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HYDROPHILIC CUTICLE – THE BASIS FOR WATER VAPOUR ABSORPTION BY THE DESERT BURROWING COCKROACH, *ARENIVAGA INVESTIGATA*

BY M. J. O'DONNELL

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

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SUMMARY

(1) A micro-method has been developed for measuring the water content of materials over a range of humidities. A vapour pressure osmometer measures the equilibrium humidity in a sealed chamber containing the sample and an accurately known volume of water.

(2) The cuticle of the hypopharyngeal bladders, which are the sites for atmospheric water absorption in *Arenivaga*, has a water affinity much greater than that of unspecialized cuticle from this and other species. This difference is also found in washed samples. The hydrophilic properties of the bladder cuticle are therefore not due to dissolved salts in the frontal body fluid which is applied to the bladders during absorption *in vivo*.

(3) Dissolved salts reduce cuticle water affinity. The basis for this effect is discussed with reference to known properties of polyelectrolytes.

(4) A model of *in vivo* absorption is proposed. It is suggested that the cyclical addition of frontal body fluid alters the water affinity of bladder cuticle so that condensed water is released. Some of the water is then swallowed.

INTRODUCTION

The desert cockroach is the first insect for which an oral site of water vapour absorption has been demonstrated (O'Donnell, 1977*a*, 1980). Vapour condenses on to two lateral diverticulae of the hypopharynx, called hypopharyngeal bladders, which protrude from the mouth (Fig. 1). An associated pair of spheroidal frontal bodies are situated beneath the frons. Cyclical contractions by muscles which connect the frontal bodies to the frons produce observable oscillations, during which the frontal bodies are distorted in such a way that they exude an ultrafiltrate of the haemolymph (O'Donnell, 1981*a*). This fluid is then conveyed by capillarity, through a groove in the epipharynx, to the bladders.

Fluid is held in the interstices of a dense mat of fine cuticular hairs which cover the bladder surface (O'Donnell, 1978, 1980, 1981*a*). Each set of 10–15 hairs arises from a blade-shaped base, 15–20 μm in length, which inserts into the epidermis

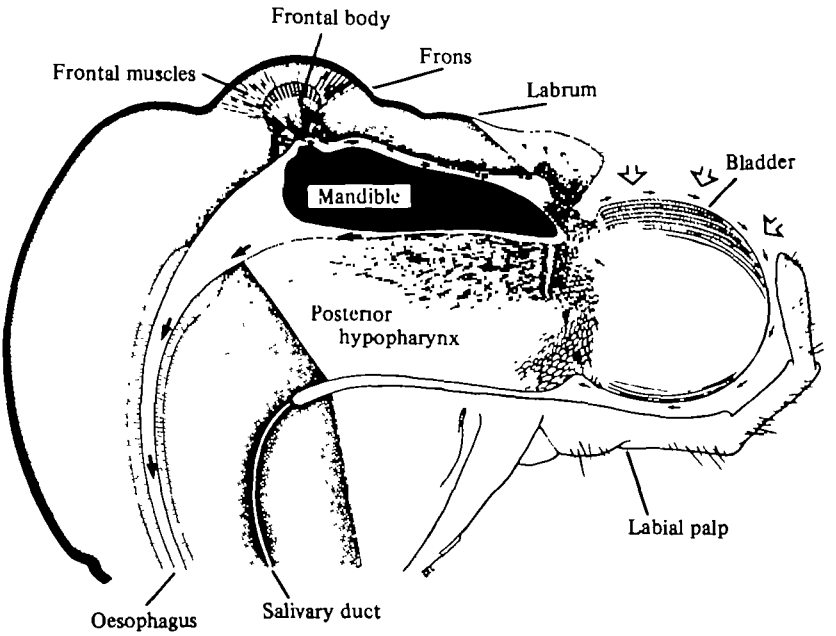

Fig. 1. Drawing of the head of *Arenivaga* with portions of the head removed to show the surface features of the distal end of the epipharynx and the hypopharynx. Parts of the head in sagittal section are stippled. Solid arrows indicate fluid movements from the site of production in the frontal bodies to the oesophagus. Atmospheric water condenses on to the bladders (open arrows) and is entrained in the fluid movements towards the oesophagus.

(O'Donnell 1978, 1981*a*). Over most of their length, hairs are 160–180 nm in diameter, and *in vivo* they bend so as to lie parallel to the epidermis.

Studies with fluorescent tracer solutions indicate that the fluid, and condensate, moves away from the end of the epipharyngeal groove, posteriorly over the hypopharynx to the oesophagus, and on to the crop. A valve, formed by the overlapping, tapering cuticular blades on the posterior hypopharynx, ensures one-way flow of condensate and prevents a backflux of water from the oesophagus to the bladder surface (O'Donnell, 1981*b*).

Any model proposed for the mechanism of atmospheric water absorption must account for several distinguishing features of the phenomenon in *Arenivaga*. (1) Absorption is energy dependent, requiring the movement of water against thermodynamic activity gradients between the atmosphere and the haemolymph as great as $2{\cdot}6 \times 10^4$ kPa. (2) Water vapour absorption involves the condensation of water on to the bladder surface (O'Donnell, 1977*a*, 1978). (3) Condensed water must continuously be removed to maintain water activity on the bladder surface below atmospheric water activity (O'Donnell, 1981*b*). (4) The frequency of frontal body oscillations increases with ambient humidity or after the addition of experimental solutions to the bladder surface (O'Donnell, 1981*a*). (5) If the epipharyngeal groove is cut, condensation on to the associated bladder surface ceases, and the bladder's surface appears dry (O'Donnell, 1981*a*).

A variety of experimental studies (O'Donnell, 1981*b*, 1982) have indicated that the

mechanism of absorption by *Arenivaga* differs markedly from the solute-dependent schemes proposed for other arthropods (Ramsay, 1964; Rudolph & Knulle, 1974; Wharton & Furumizo, 1977; Machin, 1979). These proposals require the primary generation of a water activity gradient through solute pumping, with resultant vapour absorption occurring as a secondary, passive phenomenon. However, in *Arenivaga*, neither the fluid on the bladder surface, nor the fluid source, the frontal bodies, maintain significantly elevated solute concentrations. Measured osmotic pressures in the frontal bodies, and the quantities of organic and inorganic solutes in the frontal bodies and in the fluid on the bladder surface, are 2–3 orders of magnitude below those consistent with a reduction in vapour pressure to a level equivalent with the lowest relative humidity (81 %) at which absorption is accomplished (O'Donnell, 1977*b*, 1981*a*, 1982).

The fluid applied on to the bladders from the frontal bodies, although necessary for absorption, therefore appears to play a subordinate role in whatever mechanism maintains a reduced water activity on the bladders. This suggests that the primary function of reducing water activity resides within the cuticle which forms the bladder surface.

This paper examines the water affinity of bladder cuticle. It was expected that water activity could be reduced either chemically, if the component molecules of the cuticle are remarkably hydrophilic, or physically, through capillary condensation. The latter process results from the lowering of vapour pressure above pores or spaces of very small radii of curvature. Such spaces could be found either between the cuticular hairs, or between the macromolecules which form the cuticle.

METHODS

Determination of cuticle water content by an equilibrium humidity (EH) technique

Gravimetric determination of the water content of bladder cuticle in accurately known humidities was difficult because the samples were often less than 15 μg in weight, and because currently available humidity sensors are generally only accurate to within 2–3 % R.H. A novel, non-gravimetric micro-method was therefore developed for determination of cuticle water content in different humidities. The equilibrium humidity (E.H.) technique measures water content of cuticle indirectly, through changes in relative humidity within a sealed chamber containing the sample and an accurately known quantity of water. The apparatus consists of a thermocouple sealed in an airtight Wescor water-potential cell and maintained at the dewpoint temperature by Peltier cooling. Electronic switching in a Wescor HR-33 Dew Point Microvoltmeter establishes a cycle in which the thermocouple alternately cools and measures the dewpoint temperature. The dewpoint value appears as a continuous reading on the microvoltmeter panel.

The lowest temperature to which the thermocouple can be cooled is characteristic of the materials forming the junction. This temperature represents a balance between Peltier cooling and resistive heating, and limits the lowest measurable dewpoint in 25 °C surrounding to 23·6 °C, equivalent to a relative humidity of 92 %. The instrument can measure relative humidities as high as 99·9 %, but the highest humidity

established in these experiments was 99·4 % R.H. Although absorption can be accomplished at humidities as low as 81 % R.H. (O'Donnell, 1980), the E.H. technique permitted water content measurements over a significant proportion of the range of humidities compatible with absorption *in vivo*. It was felt that the high degree of accuracy in the humidity measurement outweighed the limitations of the instrument's range.

The technique required that accurately measured volumes of water (1–200 nl) be added to the chamber. Calibrated nanolitre pipettes were inappropriate for this purpose because an indeterminate amount of water was lost by evaporation between expulsion from the pipette and sealing of the dewpoint chamber. Instead, water was retained on a strip of 0·22 μm pore size Millipore filter. Because the equilibrium humidity over pores of this size is in excess of 99·9% R.H., the strips had no significant effect upon chamber humidity. The essential feature of the Millipore material is the matt surface appearance which occurs when all the pores are filled but no excess water is present. The volume of water contained by a strip in this condition is directly proportional to its area.

A strip of known dimensions was placed in the chamber, moistened, and observed through a dissecting microscope. When the surface appearance of the strip changed noticeably from shiny to matt the chamber was quickly sealed. The volume of water retained in the chamber was calculated from the strip's area. The area–volume relationship was quantitatively determined by moistening strips of known area ($n = 14$) with tritiated water (1 mCi/ml) and then dropping the strips into 1 ml methoxyethanol in glass scintillation vials just as the excess water evaporated. The volume of water was calculated from the radioactivity, measured by a Beckman LSC 350 liquid scintillation spectrometer, and varied in a precisely linear fashion ($r^2 = 0{\cdot}997$) with the surface area of the strip according to the relation:

$$\text{volume of water (nl)} = 117{\cdot}1\ (\text{area of strip in mm}^2) - 4{\cdot}91$$

Humidity approached an equilibrium value asymptotically if a Millipore strip alone was sealed in the dewpoint chamber. Although only 0·8 nl was theoretically required to saturate the 40 μl of air in the chamber, a somewhat larger volume was required in practice because humidity was measured by condensation of water on to the thermocouple bead. In addition, minute quantities of water may also have been adsorbed on the chromium surfaces of the chamber.

The total volume of water condensed on the bead and on chamber surfaces was determined empirically by recording equilibrium humidities (E.H.) when known volumes of water alone were sealed in the chamber (Fig. 2). The volume increased with relative humidity and varied with different thermocouple beads.

Tissue placed in the chamber adsorbed water and produced a subsaturated E.H. The water content of the sample at the equilibrium humidity was calculated from the known volume of water added to the chamber, after subtraction of the volume condensed on the bead and chamber surfaces, the latter value being determined by reference to Fig. 2.

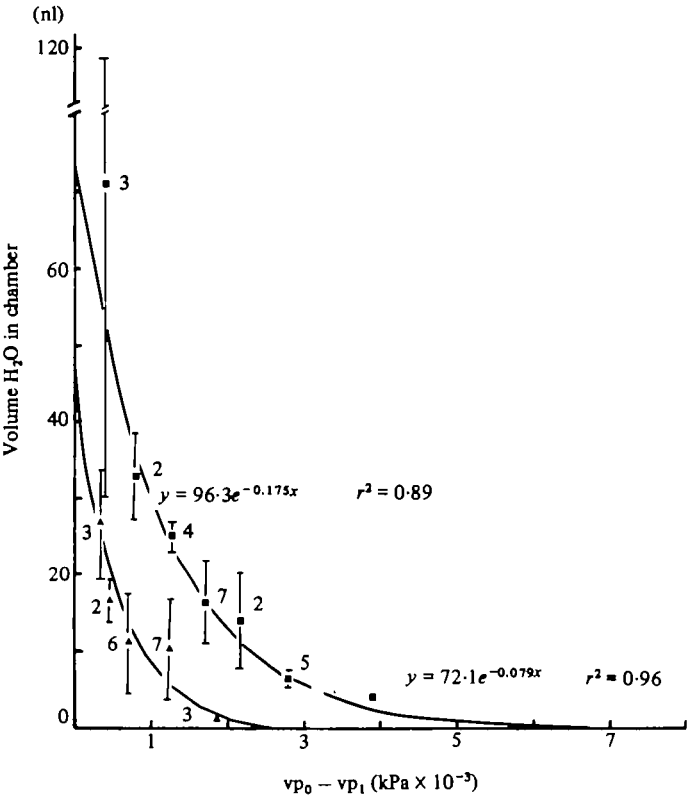

Fig. 2. Calibration curve for water retained on the thermocouple bead of the vapour pressure osmometer as a functon of vapour pressure lowering. Data are shown for two different beads (triangles, squares) used in the present study. Water contents of samples in chamber were calculated after subtraction of the volume retained by the bead at the equilibrium humidity.

Dry weight measurements

Cuticle dry weights, to the nearest 0·1 μg, were measured above silica gel in a Mettler ME22 electronic microbalance. Dry weights of bladder samples less than 10 μg were difficult to measure directly, and some were determined by interpolation of an empirically determined weight–surface area relationship. For this purpose, lyophilized bladder samples were weighed 3–5 times over silica gel, flattened between glass slides, and photographed. Surface areas of the samples were determined by weighing their images, which were cut from the photographic prints.

Scanning electron microscopy (SEM)

It was expected that if the water content of the hairs themselves increased when surrounding humidity was high, then dimensional changes of the hairs would be apparent. To test this hypothesis, animals were fast-frozen while absorbing water vapour, lyophilized, and the bladder cuticle dissected with tungsten needles and razor splinters (O'Donnell, 1981*a*). One or more samples of cuticle from each bladder were prepared directly for SEM (O'Donnell, 1981*a*). Other samples of cuticle from

each bladder were first washed and then fast-frozen in a drop of distilled water before being lyophilized and prepared for SEM. For both sets of bladder cuticle samples mean hair diameter was determined; the width of a group of adjacent, parallel hairs was determined from the micrographs, and divided by the number of hairs (usually 10–20). At the edge of the bladder samples some of the hairs were clearly exposed, and their diameters were measured individually.

Graphical analysis

The equilibrium humidity technique determined the water content of a sample at a particular humidity. Water contents were expressed as g H_2O/g dry weight and were plotted against humidity expressed as the reciprocal of vapour pressure lowering (RVPL) in $mmHg^{-1}$:

$$RVPL = 1/(vp_0 - vp_1)$$

where, vp_0 = vapour pressure at saturation and vp_1 = vapour pressure at an unsaturated humidity.

Vapour pressures are related to relative humidity by the formula:

$$RH = vp_1/vp_0.$$

Linear RVPL plots indicate that the sample's water content can be accounted for by colligative effects or by condensation into capillary spaces of greatly varying size. This type of plot also expanded the region of the humidity scale (90–100%) of most interest. The usable range of the E.H. method was 92–99·4% R.H., equivalent to 0·5–7·0 $mmHg^{-1}$ on the RVPL scale.

RESULTS

Dynamics of humidity change above cuticle samples

The time course of establishment of equilibrium humidity above cuticle samples within the dewpoint chamber was of two types, depending upon the volume of water added to the chamber. At high water contents, humidity approached equilibrium asymptotically (Fig. 3; upper trace). At low water contents, the curve showed a complex, biphasic nature. Humidity first increased rapidly, and then declined to an equilibrium which was maintained (Fig. 3; 2 lower traces). Humidities over inorganic salts placed in the dewpoint chamber approached equilibrium asymptotically, irrespective of the amount of water added to the chamber, confirming that the biphasic equilibration of humidity above cuticle was not simply an artifact of the measurement technique.

The biphasic pattern of humidity equilibration suggested that there was a delay in the wetting of the sample. The transient high-humidity phase apparently resulted from evaporation of water from the Millipore strip proceeding more rapidly than incorporation of water into the cuticle sample. Once the sample had been wetted, further condensation reduced surrounding humidity. At high water contents, wetting occurred before all the water evaporated from the Millipore strip, and the E.H. was approached asymptotically. Higher water contents in porous materials which have been previously wetted can result from capillary condensation, discussed below.

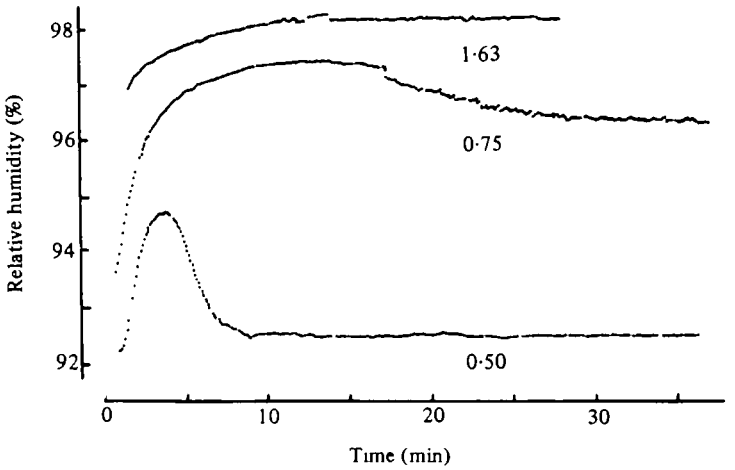

Fig. 3. Representative records of humidity equilibration over the same piece of bladder cuticle enclosed in the dewpoint chamber with different quantities of water. Numbers adjacent to the curves indicate cuticle water content (g H_2O/g dry weight).

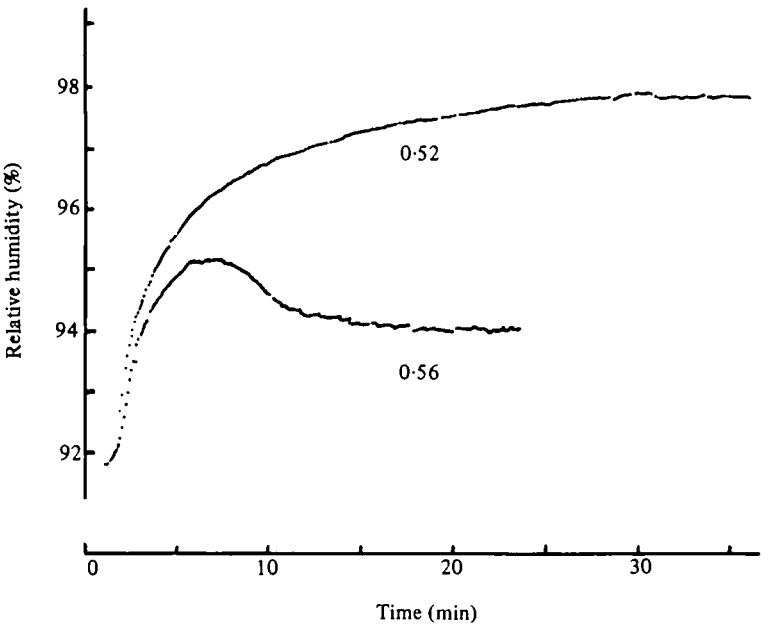

Fig. 4. Records showing different uptake kinetics and equilibrium humidities in bladder (lower) and abdominal (upper) cuticles with similar water contents (values indicated in g H_2O/g dry weight adjacent to each curve).

Water contents of cuticles under varying humidities

At comparable water contents, there was a much lower E.H. over bladder cuticle (Fig. 4; lower trace) than over hair-free abdominal cuticle (Fig. 4; upper trace). Complex interactions of water with bladder cuticle were also evident when water contents were determined over a wide range of humidities (Fig. 5). Available data for other insects have been included for comparison. Water contents of bladder

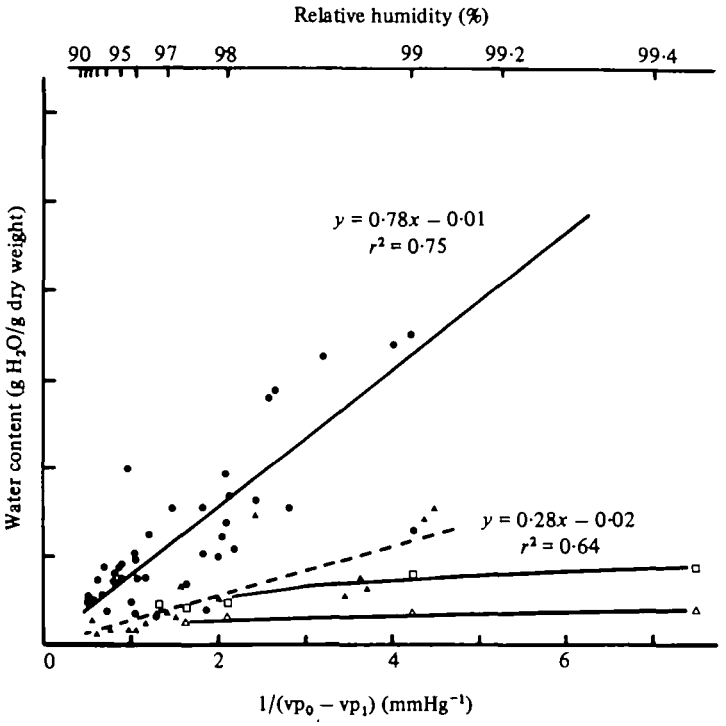

Fig. 5. Water content, determined by the equilibrium humidity technique, plotted against the reciprocal of vapour pressure lowering for hypopharyngeal bladders from absorbing animals which were quick-frozen and lyophilized (●), and abdominal cuticle of *A. investigata* (▲). Water contents of cuticle from *Periplaneta* (△), and *Locusta* (□ have been calculated from the data of Winston & Beament (1969). The corresponding regression equations are:

Locusta ($y = 0{\cdot}29 \ln x + 0{\cdot}32$; $r^2 = 0{\cdot}95$)
Periplaneta ($y = 0{\cdot}081 \ln x + 0{\cdot}22$; $r^2 = 0{\cdot}99$)

cuticle increased linearly over the entire RVPL range and were much greater than those of cuticle from other body surfaces. These differences cannot be attributed to the colligative effects of solutes of low molecular weights, because solute levels in bladder cuticle have been shown to be unexceptional (O'Donnell, 1977*b*, 1982).

Water content of washed cuticle

Further experiments were devised to determine the basis for the remarkable water affinity of bladder cuticle. The water content of cuticle can be divided into three components. Water may be: (1) associated with diffusible solutes of low molecular weight, (2) associated with non-diffusible macromolecules, and (3) condensed into capillary spaces either between the macromolecules which form the cuticular matrix, or between the hairs.

As a first step, the contribution of solute-associated water was determined from the difference in water contents before and after removal of solutes by washing in distilled water (Fig. 6). Although the quantities of water associated with solutes (upper shaded region of Fig. 6) appears large, much of it is attributable to solutes in

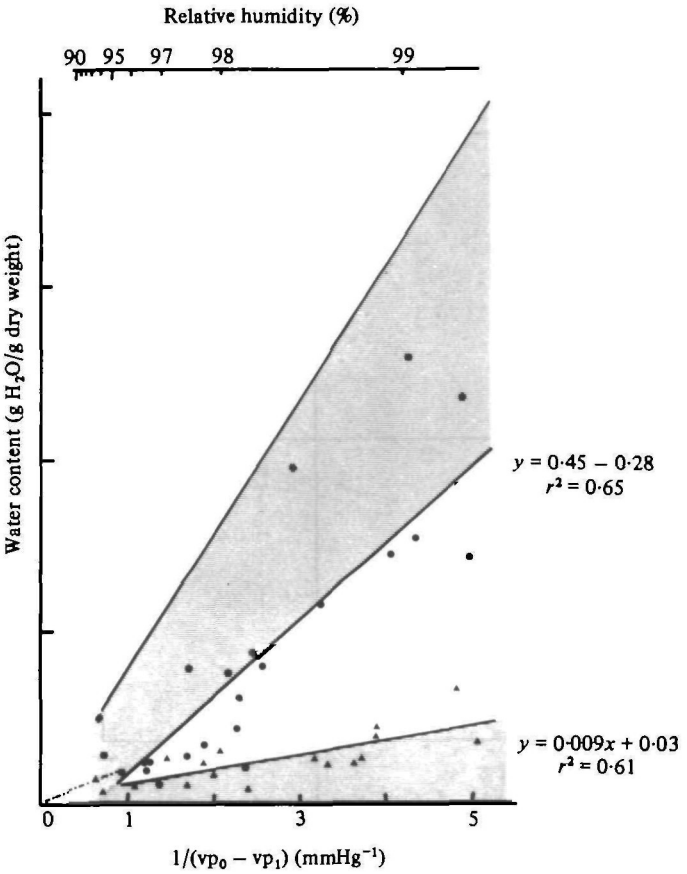

Fig. 6. Relationship of water content to humidity for washed cuticle from the bladders (circles) and the obdomen (triangles). The upper line is redrawn from Fig. 7. The water associated with diffusible solutes corresponds to the upper shaded region; water content of washed abdominal cuticle is given by the lower shaded region. The width of the unshaded region is the difference in bladder and abdominal water contents.

epidermal cells and adhering haemolymph (O'Donnell, 1982), rather than inherent to cuticle. Bladder cuticle had a much higher water content than abdominal cuticle at the same humidity, even after solutes had been removed. Cuticle of the posterior hypopharynx also contained a significant amount of water that was not associated with low molecular weight solutes (Fig. 7).

Volume changes of bladder hairs

In preliminary examinations of bladder cuticle, it appeared that hair diameters were greater if the cuticle had been washed and frozen in distilled water before lyophilizing (Fig. 8). The coiling angle of the helicoid groove which extends along each hair also appeared to be less in washed cuticles (Fig. 8). Further experiments verified that there was a significant increase in the radius of bladder hairs in the samples frozen in distilled water (Table 1). These results indicated that bladder hairs

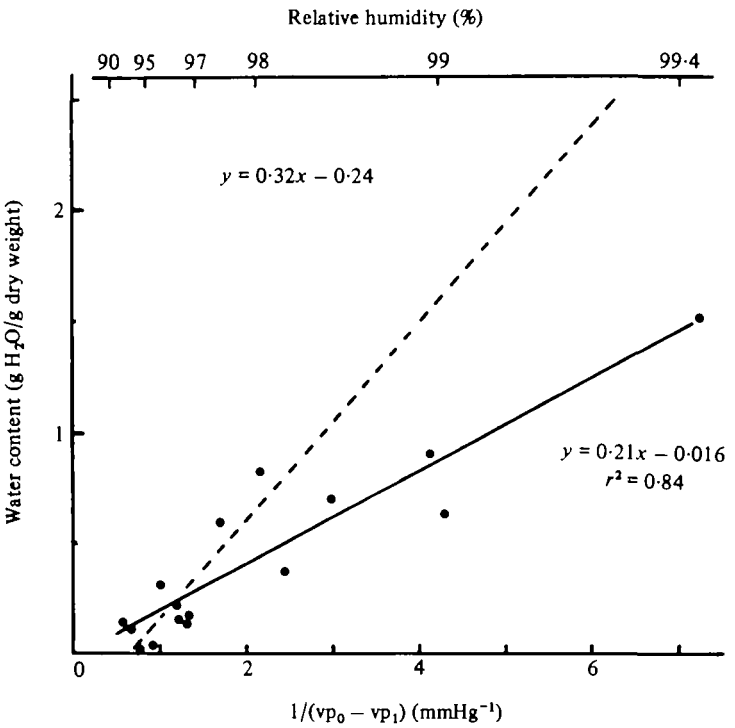

Fig. 7. Relationship of water content to humidity for washed cuticle from the posterior hypopharynx (unbroken line). The dashed line is the corresponding relationship for bladder cuticle, replotted from Fig. 6.

swelled in conditions of low tonicity. The hairs are therefore both hydrophilic and deformable. There is also some evidence for a decrease in the coiling angle (Table 1).

Effects of native solutes and NaCl on water affinity of bladder cuticle

The volume changes of hairs in conditions of varying tonicity suggested that an interaction between solutes and bladder cuticle might affect the water affinity of bladder cuticle. *In vivo*, the frontal bodies supply an ultrafiltrate of the haemolymph to the bladders. They might therefore be involved in modulating cuticle water affinity in such a way that absorbed atmospheric water could be released. Solute–cuticle interactions were therefore examined by comparing equilibrium humidities above samples of cuticle from absorbing animals and above the same samples after solutes had been removed from the sample but retained within the chamber. This was accomplished by washing the bladder tissue in several microlitres of water within the E.H. chamber, removing the cuticle to an adjacent part of the chamber, and allowing the wash water to evaporate. This process was repeated 2–3 times, the end result being that solutes were removed from the tissue but retained within the chamber. The net amount of material capable of reducing vapour pressure in the chamber was therefore unchanged. It was expected that if the macromolecules of the cuticle and the small-molecular-weight diffusible solutes exerted their effects upon vapour pressure

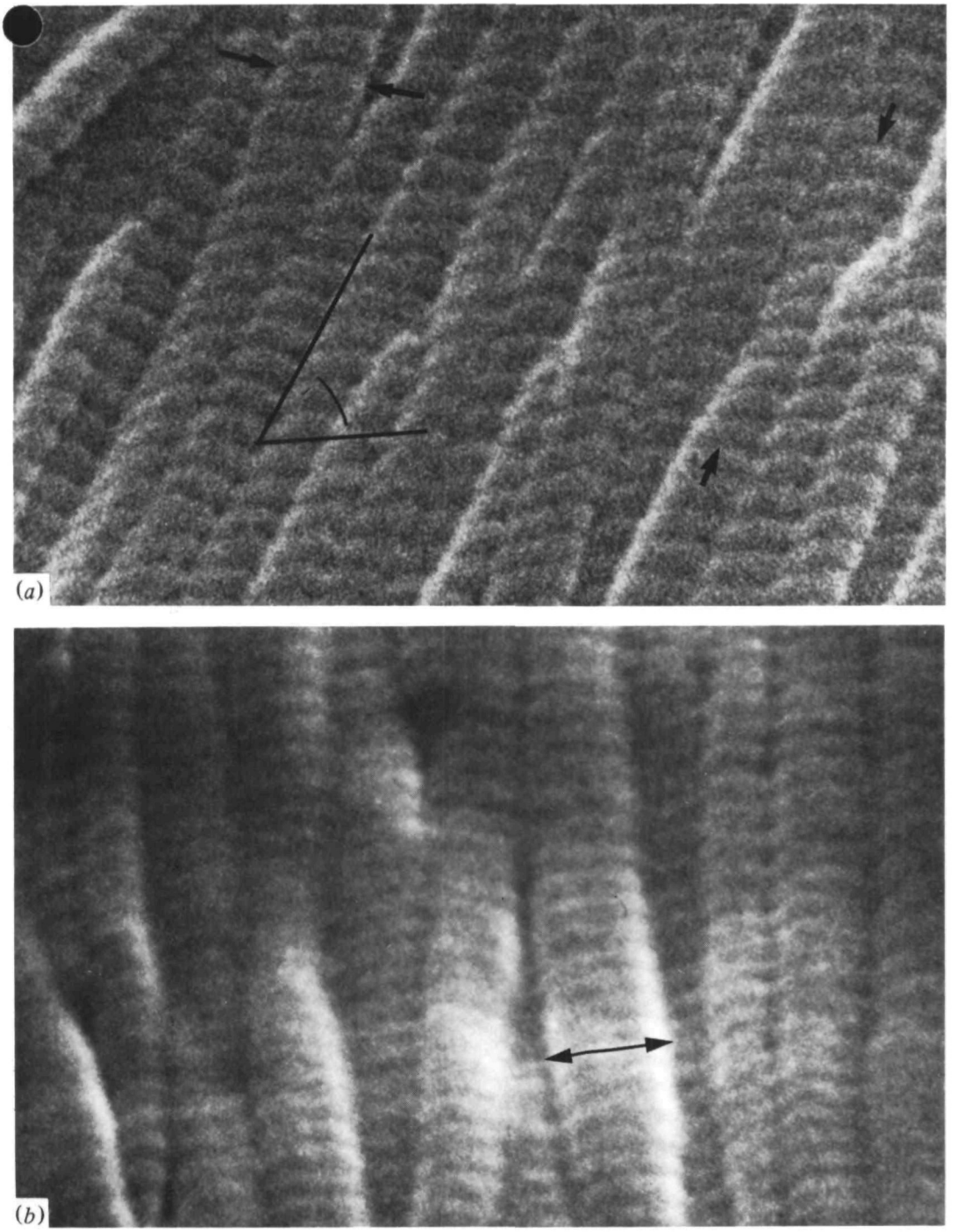

Fig. 8. (*a*) S.E.M. of the bladder surface of an absorbing animal which was quick-frozen and lyophilized. The longer of the intersecting lines is aligned parallel to the long axis of the hair, the shorter to the coils of the hair. The coiling angle is measured between the two lines. The arrows at top left indicate the diameter of one hair. The arrows at bottom right enclose 10 turns of the coil; the pitch of the helical groove is thus 1/10 of this distance. × 53800. (*b*) S.E.M. of the bladder surface of an absorbing animal which was quick-frozen, lyophilized, washed and frozen in a drop of distilled water and lyophilized a second time. The increase in diameter and coiling angle indicate swelling of the hairs primarily in the direction of the arrows on the bar. × 51600.

Table 1. *Dimension of cuticular hairs on washed bladders and absorbing bladders*

Condition	Radius (nm)		Pitch (nm)	Coiling angle (degrees)
	Individual	Group†		
Absorbing	91 ± 3 (6)*	84 ± 2 (6)	100 ± 5 (6)	71 ± 3 (6)
Washed	118 ± 7 (6)	112 ± 9 (6)	89 ± 6 (4)	75 ± 2 (8)
Student's *t*	3·96**	3·11***	1·32	1·48

† Determined from the width of a group of hairs divided by twice their number.
* $x \pm$ S.E.M.; number of animals in parentheses.
** $P < 0{\cdot}005$.
*** $P < 0{\cdot}01$.

independently, the E.H. in the chamber with the same amount of water would be the same whether or not the solutes were in contact with the bladder tissue.

In fact, when samples of cuticle were washed and the solutes retained within the Wescor chamber, equilibrium humidities at the same water content were lower than those found prior to washing. This indicated that the water affinity of the cuticle was higher when the solutes were in the chamber but not in contact with the tissue. Such results were found in 43 of 45 experiments with cuticle from 6 animals. The data are summarized in Fig. 9 (two upper lines).

The washing procedure disrupted the ordered arrangement of the bladder hairs, and might, therefore, also affect the tissue's capacity for capillary condensation. Also, the quantities of solutes involved are not exactly known, and are presumably larger than those added to the bladder surface *in vivo* by the frontal bodies, because of solutes contained within the epidermal cells of the bladder (O'Donnell, 1980, 1981*b*).

Equilibrium humidities were, therefore, also measured when known quantities of NaCl were added to the chamber along with bladder cuticle previously washed so as to remove native solutes. The quantities were chosen so as to be approximately equal to the quantities of NaCl and KCl known to be present on the bladder surface during absorption (O'Donnell, 1982). Equilibrium humidities were first measured when the NaCl was not in contact with the cuticle sample. The NaCl was then moistened with a drop of water, and the cuticle was placed in the drop, which was then allowed to evaporate. In this case, the same quantity of NaCl was then in contact with the bladder tissue. As for the experiments with the native solutes, equilibrium humidities were higher (35 of 37 experiments; bladders from 5 animals) when the solutes were not in contact with the tissue (Fig. 9; two lower lines). These results suggests that electrolytes (either native solutes or added NaCl) reduce the water affinity of bladder cuticle, and confirmed that this reduction was not due to the disruption of the ordered arrangement of bladder hairs which occurred during washing. A possible role for the frontal bodies in controlling the water affinity of bladder cuticle during absorption *in vivo* will be discussed.

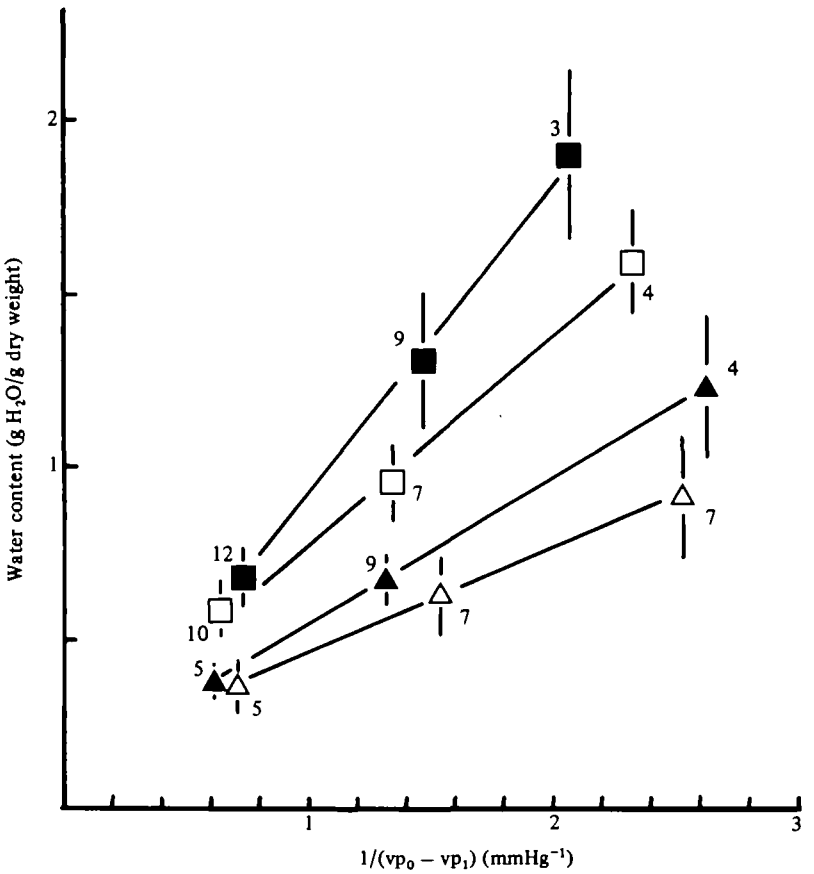

Fig. 9. The effects of electrolytes on the water affinity of bladder cuticle. Native solutes were either in contact with the bladder cuticle (□) or removed by washing but retained within the chamber (■). Small quantities of NaCl were added to previously washed samples (△) and were then removed from the cuticle by washing but retained within the chamber (▲). The numbers of samples are given adjacent to each point; lengths of the bars correspond to ± 1 S.E.M.

DISCUSSION

Water affinity of the bladder cuticle

This study has demonstrated the remarkably hydrophilic nature of cuticle from the hypopharyngeal bladders and the posterior hypopharynx. Moreover, the higher water affinities of washed samples of these cuticles, relative to the water affinity of unspecialized abdominal cuticle, indicates that their hydrophilic properties cannot be accounted for by the colligative properties of diffusible solutes. These results are consistent with those of previous studies (O'Donnell, 1980, 1981*a*, *b*, 1982) which have indicated that water vapour absorption in *Arenivaga* is not solute-dependent.

Condensation on to the bladders requires that their surfaces be wettable, and therefore hydrophilic. Wettability has been confirmed by the wet glistening appearance of the bladders during absorption (O'Donnell, 1977*a*), by the rapid spreading

and disappearance of aqueous solutions applied to the bladder surface (O'Donnell, 1981*b*), and by the swelling of hairs in conditions of low tonicity.

The hydrophilic properties of the bladder hairs contrast sharply to the water-repellency of the cuticular hairs which form the plastrons of other species. Plastron hairs are hydrophobic because of their small size and the hydrocarbon groups on their outer surfaces (Thorpe & Crisp, 1947). Bladder hairs, which are even smaller than plastron hairs, are wettable possibly because they lack the hydrocarbon groups which normally impregnate the insect epicuticle.

Possible mechanisms of water absorption

The experimental results prompt an important question concerning the mechanism of water vapour absorption: how much of the water content of bladder cuticle results from hydrophilic groups within the macromolecules which form the cuticle, and how much results from condensation into capillary spaces, either between the cuticular hairs or between the macromolecules which comprise bladder cuticle?

Capillary condensation is quantitatively described by the Kelvin equation (Defay & Prigogine, 1966), which relates the vapour pressure lowering above a fluid-filled space to the dimensions of the space. For a trough-shaped space, as is found between the cuticular hairs which form the bladder surface, the equation is:

$$\ln (vp_0/vp_1) = \ln (\text{R.H.}) = -SV \cos\theta\, (rRT)^{-1}$$

where S = surface tension, V = partial molar volume, θ = contact angle, r = radius of curvature of the fluid, R = gas constant and T = absolute temperature. Condensation will occur when $\ln (\text{R.H.}) < -SV \cos\theta\, (rRT)^{-1}$. For example, absorption at 81% R.H. would require the fluid between the bladder hairs to have a radius of curvature no greater than 3 nm.

Observations of bladder cuticle when the Wescor chamber was opened suggested that some water was retained in capillary spaces. As the cuticle dried, there was a lightening in colour and two-stage change in texture from shiny to matt. This behaviour suggests that drying occurred in two stages, and is consistent with the presence of water in two compartments; one compartment comprises the cuticle of the hairs, and the other is formed by the spaces between the hairs.

The delayed wetting of bladder cuticle samples in the Wescor chamber, mentioned with reference to Fig. 3, is also evidence that the bladder cuticle, *in vitro*, behaves as a porous structure and that some water is condensed in capillary spaces between the hairs. Delayed wetting of porous materials is attributed to interference by air adsorbed in capillary spaces (Defay & Prigogine, 1966). Wetting can also be impeded by geometric irregularities in a surface (Bikerman, 1950, 1958); the complex shapes of the capillary spaces between the helicoid bladder hairs may be important in this regard. Such effects will only be observed *in vitro*; *in vivo*, the bladders are wetted before they are protruded from the mouth (O'Donnell, 1977*a*).

There is, therefore, strong evidence for capillary condensation onto bladder cuticle *in vitro*. However, for bladder cuticle to absorb water vapour from relative humidities as low as 81%, vapour pressures in the spaces between the bladder hairs must be greatly reduced. If absorption proceeded solely through capillary condensation into

these spaces, and did not involve volume changes of the hairs themselves, some mechanism would be required to generate sufficient negative pressure ($-2{\cdot}63 \times 10^4$ kPa) to remove the condensate. Otherwise, the space would begin to fill, ln(R.H.) would equal $-SV(rRT)^{-1}$, and condensation would cease.

It is unlikely that the required negative pressures could be generated mechanically in *Arenivaga*. Although there is a large mass of muscle beneath the frons which contracts rhythmically, thereby distorting the frontal bodies and lifting the epipharynx towards the frons (O'Donnell, 1981*a*), a suction pump would require close application of the epipharynx to the hypopharynx. However, the mandibles are interposed between the epipharynx and the posterior hypopharynx, so there is little area for contact between the two structures. As well, the elasticity of the epipharynx (O'Donnell, 1981*a*) would make it unsuitable for use in a suction pump.

In summary, although a number of experimental observations suggests that some water is condensed in capillary spaces *in vitro*, it is unlikely that capillary condensation is the basis of absorption *in vivo*.

Towards a model of continuous atmospheric absorption

The swelling of the bladder hairs in conditions of low tonicity suggests that changes in the water content of the hairs are an integral part of the absorption mechanism.

Hydration and consequent swelling of macromolecules, such as chitin and protein in cuticle, are well-known phenomena (Ling, 1972). In proteins, water contents of 0·2–0·4 g H_2O/g dry weight at 90% R.H. are typical (Kuntz & Kaufmann, 1974). Variations in the water contents of cuticles have been demonstrated before (Fraenkel & Rudall, 1940; Hepburn & Joffee, 1976; Reynolds, 1975) and have been implicated in changes in the mechanical properties of the cuticle (Reynolds, 1975; Hillerton & Vincent, 1979; Vincent & Hillerton, 1979), and in the antennal hair-erection mechanism in mosquitoes (Nijhout & Sheffield, 1979). Reynolds (1975) suggested that the water content, and hence the extensibility, of *Rhodnius prolixus* abdominal cuticle is controlled through alterations of intracuticular pH.

The water content of *Arenivaga* bladder cuticle is more likely to be controlled through changes in ionic strength. Electrolytes, although they are not responsible for a significant lowering of vapour pressure during absorption (O'Donnell, 1982), appear to influence the volume of bladder hairs. The water affinity, and hence water content, of bladder cuticle is actually reduced by the presence of electrolytes such as NaCl.

It is well known that the water relations of polyelectrolytes, such as the chitin and protein of bladder cuticle, can be altered through variations in ionic strength. Salting-in and salting-out techniques are commonly used to alter the solubilities of proteins in aqueous solutions. An increase in ionic strength reduces the apparent osmotic pressure, and hence the swelling, of polyelectrolyte gels (Katchalsky, 1954; Robinson, 1965). Much of this effect arises because increasing salt concentration reduces the mutual repulsive forces between like charges on a protein or gel structure; a greater number of cross-links, possibly of the van der Waals type, is then possible, and shrinkage occurs (Katchalsky, 1954). The swelling of a gel, which increases with the square of the amount of charge placed on the gel, therefore varies inversely with the ionic strength of the surrounding medium (Hill, 1962).

Experiments with synthetic gels (summarized by Tanaka, 1981) indicate that the swelling of a polyelectrolyte is fully reversible and also discontinuous over a particular range of ionic strengths. For example, shrinkage of a polyacrylamide gel of more than 20-fold can be brought about by a change in NaCl concentration of less than 10 mM. Tanaka (1981) estimates that cylindrical gels of 1 μm in diameter could shrink in a few milliseconds.

It appears likely that absorption in *Arenivaga* involes swelling and then shrinkage of cuticular hairs, mediated through cyclical changes in ionic strength of the surrounding fluids. These changes are caused by the cyclical addition of frontal body fluid (O'Donnell, 1981*a*) to the bladder surface. The required change in volume of the hairs can be estimated from measured condensation rates. At 96% R.H., a 500 mg adult female absorbs 210 pl s^{-1} (O'Donnell, 1977*a*). For the same size animal, the bladders are approximately 1 mm in diameter, and their circumference is therefore 3·14 mm. About 80% of the circumference of the bladder, 2·5 mm ($= 3{\cdot}14 \times 0{\cdot}8 =$ L), is covered with cuticular hairs (O'Donnell, 1980). Dividing this length by the mean hair diameter gives the number of hairs in the surface layer, $N = (2{\cdot}5 \times 10^{-3}/2 \times 91 \times 10^{-9}) = 13700$. If each hair is approximated as a right cylinder, 91 nm in radius (r), the volume of the surface layer is $\pi r^2 LN = (3{\cdot}14)(91 \times 10^{-9})^2(13700)(2{\cdot}5 \times 10^{-3}) = 8{\cdot}91 \times 10^{-13}$ m^3(891 pl). If the hairs swelled to a radius of 94 nm, the corresponding volume is 951 pl, and the volume difference is therefore about 60 pl, or 120 pl for both bladders. If these volume changes occurred in phase with the frequency of frontal body pumping (2 s^{-1}; O'Donnell, 1981*a*), the animal could absorb 240 pl s^{-1}.

A small increase in the radius of the hairs is therefore sufficient to account for the volume of fluid which must be removed. An increase in 3 nm in the radius of all the hairs would produce, at most, only a 13 μm increase in the radius of each bladder. If such changes occurred, they would not be detectable through a dissecting microscope due to their small size and high frequency.

It appears that the hairs swell primarily through an increase in diameter (Table 1) rather than length. Anisotropic swelling of the hairs is consistent with the properties of fibrous materials such as chitin (Hepburn & Joffe, 1976). Marked swelling of chitin in a direction perpindicular to the fibre axis is observed (50%), whereas only small changes (10%) occur parallel to the fibres (Fraenkel & Rudall, 1940, 1947; Hepburn, 1972). Anisotropic swelling may be the result not only of the properties of the materials comprising the hairs, but also the helicoid nature of the hairs. The ratio of diameter to length will increase as the coiling angle approaches 90°.

A possible sequence of events during absorption, at the level of the cuticular hairs, is given in Fig. 10. At the start of a pump cycle, frontal body fluid increases the ionic strength of the fluid surrounding the hairs, thereby lowering their water affinity. The hairs release water and decrease in volume. The fineness of the hairs, and the helical groove which increases the surface area of each hair, facilitate a rapid response of the hairs to a change in ionic strength.

Next, negative pressures produced by swallowing may be sufficient to remove water from the posterior hypopharynx, which in turn would absorb some of the water and solutes from the bladder surface. Experiments in which finely porous materials were applied to the bladders (O'Donnell, 1981*b*) suggest that significant negative

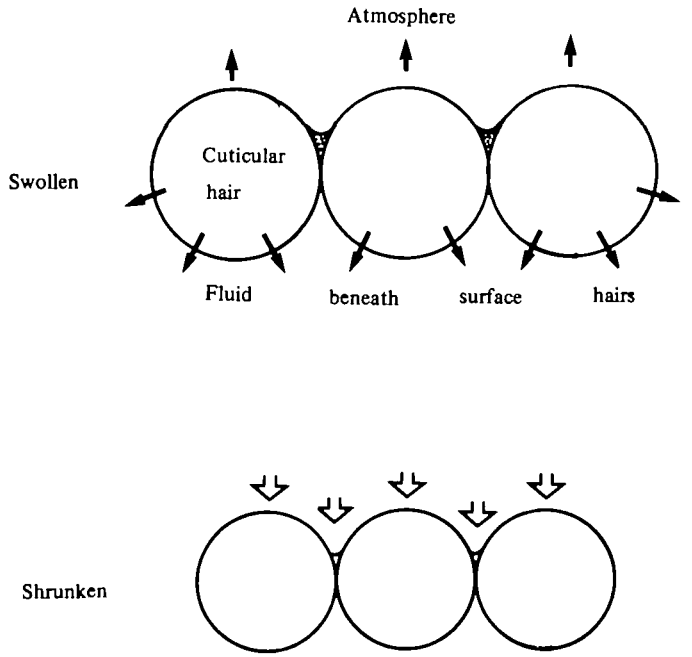

Fig. 10. Schematic diagram indicating the suggested sequence of events during absorption. Bladder hairs are depicted in cross-section. Addition of frontal body fluid (stippling) to the bladder surface decreases the water affinity of the bladder hairs, causing them to release fluid (solid arrows) and shrink. Some of the water released may be lost by evaporation, but most of it, and the frontal body fluid, is pulled away from the outermost layer of bladder hairs by the negative pressures generated by swallowing. The release of water as the hairs shrink lowers the ionic strength around them. Random condensations further reduce ionic strength around the hairs, making them sufficiently hydrophilic to promote net condensation of atmospheric water vapour (open arrows). The hairs swell, and the reduction in ionic strength as water condenses maintains the high water affinity of the hairs until the next addition of frontal body fluid.

pressures are, in fact, maintained between the hairs forming the bladder surface. Some of the water released by the hairs might also evaporate; in other words, the pump may be leaky. However, providing the amount of water swallowed is greater than that lost by evaporation, net absorption results.

Release of water from the hairs dilutes the surrounding fluids, decreasing the ionic strength in the region of the hairs. This prevents further reduction in the water affinity of the hairs. The water activity in the hairs will tend towards an equilibrium with atmospheric water activity. However, no equilibrium is reached, since the water affinity of the hairs at this point is unstable; thermal fluctuations and the randomness of molecular motions will result in some condensation on to the bladder surface, thereby further decreasing ionic strength and increasing the water affinity of the hairs. Net condensation begins, and the increase in the water affinity of the hairs continues until the situation is disturbed by the next addition of frontal body fluid.

This scheme implies a cycle in which a period of absorption is followed by a period of a smaller weight loss. A 500 mg female absorbs about 0·21 μg s^{-1}. If the gain/loss cycles occur in phase with frontal body pumping (2 s^{-1}), weight gain would

be of the order of 0·1 μg each cycle. Unfortunately, changes of this magnitude occurring at these frequencies cannot be resolved by currently available electronic microbalances.

It is important to note that fluid in the spaces between bladder hairs is in equilibrium with intracuticular water. Condensation will therefore occur both on to the surface of each hair and on to the surface of the fluid held between the hairs. However, because the area of the exposed upper surface of a hair of 90 nm radius is many times larger than the surface area of the fluid in a trough of 3 nm radius of curvature, most of the water will condense on to the cuticle directly. The contribution of capillary condensation to water absorption *in vivo* is likely, therefore, to be minimal.

When the flow of frontal body fluid is interrupted, the bladder surface dries instantly (O'Donnell, 1981*a*). In the absence of a supply of frontal body fluid, continued condensation will excessively dilute the fluid in the interstices of the uppermost layer of hairs. Swelling of the hairs may then be great enough to cause a rapid and dramatic increase in the radius of curvature of the interstitial fluid, which would be squeezed towards the surface. If its radius exceeds that which would be in equilibrium with the ambient humidity, the interstitial fluid would evaporate. Because it is such a small volume of fluid in relation to the surface area of the bladders, evaporation will be extremely rapid and the bladders will suddenly change from a wet to a dry appearance, with no intermediate condition. When this occurs to both bladders, the animal withdraws the hypopharynx, wets the bladders by salivating, and protrudes them once again (O'Donnell, 1980).

Further study, preferably with techniques such as nuclear magnetic resonance, will provide direct information on the state of water within the cuticle. Future work must also be directed at the mechanism by which the animal swallows fluid from the bladder surface and the forces generated by such a mechanism.

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