

# Advances in Insect Physiology

*edited by*

J. E. TREHERNE

M. J. BERRIDGE

and V. B. WIGGLESWORTH

Volume 14



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# Atmospheric Water Absorption in Arthropods

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## 1 Introduction

“There are temptations, on aesthetic grounds, to give too much weight to broad unifying principles which deserve to be used only as clues for suggesting further enquiry ...”

*Professor Sir Andrew Huxley,  
Presidential Address British Association  
for the Advancement of Science, 1977*

As Huxley pointed out the principle of uniformity in nature has been applied too enthusiastically in many branches of science. In the study of atmospheric absorption a cuticular theory for uptake of water vapour has been uniformly applied to a wide variety of insect and acarine species. At an early stage, water vapour absorption was treated as separate from other examples of water transport involving the liquid phase only. The proponents of a unique transport mechanism were persuaded by the much greater differences in water activity faced by animals absorbing water from the atmosphere and the fact that the humidity dependent weight adjustments observed in some of the smaller organisms resembled the behaviour of non-living hygroscopic materials. Since all of the known atmospheric absorbers were arthropods, it seemed most reasonable at the time to explain these observations in terms of a cuticular-epidermal pump in which the physical properties of solids played an important part. Indeed the popularity of this concept and its durability is indicated by the numerous reviews which accept some form of cuticular pump model (Beament, 1954; Edney, 1957; Beament, 1961, 1964; Knüle and Wharton, 1964; Lees, 1964; Locke, 1964; Beament, 1965; Edney, 1967a, b; Winston, 1967; Noble-Nesbitt, 1968; Winston, 1969; Ebeling, 1974; Locke, 1974; Neville, 1975).

This reviewer is persuaded by recent work with *Tenebrio molitor* larvae and *Arenivaga investigata* and by weaknesses in some of the evidence favouring the cuticular model that atmospheric absorption in all animals may fit instead within the framework of a water transport paradigm based on conventional cell structure and physiology. It is the purpose of this chapter to critically evaluate the original arguments in support of the cuticular absorption theory and to reinterpret the data in terms of conventional epithelial water transport. Such a reinterpretation has never been made and is now long overdue. It is hoped that the combination of both old and new data with current ideas might suggest to all workers in this field new and productive directions in which to proceed in the future.

## 2 The site of atmospheric absorption

### 2.1 THE EXTERNAL CUTICLE MODELS

*Tenebrio molitor* larvae were the first animals whose atmospheric water exchanges appeared to depart from equilibria dictated by the vapour pressure of the haemolymph (Buxton, 1930; Mellanby, 1932). The earliest explanations (Mellanby, 1932) suggested that water vapour could be absorbed by way of the tracheal system, seemed to be confirmed when Lees (1946) and Browning (1954), working with different acarines, showed that atmospheric absorption was prevented by blocking the spiracles. The observation that

dehydrated ticks steadily gain weight in high humidity, a process which was inhibited by cuticular abrasion, together with the fact that cuticular water loss in gas or liquid form could apparently be increased when the animal was over-hydrated, convinced Lees (1946, 1947) of the existence of a sophisticated cuticular-epidermal water regulatory mechanism. The apparent connection between an aging tick's loss in absorptive capacity with the filling of the cuticular pore canals with wax also seemed to support this interpretation.

Beament was much impressed by Lees' evidence and greatly extended and elaborated the cuticular absorption theory (1954, 1961, 1964, 1965), basing his arguments on experiments with both insects and acarines and extending his theories uniformly to both groups. His reports of experiments (1964) in which he demonstrated that air in the tracheal system of cockroaches (*Periplaneta*) remained in passive equilibrium with the haemolymph (99% R.H.) convincingly put an end to the tracheal uptake theory. In further support of a cuticular site of atmospheric absorption Beament (1954) quoted Locke's (personally communicated) observations that mealworms stop taking up water vapour when the cuticle becomes separated from the epidermis during moulting. In addition Beament (1965) reported, with somewhat obscure and unsubstantiated authority, that blocking the mouth and anus of mealworms failed to prevent atmospheric uptake.

The Lees-Beament cuticular model of atmospheric absorption proposes that surface water activity is lower than haemolymph levels because of the structure and diameters of the pore canals. It was argued that the water repellent nature of the canal walls together with their small diameter combine to induce the contained water to form a concave meniscus of small radius of curvature at their free outside ends. The lowered vapour pressure at the surface of each meniscus ( $p_1$ ) (Fig. 1a) varies with the radius of curvature ( $r$ ) according to the following relationship, expressed graphically in Fig. 2

$$\ln \frac{p_1}{p_0} = \frac{-2\sigma}{r} \cdot \frac{M}{RT\rho}$$

where  $p_0$ , the saturation vapour pressure, which exists at a flat free-water surface, 17.54 mmHg at 20 °C;  $\sigma$ , the surface tension of water, 75 dynes cm<sup>-1</sup>;  $\rho$ , the density of water, 1 g cm<sup>-3</sup>;  $M$ , the molecular weight of water, 18;  $R$ , the gas constant,  $8.4 \times 10^{-7}$  ergs mole<sup>-1</sup> degree<sup>-1</sup>;  $T$ , absolute temperature, 296 °K.

The fact that ( $p_1/p_0$ ) is determined by the structure of the cuticle, explains why the ambient humidity from which absorption is possible is also a constant relative humidity at different temperatures despite widely varying saturation deficits. It was recognized that absorption into the cuticle was only part of the mechanism. The other half consisted of transferring the condensed water into the haemolymph, a process which would require osmotic forces



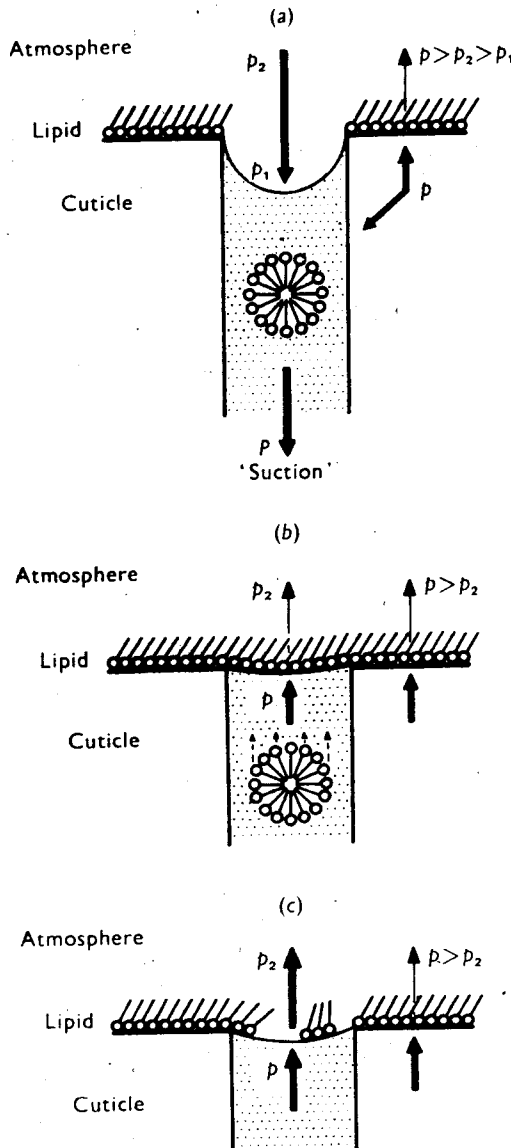


Fig. 1. Diagram of the cuticular absorption model showing the function of the pore canals. (a) High permeability phase during condensation on to the open curved surface of the meniscus. (b) Low permeability "recovery" phase with a complete protective layer of orientated lipid. This phase presumably coincides with absorption into the haemolymph. (c) Breakdown of the pump in the dead insect. (Taken from Noble-Nesbitt, 1969.)

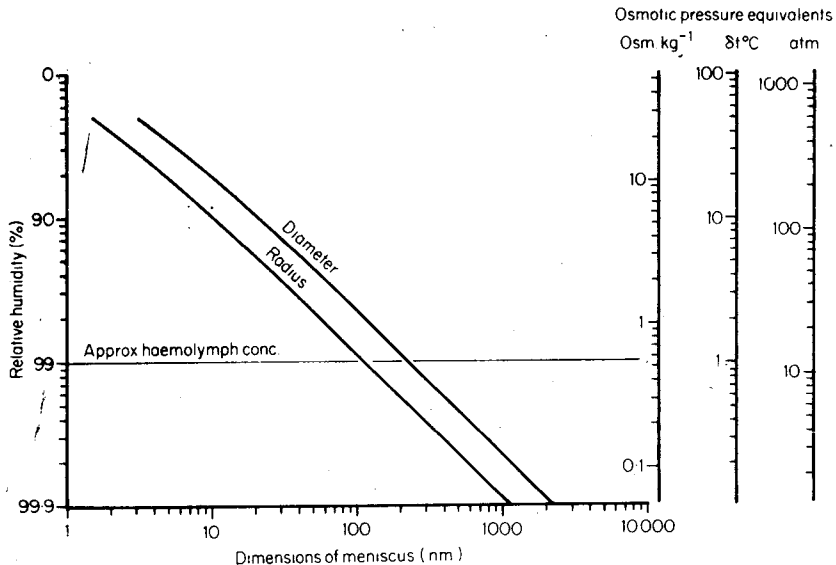


Fig. 2. Calculated relationship between curvature of a concave water surface and the relative humidity just above it at 20 °C (log scales). Equivalent water activities in osmotic units are also given.

equivalent to tens, even hundreds of atmospheres (Fig. 2). Beament exploited the well-established fact that insect cuticles and many other complex non-living membranes, show asymmetrical permeabilities favouring inward water movement. He proposed that inner cuticular layers underwent a cyclical change in water affinity, controlled and energetically dependent on the epidermal cells beneath. At the same time the impermeable, orientated layers of epicuticular lipids became disorientated or were otherwise displaced during suction and condensation (Fig. 1a) only to reform preventing outward water loss during the recovery phase of the cycle (Fig. 1b).

In support of a generally applicable absorption theory Beament (1964, 1965) cited experiments which showed that local areas of living cockroach cuticle (*Periplaneta americana*) rapidly absorbed liquid water drops leaving precipitated salts behind. In a related observation, Winston and Beament (1969) reported that the water content of freshly excised cuticles of *Periplaneta americana* and *Locusta migratoria* are below that required for passive equilibrium with haemolymph.

In the light of recent work which more convincingly establishes the rectum and other localized structures as being the site of atmospheric uptake, the evidence supporting external cuticular absorption does not appear so strong. Although Lees' evidence for cuticular-epidermal control of water loss is convincing, the next step in the argument, that various cuticular treatments or

conditions actually prevent water vapour absorption cannot be considered to have been established. Abrasion of the cuticle is harsh treatment and Beament (1961) admits increased water loss could easily mask weight gain due to absorption. The observation that moulting insects are unable to absorb water vapour has been made a number of times (Edney, 1966; Noble-Nesbitt, 1970b; Machin, 1975) and seems a widespread phenomenon. However this is not due to cuticle separation, but rather that rectal concentration gradients necessary for absorption dissipate during the moult (Machin, 1975). Ryerse has observed (personal communication) that the Malpighian tubules of *Calpodes* also cease transporting during moulting.

Although Beament (1964, 1965) placed great emphasis on the temperature insensitive nature of the relative humidity threshold for absorption, it is significant that the alternative mechanism, lowering solvent activity by high solute concentrations, also shows the same characteristics. It is well known that the relative humidities above the saturated solutions of many salts and organic solutes are remarkably temperature insensitive (Winston and Bates, 1960). So too are the relative humidities above subsaturated solutions of, for example, NaCl (Fig. 3) and KCl. It is interesting to note that Beament (1961) was so convinced that absorption took place over the entire external surface and that simultaneous gains and losses were thereby impossible that he stated

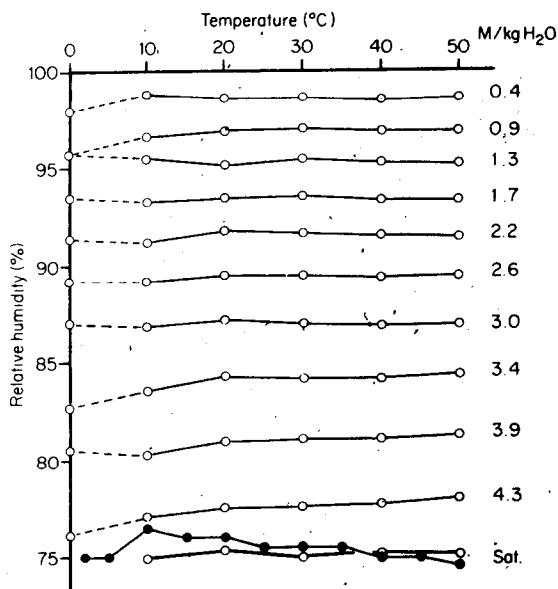


Fig. 4. Rates of weight loss (○) from *Periplaneta* with water drops placed on the cuticle in ambient chloride at different temperatures (calculated from data of Kracek, 1928). Saturated data (◐) from Brönsted, 1928; (●) from Winston and Bates, 1960.

"... it seems obvious that it [absorption] will not be achieved by direct application of massive osmotic solutions, which are actively maintained, otherwise, the rate of evaporation from living and dead insects would be very different".

The results of ultrastructural studies of insect cuticle are equivocal. Although the necessary elements of a cuticular pump may be found in the epicuticle (Locke, 1964, 1974), direct proof of its operation which must be at the level of molecular interaction between water, lipids and cuticular proteins will, of course, never be obtained. The available data suggests that pore canals are generally greater in diameter than required by the Lees-Beament model. Noble-Nesbitt (1968, 1969) states that they are 80–100 Å (8–10 nm) in diameter in *Thermobia* whereas at 45% R.H. absorption threshold is consistent with a diameter of 3 nm (see Fig. 2). In *Tenebrio* an 88% R.H. threshold implies a 20 nm diameter where observed values are 90 to 100 nm (Glued, 1968) or about 55 nm (Locke, 1974). In the cuticle of *Laelaps echidnina*, an animal with roughly the same threshold as *Tenebrio* (Wharton and Kanungo, 1962) the pore canals are about 30 to 50 nm in diameter (Wharton *et al.*, 1968). Beament (1965) explained the discrepancy by doubting whether numerical extrapolation could be made to tubes of very small diameter, suggesting that bound water molecules might reduce the effective diameter.

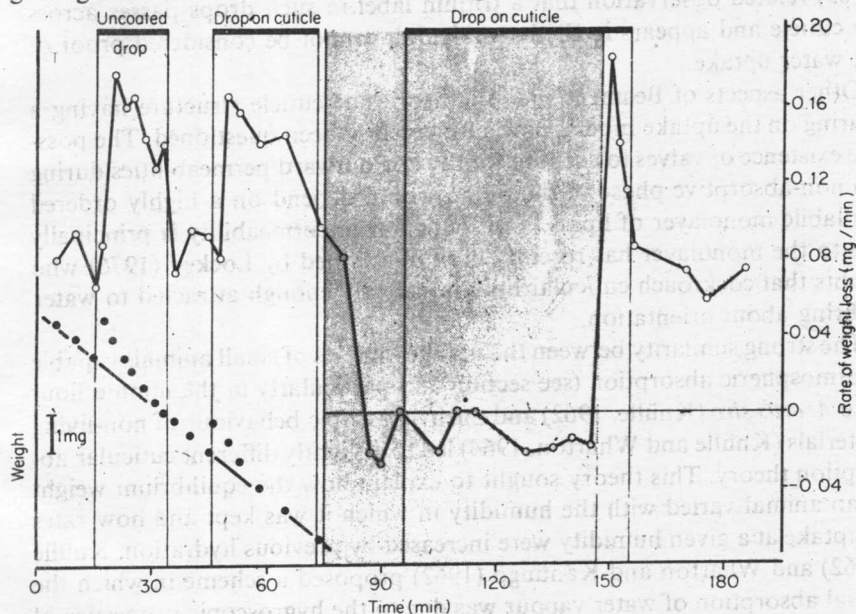


Fig. 4. Rates of weight loss (○) from *Periplaneta* with water drops placed on the cuticle in ambient humidity and in saturated air (shaded area). Negative values indicate gains. Weight measurements from which the rates were calculated, are partially indicated (●).

Noble-Nesbitt (1968) thought the answer might lie in the pore canal breaking up into finer tubules in the epicuticle.

Beament (1964, 1965) considered that his observations and experiments on liquid water drops placed on the cuticle of restrained *Periplaneta* supported the cuticle absorption theory. Even though his general observation that the rate of disappearance of the drops is very variable has been confirmed by the author, this does not necessarily mean that drops are short-lived because they pass into the cuticle. It can be seen in Fig. 4, which summarizes the author's experiments, that all drops placed on the balance with the cockroach, produce an increase in rate of weight loss, whether or not they come in contact with the cuticle. If water in the drops on the cuticle were substantially absorbed by the animal, no such increases in weight loss rates would have been observed. It was also observed that the rate of weight loss of drops on the cuticle never exceeded but sometimes approached the rate of loss of uncoated drops placed on wax beside the cockroach. The inescapable conclusion that the disappearance of the drop is caused by evaporation, presumably affected by variable lipid coating, is confirmed by the observation that a drop which stops evaporating in saturated air very rapidly disappears when the humidity is again lowered. Such marked humidity dependence would not be found if drops were actively transported across the cuticle. Beament's (1965) related observation that a tritium label in such drops passes across the cuticle and appears in the haemolymph cannot be considered proof of net water uptake.

Other aspects of Beament's (1961) theories of cuticle structure having a bearing on the uptake model, have also recently been questioned. The possible existence of valves to account for the low outward permeabilities during the non-absorptive phase of the pump would depend on a highly ordered but labile monolayer of lipids. That cuticular impermeability is principally due to the monolayer has recently been questioned by Lockey (1976) who doubts that cockroach cuticular lipid is strongly enough attracted to water to bring about orientation.

The strong similarity between the uptake kinetics of small animals capable of atmospheric absorption (see section 3.2) particularly in the minute flour mite *Acarus siro* (Knülle, 1962) and the hygroscopic behaviour of non-living materials (Knülle and Wharton, 1964) led to a slightly different cuticular absorption theory. This theory sought to explain how the equilibrium weight of an animal varied with the humidity in which it was kept and how rates of uptake at a given humidity were increased by previous hydration. Knülle (1962) and Wharton and Kzungo (1962) proposed a scheme in which the initial absorption of water vapour was due to the hygroscopic properties of the cuticle. These workers were aware that uptake in animals could not be explained by purely passive mechanisms, but did not explain why.

The arguments against exclusively physical exchanges of water vapour in small animals, without the use of energy, may be presented as follows. Animals could change their equilibrium weights with ambient humidity by means of a hygroscopic compartment separated from the rest of the body fluids. Unfortunately, the external cuticle alone is not sufficiently large in volume to account for the observed changes in weight. Then perhaps a second hygroscopic compartment, still isolated from the haemolymph exists in the animal. Experiments with tritiated water indicate that, although the body water is compartmentalized, each compartment readily exchanges with atmospheric water (Wharton and Devine, 1968; Knülle and Devine, 1972; Devine and Wharton, 1973; Arlian and Wharton, 1974; Arlian, 1975a, b; Ellingsen, 1975). The alternative possibility, that all fluid compartments in the body behave hygroscopically, also does not fit with the observations. The increase and decrease of equilibrium weights are not large enough to bring about the required changes in concentration of the haemolymph for completely passive movement of water. The loss in weight of dead animals in humidities where weight is regulated in live animals (Knülle, 1967) confirms that the haemolymph is not in passive equilibrium with ambient humidity, unless it is, very high (about 99% R.H.).

As in the case of the earlier model, a hygroscopic theory of absorption from subsaturated atmospheres which is consistent with experimental observations, will only work if a substantial osmotic imbalance is actively maintained between cuticle and haemolymph. At the same time this imbalance must permit a unidirectional flow of water against the gradient. Once again this most essential aspect of any cuticular pump remains obscure.

There is some evidence that the cuticles of *Locusta migratoria* and the cockroaches *Periplaneta americana* and *Leucophaea maderae* (Winston, 1967, 1969; Winston and Beament, 1969; Winston and Hoffmeier, 1968), animals which are not known to take up water from the atmosphere, are not in equilibrium with the haemolymph. Rapidly excised cuticles from animals kept in a wide range of humidities all show gains in weight when placed in humidities equivalent to that of the haemolymph. The critical experiment (Winston and Beament, 1969) which seeks to establish the equilibrium humidity of the living cuticle shows that it is 98.5% R.H. (0.42 M NaCl) for *Periplaneta* at 25 °C and 98.2% R.H. (0.50 M NaCl) for *Locusta* compared with 99.4% for the haemolymph in both animals. Clearly this difference is slight compared with the imbalance required for absorption for much lower humidities. In this reviewer's opinion the disequilibrium between the cuticle and the haemolymph may be explained by the cuticle being partially dehydrated during life by the surrounding air, since the amount absorbed increases with humidity.

## 2.2 EVIDENCE IN FAVOUR OF LOCALIZED ABSORPTION SITES

Perhaps the principle argument against the general use of the external cuticle is the discovery that absorption occurs at localized sites. The finding by Noble-Nesbitt (1970a, b) that blocking the anus prevented water vapour absorption in *Thermobia domestica* (= *Lepismodes inquilinus*, see Noble-Nesbitt, 1970b) and *Tenebrio molitor* larvae represented an important change in direction. These results were later confirmed in *Tenebrio* by Dunbar and Winston (1975) and Machin (1975). Surprisingly, the identification of a rectal site of absorption was not seen at first as a threat to the cuticular absorption theory; the model was simply transferred to the rectal cuticle and epidermis (Noble-Nesbitt, 1970b). Those who have attempted to occlude a specific area of an arthropod's body with wax will be aware of the unsatisfactory nature of this technique. One can never be sure whether the failure of the application is due to minute cracking or an imperfect seal or whether its apparent effect is really due to the damage caused by applying the wax at too hot a temperature. Okasha (1971) questioned Noble-Nesbitt's results on the grounds that the application of wax in the anal region might inhibit sensory processes associated with atmospheric uptake. An alternative technique, evolved independently by Rudolph and Knülle (1974) and Noble-Nesbitt (1975) in which the wax simply provides an air-tight seal between a head and tail chamber, is much more satisfactory since the uptake of water vapour can be directly associated with exposure to high humidity. The disappearance of water from a drop of saturated  $\text{KNO}_3$  (93% R.H. at 20°C) observed only in the head chamber, together with various experiments in which wax was applied to the mouth parts (Rudolph and Knülle, 1974) conclusively demonstrated an anterior site of atmospheric uptake, which was close to the mouth in several ixodid ticks. On the other hand a posterior site of atmospheric absorption was confirmed in *Thermobia* when weight gains were found to occur only when the tail end of the animal was exposed to high humidity (Noble-Nesbitt, 1975). Machin (1976) and O'Donnell (1977a, 1978) have exploited an alternative technique for testing whether or not the rectum is capable of atmospheric absorption which does not involve restraining the experimental animal. The technique is based on Ramsay's (1964) method of determining the humidity in the rectal lumen of mealworms by measuring weight changes of freshly eliminated faecal pellets in known humidities. Machin found that faecal pellets produced by mealworms in high humidity before absorption began, gained weight above a threshold of about 90% R.H., demonstrating that conditions in rectum were compatible with atmospheric uptake. Pellets produced by mealworms during absorption did not subsequently change weight, demonstrating that luminal and ambient

humidity differences were abolished by the inward diffusion of water vapour associated with absorption. On the contrary, O'Donnell (1977a, 1978) found that faecal pellets of *Arenivaga* lose weight in all humidities from which uptake is possible, indicating that absorption does not take place in the rectum. Using a technique similar to that of Rudolph and Knülle (1974) and Noble-Nesbitt (1975) he went on to demonstrate that water vapour was absorbed in the mouth region. The correspondence between weight gain and eversion of two bladder-like structures on either side of the mouth together with their surface temperatures measured by thermocouples, unequivocally identify these structures as the site of absorption in *Arenivaga*.

Clearly arthropods display a great variety of uptake mechanisms at different specific locations on the body. None of the examples just described involve the general external cuticle.

### 3 The kinetics of atmospheric absorption

The change from general surface absorption model to one involving a localized area is, at first sight, a minor one. Such a change was made by Noble-Nesbitt (1970b) who simply ascribed the Lees-Beament absorption model to the rectal cuticle without exploring its implications. The results of studies which identify limited sites of absorption are important because they require a rethinking about the forces involved in arthropod water exchange in the achievement of water balance. The rediscovery that the components of an animal's external surface can have different properties which define different simultaneous exchange processes, demands that we again view arthropod water relations in conventional terms of balancing separate gains and losses. Since water vapour absorption kinetics have previously been interpreted with cuticular absorption theories in mind, it is useful and hopefully revealing to reappraise the data knowing that several simultaneous exchange processes are possible in the same animal.

#### 3.1 UPTAKE BY LARGER INSECTS

Because of their comparatively large size and low integumental permeability (see Table 1) *Tenebrio molitor* larvae, of this size range, usually chosen for study, lose weight in dehydrating conditions only very slowly (Buxton, 1930; Mellanby, 1932; Machin, 1975). The mealworms' ability to oxidize stored fat during food deprivation to produce significant amounts of metabolic water (Johansson, 1920; Mellanby, 1932) permits the proportion of water to dry weight in these animals to remain almost constant during long periods



without food. Their levels of hydration are rather consistent over a wide range of sub-absorption humidities (Buxton, 1930).

In favourably high humidities water vapour uptake is rapid enough to markedly increase weight (Dunbar and Winston, 1975) and cause measurable haemolymph dilution (Machin, 1975). Mealworms are capable of responding to existing levels of hydration and modulating the amount of atmospheric absorption accordingly. For example, animals taken from a culture where water is available from fruit and vegetables, will rarely absorb from the atmosphere unless they are first dehydrated. Animals from a dry meal culture will absorb water vapour almost immediately upon exposure to high humidity, the amount taken up increasing with animal size. It is important to note that dehydration increases the amount absorbed but not its rate of uptake (Machin, 1975). Water content and variability in osmotic pressure of the haemolymph (Buxton, 1930; Machin, 1975) together with the tendency for absorption following a period of dehydration to overshoot previous hydration levels (Dunbar and Winston, 1975) suggests the mealworm's capacity to regulate water content is rather poorly developed.

Continuous recording of the weight of intermoult mealworms indicate that the physiological parameters of the pump are remarkably consistent. Atmospheric uptake is primarily determined by the amount by which ambient humidity exceeds a threshold close to 88% R.H. Only slightly higher thresholds (90% R.H.) are observed at the onset and termination of prolonged uptake periods lasting many days. In animals of similar size, uptake rates, but not thresholds, apparently vary with the amount of faecal material in the rectum which interferes with absorption (Machin, 1976, 1978). Uptake rates in all animals increase linearly with ambient humidity above the threshold. For this reason there is no sign of pump saturation at high absorption rates, at least up to 98.7% R.H. ( $14.9\%$  body wt  $\text{day}^{-1}$  at  $20^\circ\text{C}$ ). Since large variations in uptake rate in the same animal are only seen in studies using intermittent weighing techniques such variations must be experimental artefacts due to the adverse effects of handling the animal. It may be concluded that the modulation of atmospheric absorption in *Tenebrio* larvae is therefore brought about by an all or none switch, presumably the opening and closing of the anus, and not by changes in absorption rate. It follows that control of uptake appears to occur at a sensory level rather than at the level of the pump's physiology.

Using special experimental techniques water loss from mealworms can be seen to follow a profoundly different pattern from that normally observed. Weight loss in previously absorbing animals which are suddenly exposed to sub-absorption humidities with no further disturbance, occurs much more rapidly than is normally found of those humidities. The trend of such losses indicates that water is transpired from a fluid compartment of significantly