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PASSIVE EXCHANGES DURING WATER VAPOUR ABSORPTION IN MEALWORMS (*TENEBRIO MOLITOR*): A NEW APPROACH TO STUDYING THE PHENOMENON

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SUMMARY

The weights of single mealworms were continuously recorded at 20 °C during exposure to periods of constant humidity and to abrupt changes in atmospheric vapour pressure. Two exchange stages were recognized in each animal. Weight changes were either limited to slow losses, suggesting transpiration through the external cuticle, or showed more rapid humidity-dependent gains as well as losses. Rapid exchanges indicated that water was gained or lost through permeable barriers, from a fluid compartmet of significantly lower vapour pressure than the haemolymph, equivalent to about 90% R.H. Weight gains and losses during humidity changes provided evidence of a significant, passively exchanging fluid compartment located between the exchange surface and absorbing mechanism. Weight changes in faecal pellets following their elimination provide further support for a rectal site of atmospheric absorption.

INTRODUCTION

The first indication in some terrestrial arthropods, that water exchange does not always follow simple concentration differences between the haemolymph and the atmosphere, was obtained by Buxton (1930) using *Tenebrio* larvae. His results, which were subsequently confirmed by Mellanby (1932), showed that mealworms gain weight in humidities exceeding 90% R.H. In a related observation, Kalmus (1936) found that healthy *Tenebrio* held in restricted spaces, bring their surroundings to about 90% R.H., whereas those that die reach an equilibrium with almost saturated air. Beament (1954, 1961, 1964, 1965) realized the physiological significance of these and similar observations, describing them as unusual and spectacular examples of active transport which he attributed to the epidermis and external cuticle. Despite the continued support of Beament's theories, notably by Locke (1974), the discovery by Ramsay (1964) that the faecal pellets of *Tenebrio* were equilibrated to 90% R.H., heralded a change in emphasis to the rectum as a possible site of atmospheric absorption in this animal. Subsequently, Noble-Nesbitt (1970 *a*) was able to demonstrate that weight gain in high humidities was prevented by blocking the anus with wax. Two recent studies have both supported Noble-Nesbitt's findings, these are Dunbar & Winston

(1975), using ligaturing techniques and Machin (1975) using wax. Machin also demonstrated that water vapour absorbed through the rectum has a profound effect on haemolymph water content which subsequently leads to increased growth.

The number of other insects and arachnids known to be able to absorb water vapour from subsaturated atmospheres is now quite considerable (Noble-Nesbitt, 1969). Although early studies of these animals were concerned mainly with the biological aspects of this phenomenon, the emphasis is now changing to more physiological problems. Using wax blocking techniques or variants of them, Noble-Nesbitt (1970*b*, 1975) has convincingly established the rectum as the site of water vapour uptake in *Thermobia*. The same technique has implicated the salivary glands as the site of absorption in ticks (Rudolph & Knülle, 1974). The high solute concentrations in the saliva are consistent with this idea but the route of water transport requires further investigation. Ramsay's (1964) analysis of concentration in the various fluid compartments of the rectal complex in *Tenebrio* suggests a number of possible uptake mechanisms based on high solute concentrations either alone, or combined with more complex, as yet unknown pumping mechanisms. The experimental evidence collected so far is not sufficiently convincing to distinguish between these.

Techniques already developed to estimate the volume of a solvent compartment in a complexly exchanging molluscan epithelium (Machin, 1972) suggest a new approach. It seems possible that the appropriate analysis could provide information concerning the location of the pumping mechanism within the rectal complex of an intact animal. The techniques have the advantage of not interfering with the experimental animal beyond obtaining a continuous record of its weight. They are concerned with the interpretation of non-equilibrium weight adjustments which take place in a series of barriers and compartments in the change from one steady state to another.

MATERIALS AND METHODS

Mealworms were kept in dry bran cultures at laboratory temperatures (20–24 °C) and humidities (30–60% R.H.). Experiments were performed on animals weighing between 75 and 100 mg. They were normally removed from the culture at least two days before use and kept individually to clear their guts of food in inverted glass vials with stainless-steel gauze lids. The production of faecal pellets, which fell through the gauze and thus could not be reingested, was reduced to a minimum in this time. Several experimental runs were also performed on animals taken direct from the culture in order to study the equilibration of faecal pellets.

Apparatus design

Studies on mealworms undergoing atmospheric absorption are restricted to a narrow range of ambient humidities exceeding 90% R.H. Precise humidity control and measurement was therefore essential. The technique further required that humidities be abruptly changed without disturbing the experimental animal.

Fig. 1 indicates the general arrangement of the various components making up the experimental system. A filtered, regulated flow of air was pumped through a distilled water column to saturate it at the roughly controlled room temperature of 23 °C. The humidified air was adjusted to the chosen vapour pressure by passing it through one of

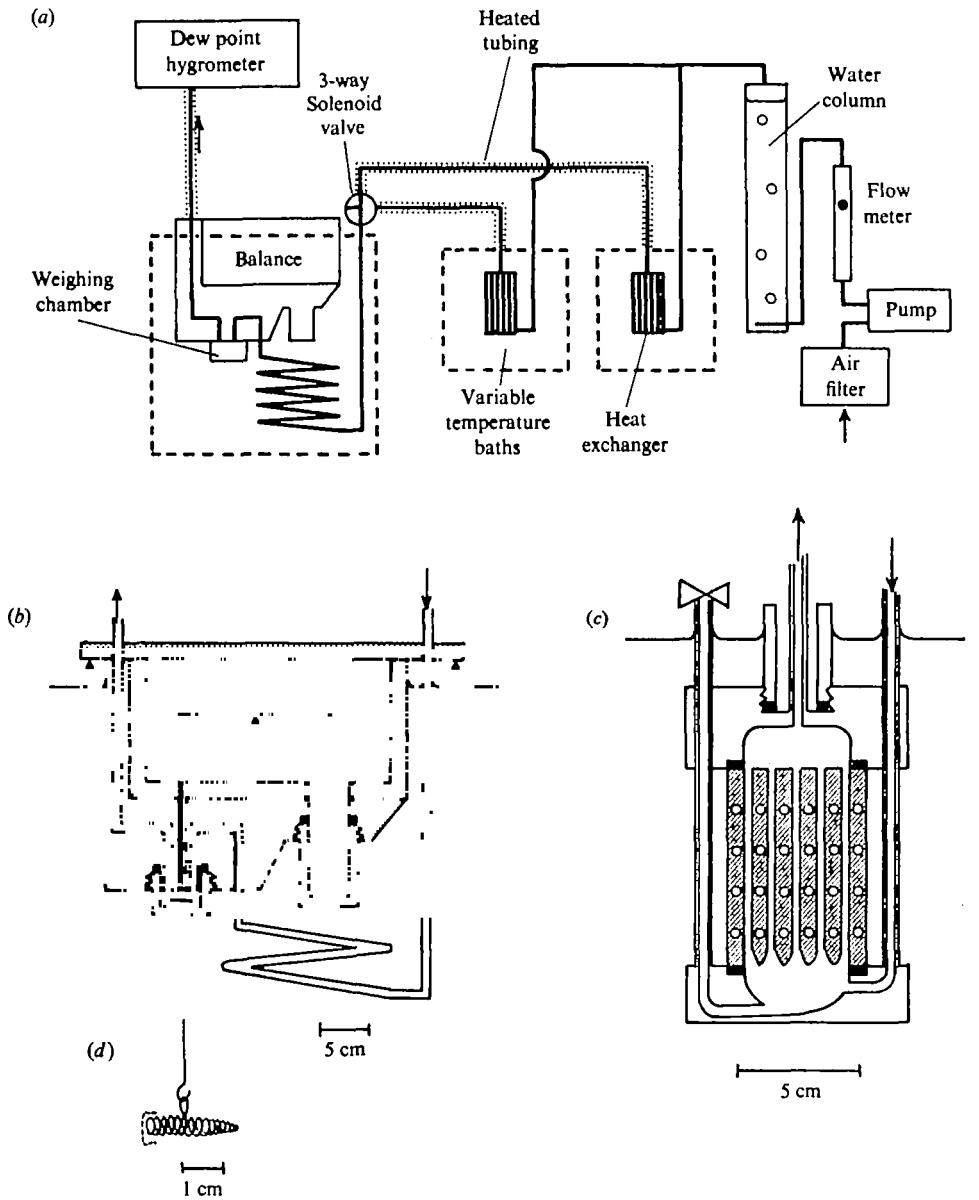

Fig. 1. (*a*) Diagram illustrating general layouts of apparatus. Dotted lines indicate heated tubing, dashed lines indicate temperature controlled water baths. (*b*) Details of balance construction. Plexiglas components are stippled. (*c*) Vertical section of humidifier with copper slabs cross shaded and rubber gaskets and 'O' ring seals in black. (*d*) The mealworm weighing cage.

two heat exchangers immersed in separate Brinkman circulating water baths, maintaining different set temperatures to ±0·1 °C. The heat exchangers were constructed of 1 cm thick perforated copper slabs, bolted together in a sandwich arrangement to give a large surface area for condensation. Condensed water droplets were conducted down vertical grooves milled in the copper to a reservoir beneath, which was

periodically drained. The large mass of the copper damped the thermal oscillations of the water bath.

Air was conveyed to the balance chamber through copper tubing, heated to about 30 °C with heating tape to eliminate the risk of condensation. The flow could be switched alternately from either of the heat exchangers by a manually or solenoid-actuated 3-way valve. Before passing through the weighing chamber the air stream was brought to balance temperature by conveying it through a long copper coil immersed in a water bath containing the balance housing. This bath was set at 20 °C and regulated remotely by a third circulating temperature bath to ±0·01 °C, a level of control made possible by the greater water volume involved.

Weight changes in the mealworm were measured using a Cahn R.H. electro-balance movement, mounted in a transparent Plexiglas housing immersed in the bath. Non-permanent plastic to plastic seals were made with silicone-greased rubber gaskets or 'O' rings. Coupling between copper tubing and plastic were made with brass 'Swagelok' tube fittings. The time required to make an experimental humidity change was reduced to a minimum by keeping the volumes of through-flow space of the housing as small as possible and the weighing chamber itself to 35 ml. Although the balance movement occupied a space of about 1 l, bulk flow between this volume and the weighing chamber was prevented by keeping the balance housing air-tight. Diffusive exchange between the two chambers was minimized by connecting them with a long narrow hang-down tube. The fact that humid air is more dense also contributes to the separation of air masses in the two chambers. It was found by some initial experimentation that mealworms were least agitated when the animal holder maintained physical contact with large areas of the animal's body, particularly at the extremities. The most suitable animal holder proved to be a tapered coil of thin nichrome wire about 25% longer and wider than the animal it contained, closed at the anterior end with aluminium gauze.

Air from the weighing chamber was conveyed by a further heated copper tube to a dew-point hygrometer (Cambridge Systems, Newton, Massachusetts) with a modified high sensitivity output. This, together with the balance output, were simultaneously recorded on a 10 in, two-channel chart recorder.

Operating details

Experiments were performed at 20 °C in order to make the results compatible with previous experiments performed at room temperatures (Machin, 1975). Air was passed through the apparatus at 270 ml min^{-1}. At this flow rate the pressure in the balance chamber exceeded atmospheric by about 4 mmHg. Evaporation from water drops placed in the weighing chamber indicated that the change in humidity began 24 s after the switch in air streams and was completed within 2 min. Although the hygrometer was sensitive enough to distinguish reliably between the various experimental humidities to be used, there was some uncertainty about its actual reading since its mode of operation and display are based on the oscillation of a mirror's temperature about dew point. The correction factor necessary to bring the average hygrometer reading into line with actual dew point in the weighing chamber was determined directly by plotting observed evaporation rates of a 5 μl droplet of distilled water against mean indicated dew point. The calculated dew point for zero evaporation

was found to be 0·3° lower than 20 °C. There was some concern that gains or losses of water might affect the prevailing weighing chamber humidity, particularly during hygrometer calibration where the water droplet evaporated up to ten times more rapidly than the fastest rates of exchange observed with the mealworm. The error, however, was considered negligible after the most rapid rates of evaporation were found to add only 2·6% of the water vapour already present at the lowest experimental humidity.

Experiments were performed in darkness or subdued light, usually at two set humidities, one just above 90% R.H. and the other just below saturated. Timing controls were set to alternate from one to the other at 3 h intervals. A few experiments, however, used a series of smaller humidity changes. In this case the required temperature adjustments were made on the humidifier not in use at the time. Experimental humidities in the region of 60% R.H. were obtained by pumping ambient air through the apparatus.

Blank runs with the animal cage empty were performed to check the stability and reliability of the weighing system. Significant weight changes were recorded whenever the humidity changed, emphasizing the importance of making corrections for adsorption and perhaps changes in air density. In some cases the blank error increased with handling and use of the cage, presumably because of the accumulation of dirt. To keep this variation to a minimum, the cage was routinely handled with gloves or forceps and washed periodically with water then acetone. The necessary weighing corrections were predictable from one or two blank measurements made at the end of each experiment.

RESULTS

General characteristics of weight records

Weight traces of fasted mealworms after an initial period of mechanical interference due to activity, showed stable rates of weight change in constant humidity for prolonged periods. The existing equilibrium was noticeably disturbed by humidity changes, ultimately leading to a new steady state. This transition, lasting up to 1 h, greatly exceeded the actual period of humidity change in the weighing chamber. An increase in humidity was associated with a net weight gain and a lowering with a weight loss. Fig. 2, which shows typical weight traces obtained in this study, also illustrates the method by which the transitional weight changes were quantified by extrapolating steady state trends back to the original weight disturbance. The method easily and logically separates steady state and non-equilibrium phenomena.

Weight traces fell into one of two distinct categories, identified below as types I and II, with no intermediates. In type I traces, steady state trends showed zero or low loss rates (less than 10 $\mu g \cdot h^{-1}$) and no steady state gains. Weight adjustments associated with humidity changes were comparatively small and symmetrical, gains and losses being identical for a given change. Type II traces usually showed much more rapid weight losses or substantial weight gains, depending on humidity. Transitional exchanges for a given humidity change were somewhat larger than in type I. Adsorption or buoyancy effects obtained during blank runs could not be distinguished from transitional weight changes in type I recordings with a mealworm.

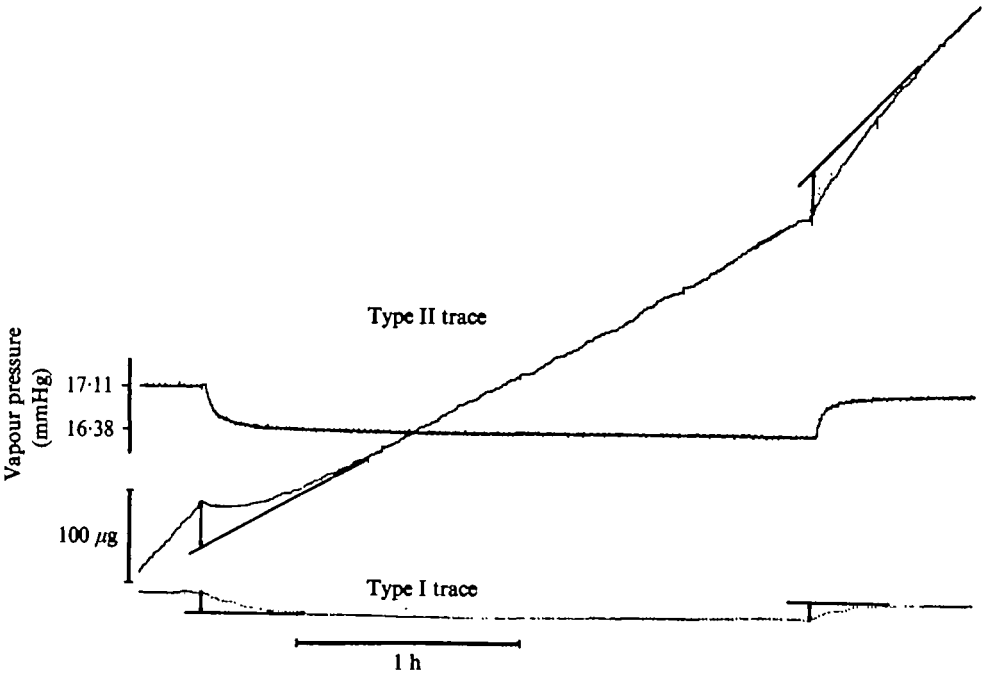

Fig. 2. Representative type I and type II weight traces during a single vapour pressure change cycle. The vertical weight scale, 100 μg, is identical for both traces. Extrapolated lines on the traces illustrate the method of determining transitional weight changes.

Experiments in humidities exceeding 90% R.H. invariably commenced with type I traces, which usually persisted for a few hours. Transition from a type I to a type II trace was clearly marked by a change from slow weight loss to rapid weight gain in constant humidity. Undisturbed animals continued to gain weight in favourable humidities for several days before returning to type I traces. The introduction of air below 90% R.H. into the weighing chamber during absorbtion, sometimes brought a change to a type I trace within minutes. However, in most instances type II traces persisted for several hours, showing rates of weight loss which were much larger than found in type I recordings. Mechanical disturbance of the balance caused premature interruption of absorption and an immediate return to a type I trace.

Equilibration of faecal pellets following elimination

A series of experiments was performed in which the rear of the mealworm cage was provided with a foil floor to retain faecal pellets in order to study their re-equilibration to ambient humidities, after the method of Ramsay (1964). Type I traces in humidities suitable for absorption showed small, comparatively rapid step-like weight increases, up to 10 μg, associated with faecal elimination. The size range of these increases agreed well with estimates of the amount of water required to bring pellets from equilibrium with 88% R.H. to the ambient humidity, based on data in Ramsay (1964) and Machin (1975). This indicated that the humidity in the rectum was below that of the animal's surroundings. By contrast, type II traces, even though defaecation occurred, showed

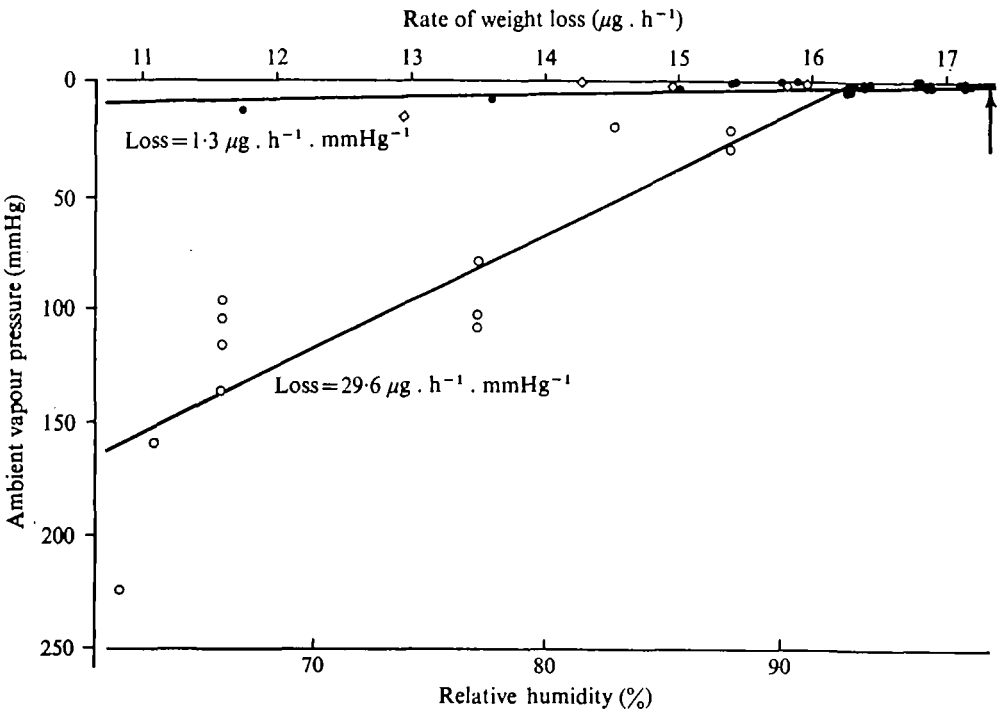

Fig. 3. Summary of weight loss data in animals before (closed circles) and after (open symbols) a period of absorption. Post-absorption points fall into two groups, a few that follow pre-absorption, type I evaporation characteristics (◇) and those that show more rapid weight loss rates (○). The regression lines were fitted to the open and closed circles by the least squares method. The arrow indicates estimated haemolymph vapour pressure.

no such adjustments in weight, indicating that the rectal contents were already equilibrated to the humidity surroundings of the animal.

Steady state exchanges

The observation that rates of weight gain increase proportionately with ambient vapour pressure above a certain threshold is well established and was confirmed by type II recordings. Rates of weight loss, summarized in Fig. 3, were generally lower than comparable weight increases. Weight loss rates of type I animals taken exclusively from the period preceding absorption conform to the expected evaporation characteristics of an insect of low cuticular permeability at sub-critical temperatures. The extrapolated humidity for zero loss, 18·6 mmHg, corresponds with reasonable error, in view of the very small amounts involved, to the expected vapour pressure of the haemolymph at 20 °C. Post-absorption loss rates, obtained by subjecting animals which had begun absorption to sub-threshold humidities, fell into type I and type II. The majority of values (type II) showed more rapid losses than type I animals, the trend calculated by least squares indicated evaporative loss principally from a compartment whose vapour pressure was below that of the haemolymph, 16·3 mmHg, or 92·8% R.H. The second group of post-absorption points, arbitrarily excluded from the regression analysis, corresponded more closely to the pre-absorption data of type I

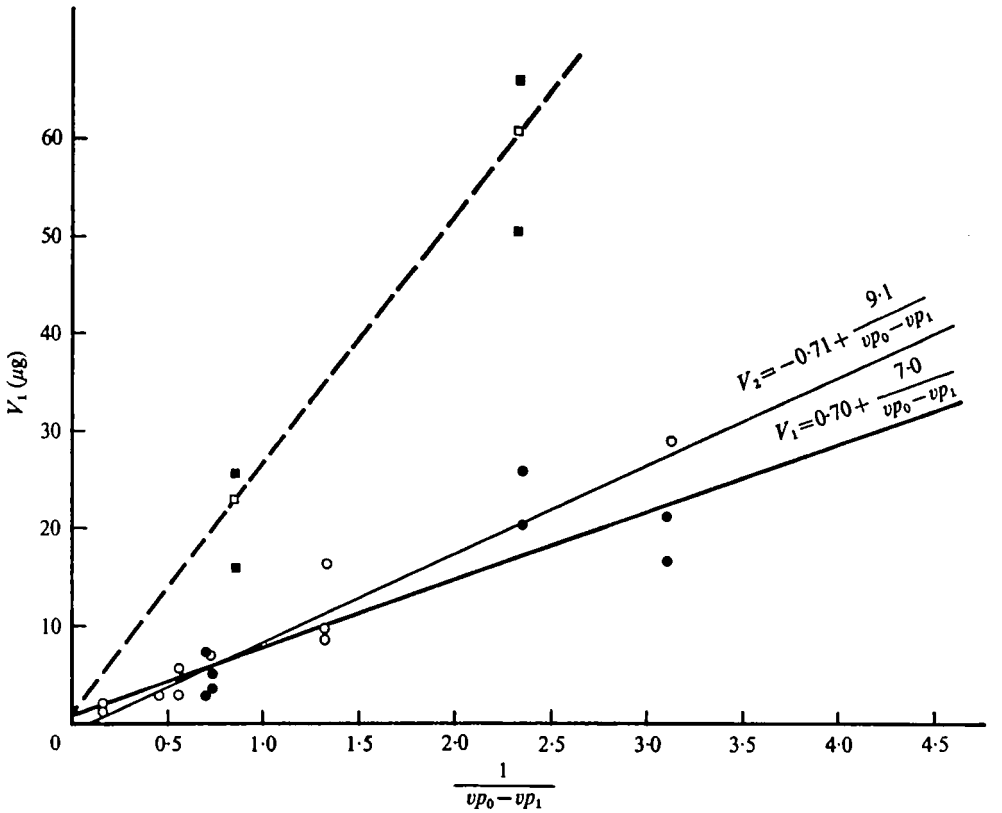

Fig. 4. Plots of calculated blank (open symbols) and corresponding type I (closed symbols) solvent volumes against the reciprocal of vapour pressure lowering. The dotted line represents data obtained with a contaminated weighing cage.

animals. It is assumed that these animals, having been exposed to sub-threshold humidities, rapidly reverted to the type I condition whereas the others did not.

Exchange following a humidity change

Weight gains and losses following known humidity changes were used to calculate the solvent volume of an exchanging compartment (V_1) within the mealworm, using the following equation based on Raoult's law, previously derived by Machin (1972):

$$V_1 = \delta V \frac{vp_0 - vp_2}{vp_1 - vp_2}, \tag{1}$$

where vp_1 and vp_2 are the vapour pressures of the compartment in two conditions, vp_0 is the vapour of the pure solvent and δV is the change in solvent volume necessary to bring about the change from one condition to the other without the movement of solute. In the present case since the solvent is water, δV can be measured by weight, as indicated in Fig. 2. For the purposes of the following analysis it has been assumed that the vapour pressures of the compartment and the ambient air with which it readily exchanges, are similar. The values of vp_1 and vp_2 are therefore given by the measured atmospheric humidity. When values of V_1 calculated from equation (1) are plotted against the reciprocal of the corresponding solute concentration, or vapour

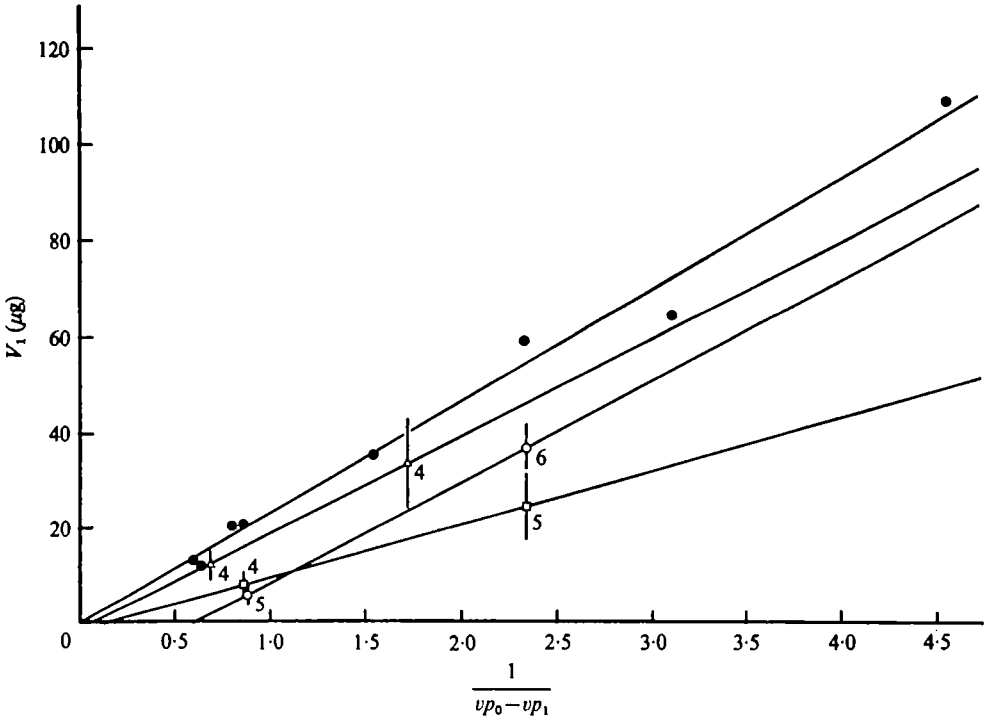

Fig. 5. Graph showing relation between calculated exchanging solvent volumes (V_1) and the reciprocal of vapour pressure lowering in four type II animals. Single data points are indicated by closed symbols, mean values of multiple replicates are given, vertical bars represent ±S.E.

pressure lowering ($1/vp_0 - vp_1$), all data sets, including those of blank runs, conform well to straight lines passing close to the graph origin. Fig. 4 shows the results of two blank runs and corresponding type I results. Close agreement between corresponding pairs of data indicates that most of the exchange of absorbed water occurs on the cage and not on the mealworm's surface. Presumably this is a consequence of the water-repellent (Holdgate, 1955) waxy nature (Locke, 1974) of the mealworm's external surface. The two lower slopes describe normal absorption and buoyancy errors while the steeper one shows the effect of faecal pellets stuck on the weighing cage.

The solvent voumes in type II recordings in all cases significantly exceeded those of the corresponding blank. The data with absorption and buoyancy errors subtracted (Fig. 5) indicate the presence of a passively exchanging aqueous compartment within the mealworm, having the volume-concentration characteristics of a simple solution. Differences in V_1 between individual mealworms are again presumably due to size.

DISCUSSION

This study has identified in weight recordings two water exchange states in experimental mealworms. The identification goes beyond the separation based on whether the animal is absorbing water vapour or not by demonstrating that several additional characteristics distinguish the two conditions. Unfortunately, it is impossible using the present apparatus to confirm definitely that the two states depend on whether or

not the anus is open. However, observations that the hydration of faecal pellets differed in the two conditions convincingly demonstrate that the rectal surfaces are in free communication with surrounding air when absorption takes place but isolated from it when uptake does not occur. Taken with the anus blocking and rectal ligaturing experiments, which prevent atmospheric absorption (Noble-Nesbitt, 1970*a*; Dunbar & Winston, 1975; Machin, 1975), the results can only mean that atmospheric absorption occurs at the rectal surface. There is close agreement between type I steady state weight loss rates (1·3 $\mu g.h^{-1}.mmHg^{-1}$) with 78–96 mg animals and those determined at the same temperature by conventional long-term weight studies (1·46 $\mu g.100\ mg^{-1}.h^{-1}.mmHg^{-1}$; Machin, 1975). Proportionality between loss rates and estimated vapour pressure gradient between haemolymph and surrounding air, strongly suggests that the source of water loss is the haemolymph. Rates of weight loss are much more rapid in type II traces and indicate a vapour pressure threshold for evaporation which is considerably below that of the haemolymph. This means that evaporation from animals with the anus open receives an additional major contribution from a compartment other than the haemolymph and by a route other than the external cuticle. There is sufficient similarity between this threshold (16·27 mmHg) and that of absorption (15·58–15·77 mmHg) to propose that exchanges in animals with the anus open represents gains or losses to the same concentrated fluid compartment located within the rectal complex. The rate and direction of net flow are presumably determined by the surface area available for exchange, the diffusion distances in the rectal lumen, and the magnitude and direction of vapour gradients existing between the compartment and the ambient air. Type II exchange rates expressed per unit concentration difference permit comparisons of flows in opposite directions. They indicate that water enters the animal (164–357 $\mu g.h^{-1}.mmHg^{-1}$) 5–12 times more rapidly per unit gradient than it leaves (29·6 $\mu g.h^{-1}$). It is premature, however, to explain this rectificaton in terms of the asymmetrical permeability of a particular barrier, without establishing its location and further clarifying the nature of the forces which drive water in and out of the animal.

Atmospheric absorption in mealworms appear to be regulated by at least two influences. Since faecal pellets eliminated before uptake begins, gain weight in humidities suitable for absorption, the rectum must normally be maintained in a potentially absorptive state. Long-term records show small variation in uptake performance which follow a consistent pattern. At the beginning of a period of absorption, uptake rates are lower than the rates established after several hours of sustained absorption, there are also related changes in absorption threshold which slightly decrease with time. This trend in uptake rates and absorption threshold is reversed during the final hours of a prolonged uptake period. These slow and comparatively minor adjustments presumably represent physiological changes in the absorption mechanism itself. On the other hand the abrupt termination of absorption suggests a different type of control. Cessation of absorption immediately following mechanical disturbance of the animal has further shown that the control is influenced by external stimuli. Again, the opening and closing of the anus seems the most likely explanation. If this is the case, an overriding regulatory mechanism under rapidly responding neuromuscular control would have the advantage over slower, purely physiological adjustments in absorbing capacity. Such a mechanism would be able to

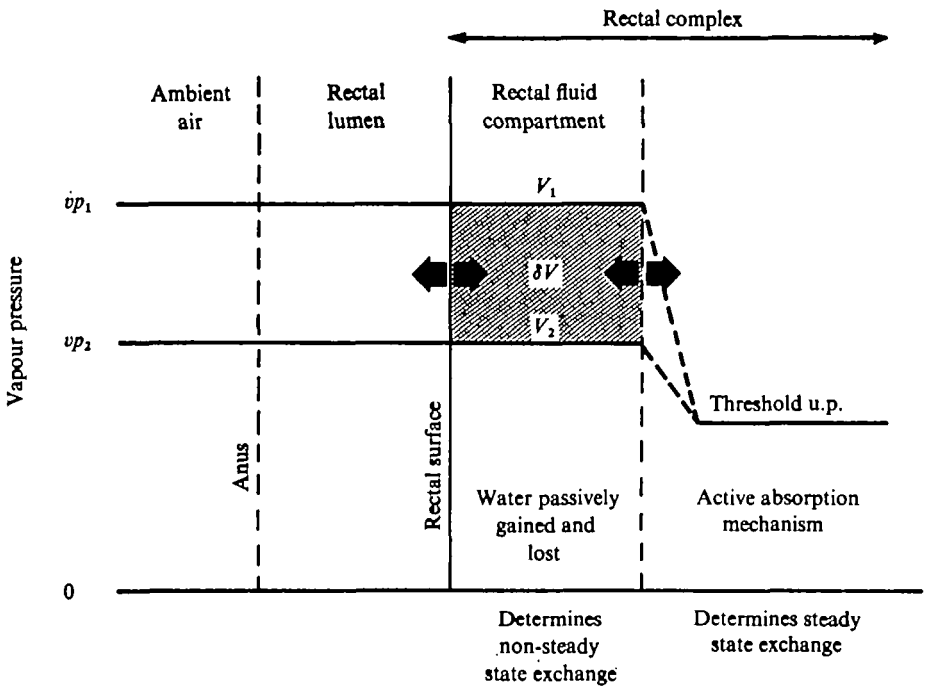

Fig. 6. Diagrammatic model of rectal absorption mechanism, showing the assumptions and simplifications used in the compartmental analysis (see text).

respond rapidly to changing ambient conditions, permitting favourably high humidities to come in contact with rectal surfaces but preventing excessive evaporation when humidity remains below threshold for long periods.

Perhaps the most significant contribution made by this study is its introduction of a technique which, with refinement, might be used to locate the driving force for atmospheric water absorption. The approach centres around the experimental identification and measurement of fluid compartments lying between the surface and the actual absorbing mechanism and their comparison and correlation with known structural features in the rectum. This study has identified in water vapour absorbing mealworms a sizeable fluid compartment which readily exchanges with the atmosphere in a predictable, passive manner whenever ambient humidity conditions change. A similar water compartment associated with the uptake mechanism has been demonstrated by a different method in the mite *Dermatophagoidex farinae* by Arlian & Wharton (1974). As in *Tenebrio* this compartment exchanges more rapidly with the atmosphere than the major fluid compartment of the body. On the other hand no such compartment associated with the absorption mechanism is apparent in Noble-Nesbitt (1975) weight traces from *Thermobia*.

The mathematical model used in the preceding analysis to describe how the solvent volume of this compartment changes with concentration, shown diagramatically in Fig. 6, contains a number of approximations or assumptions. The model assumes that solvent concentrations within the compartment at equilibrium, are equal to the prevailing ambient vapour pressures (vp_1, vp_2). This cannot be strictly true since water

would not be absorbed by the animal in the absence of gradients. However, there are two pieces of evidence which suggest that the diffusion paths into the rectal lumen and across the rectal surface are comparatively unrestricted and gradients across them are correspondingly small. The first is that the rectal contents of type II animals are equilibrated to a humidity which is very close to that of the ambient air. The second is the rapidity with which exchanges across the rectal surface begin after a humidity change, suggesting a superficial location and close coupling between the exchanging compartment and the rectal lumen. It is necessary to propose that a separate, deeper compartment system exists, capable of generating low vapour pressures to drive the steady state uptake mechanism itself. The fact that absorption increases proportionately with ambient vapour pressure above this threshold indicates that its value remains comparatively constant in varying flows of water through the system. If this is so, the measurement of superficial, passively exchanging compartment volumes may be used functionally to locate the deeper compartment. It is possible to propose more advanced models which might more accurately describe the uptake mechanism in terms of a series of concentration gradients, instead of discrete concentration steps. All of these predict somewhat higher solvent volumes than that of the simple model.

At the present time it was not felt justified to examine complex models in depth without first confirming how gradients were actually distributed in the rectal complex. Similarly, it is not possible to identify the source of passively exchanging water or to identify its precise location in the rectal complex. A study of the solvent volumes of the various compartments of the rectal complex together with the distribution of osmotic gradients is in progress.

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REFERENCES

ARLIAN, L. G. & WHARTON, G. W. (1974). Kinetics of active and passive components of water exchange between the air and a mite *Dermatophagoides farinae*. *J. Insect Physiol.* **20**, 1063–77.

BEAMENT, J. W. L. (1954). Water transport in insects. *Symp. Soc. exp. Biol.* **8**, 94–117.

BEAMENT, J. W. L. (1961). The water relations of insect cuticle. *Biol. Rev.* **36**, 281–320.

BEAMENT, J. W. L. (1964). The active transport and passive movement of water in insects. *Adv. Insect Physiol.* **2**, 67–125.

BEAMENT, J. W. L. (1965). The active transport of water: evidence, models and mechanism. *Symp. Soc. exp. Biol.* **19**, 273–98.

BUXTON, P. A. (1930). Evaporation from the mealworm (Tenebrio: Coleoptera) and atmospheric humidity. *Proc. R. Soc. Lond.* **106**, 560–77.

DUNBAR, B. S. & WINSTON, P. W. (1975). The site of active uptake of atmospheric water in the larvae of *Tenebrio molitor*. *J. Insect Physiol.* **21**, 495–500.

HOLDGATE, M. W. (1955). The wetting of insect cuticles by water. *J. exp. Biol.* **32**, 591–617.

KALMUS, H. (1936). Die Verwendung der Tracheenblasen der Corethralarve als Mikrohygrometer. *Z. wiss. Mikrosk.* **53**, 215–19.

LOCKE, M. (1974). The structure and formation of the integument in insects. In *The Physiology of Insects*, vol. VI, 2nd edn (ed. M. Rockstein), pp. 123–213. New York and London: Academic Press.

MACHIN, J. (1972). Water exchange in the mantle of a terrestrial snail during periods of reduced evaporative loss. *J. exp. Biol.* **57**, 103–11.

MACHIN, J. (1975). Water balance in *Tenebrio molitor* L. larvae: the effect of atmospheric water absorbtion. *J. comp. Physiol.* **101**, 121–32.

MELLANBY, K. (1932). The effect of atmospheric humidity on the metabolism of the fasting mealworm (*Tenebrio molitor* L., Coleoptera). *Proc. R. Soc. Lond.* **111**, 376–90.

NOBLE-NESBITT, J. (1969). Water balance in the firebrat, *Thermobia domestica* (Packard). Exchange of water with the atmosphere. *J. exp. Biol.* **50**, 745–69.

NOBLE-NESBITT, J. (1970*a*). Water uptake from subsaturated atmospheres: its site in insects. *Nature, Lond.* **225**, 753–4.

NOBLE-NESBITT, J. (1970*b*). Water balance in the firebrat, *Thermobia domestica* (Packard). The site of uptake of water from the atmosphere. *J. exp. Biol.* **52**, 193–200.

NOBLE-NESBITT, J. (1975). Reversible arrest of uptake of water from subsaturated atmospheres by the firebrat, *Thermobia domestica* (Packard). *J. exp. Biol.* **62**, 657–69.

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

RUDOLPH, D. & KNÜLLE, W. (1974). Site and mechanism of water vapour uptake from the atmosphere in ixodid ticks. *Nature, Lond.* **249**, 84–5.