverse section. Radial dimensions are drawn on an expanded scale in relation to the longitudinal to increase space for the numbers. Compartments outlined upwards from the baseline are rectal lumen, rectal epithelium, perirectal spaces, Malpighian tubules and haemolymph. Columns of figures indicate the position of each transverse section. Where values are missing reliable melting points in that part of the section could not be obtained.

Rectal cuticle volume–concentration relationship

Fig. 7 shows how the water content of rectal cuticle changes inversely with vapour pressure lowering. The relationship corresponds reasonably well to a straight line (r^2 = 0·90) in view of the small weight changes involved (tens of microgrammes).

Perirectal fluid volume–concentration relationship

Drop volumes when plotted against the reciprocal of vapour pressure lowering corresponded well to straight lines, showing no evidence of hysteresis during volume changes. Fig. 8 compares representative results for drops of similar initial size from absorbing and non-absorbing animals. The initial osmotic pressures of each sample, calculated by interpolating the initial drop volume on the regression line, are also shown. Table 2 summarizes all of the perirectal osmotic pressures obtained in this way. Values less than 1 osmol.kg^{-1} are less accurate than the others because they must be obtained by extrapolating regression lines determined at lower and more reliably known $1/v_{p_0} - v_{p_1}$ values to near saturated humidities, which were beyond control in the chamber. It can be seen that osmotic pressures from absorbing animals were generally greater than those of moulting animals. Variability in the intermoult data presumably reflects the different portions of the standing gradient sampled.

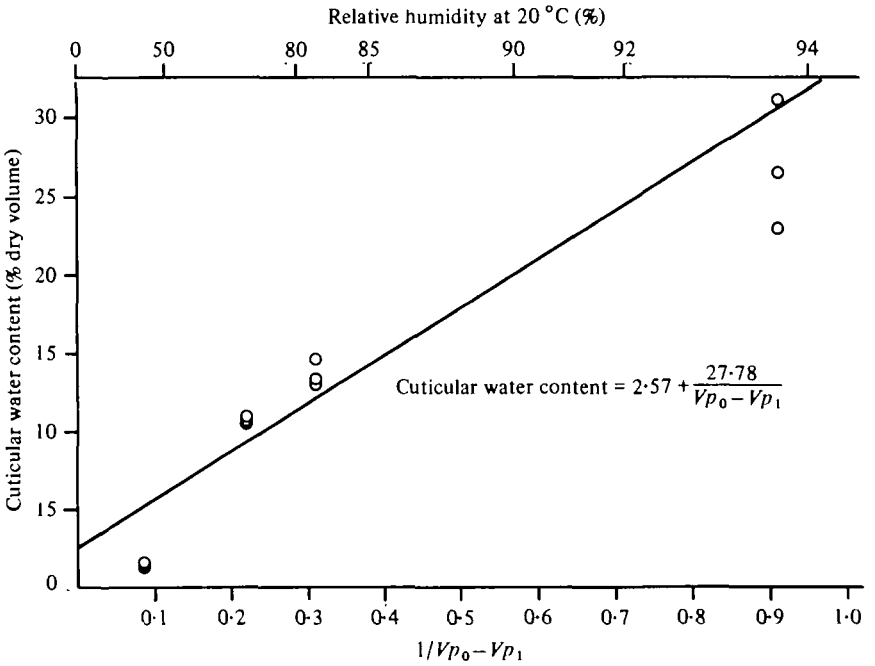

Fig. 7. Graph showing the relationships between cuticular water content and the reciprocal of vapour pressure lowering.

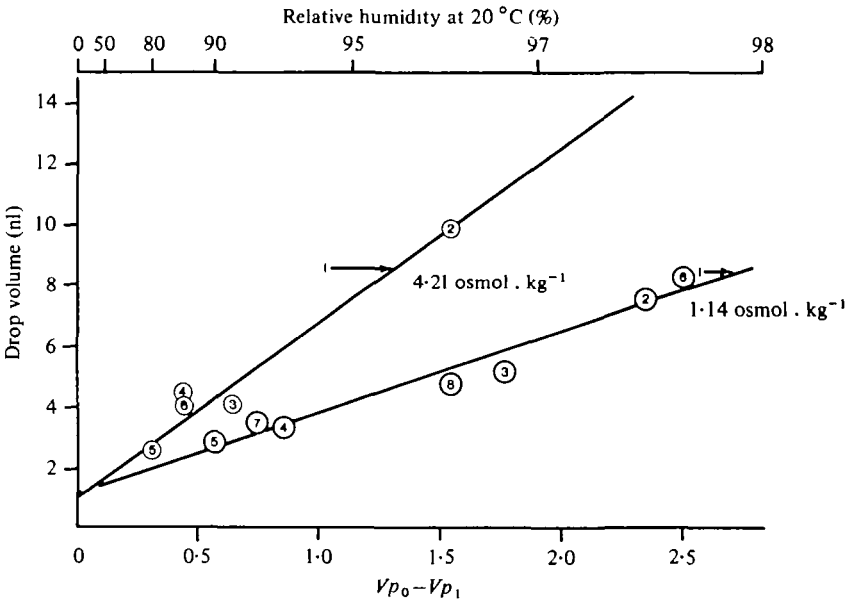

Fig. 8. Representative plots of drop volume against the reciprocal of vapour pressure lowering of similar sized samples of perirectal fluid from absorbing (fine open circles) and non-absorbing (heavy open circles) mealworms. I, Initial volume of the drops and the osmotic pressure calculated by interpolation. The other points are equilibrium values measured in the order indicated by the numbering.

Table 2. *Summary of measurements with perirectal fluid*

(Slopes and intercepts were calculated by least-squares linear regression analysis of drop volume plotted against $1/v_{p_0}-v_{p_1}$.)

Initial sample vol. (nl)	Regression constants			Ratio slope/ intercept	Initial O.P. (osmol.kg^{-1})
	Intercept	Slope	r^2		
		Non-absorbing mealworms			
11·2	0·93	1·46	0·998	1·57	0·43
7·4	1·26	1·18	0·823	0·94	0·61
13·3	0·60	3·10	0·934	5·17	0·77
5·8	0·23	1·81	0·878	7·87	1·05
*8·5	1·13	2·65	0·963	2·35	1·14
				3·58 ± 1·29	0·80 ± 0·13†
		Absorbing mealworms			
5·1	0·73	0·96	0·920	1·32	0·69
15·0	1·68	3·60	0·769	2·14	0·86
20·1	1·50	5·05	0·921	3·37	0·86
8·2	0·63	5·12	0·957	8·13	1·08
2·7	0·48	0·76	0·933	1·58	0·09
4·7	1·19	5·62	0·995	4·72	1·29
5·3	1·85	3·25	8·091	1·76	2·95
9·1	1·00	8·21	0·842	8·21	3·22
*8·6	0·93	5·78	0·924	6·22	4·21
2·5	0·24	3·77	0·973	15·71	5·19
				5·32 ± 1·42†	2·14 ± 0·51†

* Samples used in Fig. 8.
† Mean ± S.E.

Least-squares regression analysis of all data showed considerable variation in both slope and intercepts between individual samples. Differences may be expected to depend on the size and solute concentration of the sample. Significant positive intercepts suggest the presence of macromolecular solutes. Confirmation of this was obtained by microscopic examination of air-dried fluid samples following measurement in the chamber, which revealed a yellowish, gum-like residue unique to the perirectal fluid. In samples from moulting animals the residue formed a thin film with some clear areas perhaps formed by salt crystals, whereas in pumping animals the residues were more substantial with no clear areas. If the same solute was responsible for vapour pressure lowering in all samples and its osmotic coefficient remained constant, the ratio of intercept/slope should not change. One possible reason for the variability in this ratio are errors in drop volume calculation. Values of the smaller parameter, the intercept, would be least reliable because they must be determined by extrapolation. Comparing the means of different groups in Table 2, indicates that the average slope–intercept ratio is higher in absorbing animals (5·32 ± 1·42; mean ± S.E.) compared with the others (3·58 ± 1·29). Similarly, if samples are grouped by osmotic pressure, samples with osmotic pressures of greater than 2·95 osm.kg^{-1} show higher mean ratios (7·98 ± 2·91) than those of lower osmotic pressure than 1·29 osm.kg^{-1} (3·56 ± 0·78). None of the differences, however, is significant. Lower ratios would be expected in samples with a higher electrolyte content which would contribute to osmotic pressure but [illegible]t non-solvent volume as measured by regression intercept.

DISCUSSION

This paper contains new information relating to the location and nature of the water vapour uptake mechanism in mealworms. The distribution of osmotic pressures in the rectal complex resembles those obtained earlier by Ramsay (1964) and Grimstone *et al.* (1968) in showing very high values which increase towards the posterior end. The present study includes osmotic pressures of the rectal epithelium for the first time. In rapidly frozen preparations, which represent conditions in the intact living complex most closely, water activities decrease progressively from the lumen to minimum values in the Malpighian tubules. They clearly differ from the results of earlier descriptions of *Tenebrio* rectal physiology in showing higher water activities in the lumen and in the other compartments. Two of the six tubular osmotic pressures obtained from the posterior third of the complex, but not at its exteme end when the maximum values would be expected to occur, are very close (5·4–6·5, 6·5–7·0 osmol. kg^{-1}) to the well-established absorption threshold for *Tenebrio* of 88% R.H. at 20 °C (Mellanby, 1932; Locke, 1964; Machin, 1975), which is equivalent to an osmotic pressure of 6·67 osmol.kg^{-1}. The remaining freezing points were about 70% of this value, presumably because sections were not taken at the posterior end of the complex.

The discrepancy in the distribution of osmotic pressures obtained in the present study and those obtained earlier can be explained by the limitations of micropuncture sampling to obtain representative osmotic pressures in compartments showing standing gradients. Since sample volumes used by Ramsay (1964) and Grimstone *et al.* (1968) represented a significant but variable proportion of the total compartment, the osmotic pressures obtained must have been influenced by this proportion. As a result determinations from adjacent compartments may not be exactly comparable, and because of longitudinal mixing, did not give extreme values from the ends of the gradient. These limitations are overcome by using the frozen section technique.

The disappearance of radial osmotic gradients following a delay in freezing after exposure to high humidity emphasizes the critical role of the availability of luminal water in determining the distribution of osmotic pressures within the complex. It has been shown earlier (Machin, 1976) that humidity conditions in the lumen differ markedly, depending upon the particular functional mode of the rectum. During faecal dehydration with the anus closed, absorption mechanisms are apparently able to keep pace with the injection of water from the mid-gut and the faecal contents become fully equilibrated with the highest osmotic pressure of the complex. In contrast, when the anus is open during the atmospheric absorption mode, the faecal contents of the rectum reach the same humidity as that of the ambient air by diffusion. This study has demonstrated that when water is being absorbed from the atmosphere a steady state is established in which water activates progressively decrease towards the Malpighian tubules. Earlier descriptions of *Tenebrio* rectal physiology (Phillips, 1970; Maddrell, 1971) have failed to take this important change depending on the amount of water available to the rectum into account. They assumed that luminal humidities during atmospheric absorption were similar to those determined in the faecal dehydration mode by Ramsay (1964) and were left with a system in which activities apparently favoured passive water movements from the tissues to the lumen.

Uncertainties about the mechanism of initial water vapour transfer from the lum

to the rectal tissues also encouraged speculation about the role of macromolecular solutes within perirectal fluid and the possible significance of the anomalous freezing of this fluid (Ramsay, 1964; Grimstone *et al.* 1968). Despite continued interest in a more complex role for perirectal fluid (Patterson & Dunbar, 1978), there were no abnormalities apparent in the present study using isolated samples at 20 °C. Indeed, a single pump model of rectal complex operation would require that all compartments between the Malpighian tubules and the rectal lumen behave passively. Previously described anomalies may be uniquely related to the thermodynamics of macromolecular freezing, not affecting the colligative properties of the fluid at higher temperatures.

One of the more convincing confirmations of a rectal site of absorption is the correlation obtained in this study between the elevated osmotic pressures of rectal compartments and the ability of the animal to absorb water from the atmosphere. Not only, it appears, do the rectal tissues have to withstand fluctuations in osmotic pressure caused by changing external humidities but also even more extreme ones as rectal tissues become iso-osmotic with the haemolymph. There is evidence (Machin, 1976) that the loss in water vapour uptake capacity is associated with moulting. Indeed, it has recently been reported that fluid secretion, apparently inhibited by the retraction of the mitochondria from tubular microvilli in the presence of ecdysterone, ceases at the onset of population in several insects including *Tenebrio* (Ryerse, 1977, 1978). However, Ryerse reports that he did not observe the same inhibition of tubular secretion at larval-larval moults. Evidence has also been presented elsewhere (Machin, 1978) that a certain amount of osmotic swelling takes place in the tubules and perirectal space between the two physiological states but less than predicted for such large osmotic pressure changes. Possible mechanisms for reducing osmotic swelling which probably act as important safeguards against mechanical damage would be the dissipation of solutes to the haemolymph and the escape of perirectal fluid from the valve at the anterior of the rectal complex (Ramsay, 1964). Observations on the perirectal fluid in non-absorbing animals, however, suggest that significant macromolecular solute remains in the fluid and salts may even diffuse into it.

Frozen sections have provided further information about the contents and state of the rectal lumen than was previously available, suggesting considerable differences between anterior and posterior zones. Macleod (1941) put forward X-ray evidence that the rectum of starving mealworms was filled with air. In feeding or recently feeding animals this is certainly true of the posterior third to one-half of the rectum, air remains the continuous phase, though faecal pellets are also frequently present in this part of the lumen. Cuticle lining this area is presumably the site of water vapour condensation during uptake. The anterior half of the rectum on the other hand, appears completely filled with solids and some fluid, as seen by its melting, in both quick and delayed frozen specimens. The presence of this fluid suggests that mid-gut contents are being introduced even while atmospheric uptake continues. Osmotic gradients favouring the uptake of water from this fluid still exist in the anterior rectum. Ramsay's (1964) data also indicates that mid-gut fluid is about 25% hyperosmotic to the haemolymph. Since radical gradients persist in the anterior rectum it is likely that tubular fluid emerging from the complex unit is hyperosmotic to the haemolymph. This is born out by melting of fluid in the common trunk seen

Table 3. *Summary of known rectal compartment volume-concentration regression equations*

Compartment	Regression equations	Authority
Perirectal space	Relative volume $= 1{\cdot}00 + \frac{5{\cdot}32}{v_{p_0} - v_{p_1}}$	Present study
Rectal cuticle	Relative volume $= 1{\cdot}026 + \frac{0{\cdot}278}{v_{p_0} - v_{p_1}}$	Present study
Faeces	Relative weight $= 1{\cdot}00 + \frac{0{\cdot}274}{v_{p_0} - v_{p_1}}$	Machin (1975)

in mid-rectum sections. Ramsay (1964) was also able to demonstrate that fluid emerging from the severed common trunk in a dissected preparation was also more concentrated than the haemolymph. Since frozen sections show that osmotic equilibrium is established by the time fluid reaches that part of the Malpighian tubules which lie free in the haemolymph, it seems likely that coupled salt and water transport would return both water and salts to the haemolymph at this point.

If high osmotic pressure generated by the tubules is osmotically coupled to the lumen, the passive properties of the intervening compartments are of considerable interest. It follows that the quantitative relationship between total water content and solute concentration or osmotic pressure will determine the effectiveness of 'tightness' of the coupling in fluctuating external humidities. Although all compartments so far investigated will behave as osmotic couplers, since they show an inverse relationship between water content and vapour pressure lowering (Table 3), the effectiveness of the coupling varies a great deal. Rectal cuticle is an effective coupling agent because of the low slope value of the regression equation, meaning that changes from one vapour pressure to another can be brought about rapidly because relatively little water is involved. On the other hand, fluid-filled compartments like the perirectal space are less effective coupling agents because high slope values mean longer and therefore slower changes are required to bring about a given vapour pressure change. It should be noted, however, that perirectal coupling would have been less effective if it contained only low molecular weight, diffusible solutes. Non-diffusibility of the macromolecular solutes (Maddrell, 1971) together with the molecules' non-solvent volume which will contribute to a significant proportion of the total volume when concentration will both enhance the fluid's coupling agent efficiency. Using a value of 4·9 osmol.kg^{-1}, a mean of epithelial and tubular pressures (Table 1), it was calculated that solutes would occupy 23% of the total fluid volume.

Although the low relative water content of faecal pellets indicate fairly effective osmotic coupling, their presence in the rectum must be regarded as a hinderance to atmospheric absorption. They appear to obstruct diffusion and reduce the available absorptive surface area (Machin, 1978) as well as adding passively exchanging compartments of the complex. At the present time, the properties of the largest compartments of complex, cells of the rectal epithelium and Malpighian tubules are unknown. Some evidence has been presented (Machin, 1978) suggesting that epidermal cells

may not swell and shrink according to classical Van t'Hoff principles. Any capacity for cell volume regulation would enhance the osmotic coupling between tubules and absorbing surface, but this awaits further confirmation.

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REFERENCES

BEAMENT, J. W. L. (1958). The effect of temperature on the water-proofing mechanism of an insect. *J. exp. Biol.* **35**, 494–519.

GRIMSTONE, A. V., MULLINGER, A. M. & RAMSAY, J. A. (1968). Further studies on the rectal complex of the mealworm *Tenebrio molitor*, L. (Coleoptera, Tenebrionidae). *Phil. Trans. R. Soc.* B **253**, 343–382.

LOCKE, M. (1964). The structures and formation of the integument in insects. In *The physiology of insecta* (ed. M. Rockstein), vol. III, pp. 379–470. New York: Academic Press.

LOVELACE, B. F., FRAZER, J. C. W. & SEASE, V. B. (1921). The lowering of the vapour pressure of water at 20 °C produced by dissolved potassium chloride. *J. Am. Chem. Soc.* **43**, 102–110.

MACHIN, J. (1974). Osmotic gradients across snail epidermis: evidence for a water barrier. *Science N.Y.* **183**, 759–760.

MACHIN, J. (1975). Water balance in *Tenebrio molitor* L. larvae: the effect of atmospheric water absorption. *J. comp. Physiol.* **101**, 121–132.

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. (1978). Water vapour uptake by *Tenebrio*: a new approach to studying the phenomenon. In *Comparative Physiology: Water, Ions and Fluid Mechanics* (ed. K. Schmidt-Nielsen, L. Bolis and S. H. P. Maddrell), pp. 67–77. Cambridge University Press.

MACLEOD, G. F. (1941). X-ray studies of starving mealworm larvae. *Ann. ent. Soc. Am.* **34**, 696–701.

MADDRELL, S. H. P. (1971). The mechanisms of insect excretory systems. In *Advances in Insect Physiology* (ed. J. W. L. Beament, J. E. Treherne and V. B. Wigglesworth), pp. 199–331. New York, London: Academic Press.

MELLANBY, K. (1932). The effect of atmospheric humidity on the metabolism of the fasting mealworm (*Tenebrio molitor* L., Coleoptera). *Proc. R. Soc. Lond.* **111**, 376–390.

NOBLE-NESBITT, J. (1970). Water uptake from subsaturated atmospheres: its site in insects. *Nature, Lond.* **225**, 753–754.

PATTERSON, J. L. & DUNBAR, J. G. (1978). The role of the thermal hysteresis factor in *Tenebrio molitor* larvae. *J. exp. Biol.* **74**, 37–45.

PHILLIPS, J. E. (1970). Apparent transport of water by insect excretory systems. *Am. Zool.* **10**, 413–436.

RAMSEY, J. A. (1964). The rectal complex of the mealworm *Tenebrio molitor* L. (Coleoptera, Tenebrionidae). *Phil. Trans. R. Soc. Lond.* B **248**, 279–314.

RICHARDS, A. G. (1951). *The Integument of Arthropods.* Minneapolis: University of Minnesota Press.

RYERSE, J. S. (1977). Control of mitochondrial movement during development in insect Malpighian tubules. *Proc. Microscop. Soc. Can.* **4**, 48–49.

Ryerse, J. S. (1978). Ecdysterone switches off fluid secretion at pupation in insect Malpighian tubules. *Nature, Lond.* **271**, 745–746.

WINSTON, P. W. & BATES, D. H. (1960). Saturated solutions for the control of humidity in biological research. *Ecology* **41**, 232–237.