

AN ACTIVE REDUCTION OF WATER LEVEL IN INSECT CUTICLE

BY P. W. WINSTON AND J. W. L. BEAMENT

*Department of Biology, University of Colorado, Boulder, U.S.A. and
Department of Zoology, University of Cambridge, England*

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INTRODUCTION

The control of water movement through the cuticle of terrestrial arthropods is demonstrated by a number of phenomena. For example, Lees (1946) has shown an active retention of water, Winston & Nelson (1965) indicate an active regulation of water loss, and Beament (1961) reviews the evidence for active uptake of atmospheric water. These phenomena are best explained by a water pump of some kind in the cuticle (Beament, 1964). It is difficult to envisage a pump which does not, at least periodically, reduce the level of water activity in the cuticle with consequent exposure of the epidermal cells to osmotic gradients. But a pump, for example, of the kind suggested by Beament (1964, 1965) would not lead to such serious conditions for the epidermal cell as a directly acting osmotic system which is envisaged by Edney (1957). If the 'capillary suction pressure' proposal of Wharton & Kanungo (1962) is viable, it might not involve the epidermal cell in osmotic problems.

All the observed phenomena would come closer to an explanation if it could be demonstrated that the cuticle is not in equilibrium with the blood, so far as level of water activity is concerned, and much would depend on the actual level of reduction which might exist. The evidence based upon the water exchange of living animals would also lead one to suppose that the water level in the cuticle might be related to the humidity to which the animal is exposed. A preliminary report (Winston, 1967) suggests that the cuticle may not be in osmotic equilibrium with the blood; further evidence forms the substance of this paper.

MATERIALS AND METHODS

Final instar nymphs of *Periplaneta americana* and *Locusta migratoria* were used. Cockroaches were chosen 12-15 days after moulting, and starved for 24 hr.; locusts 3-5 days after ecdysis were starved for 3 hr., both at room humidity. Experimental humidity chambers, using saturated salt solutions (Winston & Bates, 1960) were set up with relative humidities of 1, 43, 55, 76 and 85% respectively. Animals were kept in these humidities for 4-7 hr. before being lightly anaesthetized with CO₂ and decapitated. In a chamber with humidity between 85-90% R.H. the prothoracic shield was cut off, scraped free of underlying muscle and vigorously wiped with filter paper to rid it of epidermal tissues and any free moisture. This procedure was reduced with practice to last only a few seconds. A disk 3.5 mm. diameter was punched from the

centre of the prothorax, wrapped in a pre-weighed cup of aluminium foil, and weighed on a torsion balance sensitive to 0.01 mg. The cups were then opened and hung in small jars containing sodium chloride solution to control humidity. The jars were placed in a water-bath ($25 \pm 0.05^\circ \text{C}$); control cups were used to check whether any precipitation occurred even with such close temperature control. Humidities were calculated from the data of Washburn (1928).

Other experiments (reported below) indicated that the average blood osmotic pressure of the animals was equivalent to 0.175 M-NaCl, and such a solution was therefore used for the exposure of disks from cockroaches. But locust cuticles proved to absorb so much water that in most experiments 0.3 M-NaCl was used, greatly reducing the chance that liquid water could form on the disks through precipitation. The disks were reweighed after equilibration for 24 hr. at blood humidity and dried at 85°C . to a constant weight to obtain their free water content.

Blood osmotic pressures were obtained by the method of Ramsay & Brown (1955). For these measurements animals were killed by inserting electrodes and exposing them to a shock at 120 V. for 5 minutes. This treatment would appear to have less disturbing effect on the composition of the blood than the use of chemicals or vapours of any kind. We appreciate that, in an ideal experiment, it would be valuable to know the blood osmotic pressure of each animal from which a prothoracic disk was obtained, and to suspend the disk in this particular humidity. But the need to be expedient with the treatment of the disk prevented such a nicety.

RESULTS

Disks from cockroaches varied in initial weight from 0.35 to 0.65 mg.; from locusts the range was 0.45–1.10 mg. The percentage free water in them (Fig. 1) varied greatly; in cockroaches it was 15–40% and in locusts 22–52%. There was no correlation between the percentage water and the initial weight of the disk. There is remarkably little evidence that the humidity to which an animal is exposed, previous to taking a disk, has any effect on the water level in its cuticle. The data could indicate that there was slightly more cuticular water in cockroaches from 85% R.H. compared with those from 1% R.H., but the surprising feature of these results is the variation of all the parameters concerned: weight of disk, free water content, blood osmotic pressure and blood volume.

On the other hand, the cuticle disk is not in equilibrium with blood osmotic pressure (Fig. 2). This was revealed in a number of ways. All the disks from cockroaches gained weight; those from locusts gained considerably in weight over 0.3 M-NaCl, and preliminary tests showed that their gain in weight over a solution representing blood osmotic pressure would have been much greater. In contrast, a number of animals were killed and then kept for 2 hr. in saturated air before disks were taken from them. Disks from these cockroaches did not change weight significantly over a solution representing blood osmotic pressure, while those from locusts lost a significant amount of weight when suspended over 0.3 M-NaCl. The conclusion is inescapable; in the dead animal the cuticular membrane has a water content in equilibrium with its blood osmotic pressure and in life the water content is much lower than this. It was not found possible to correlate the water level in life with the weight of the disk, with its

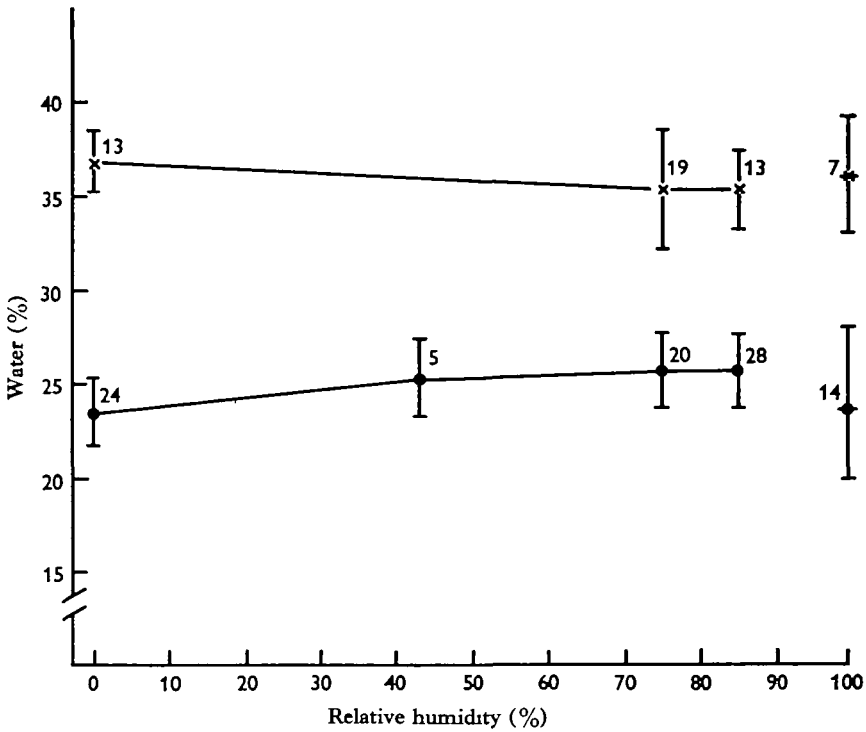


Fig. 1. Relationship between water content of cuticle and humidity to which an animal has been previously exposed. Water content is expressed as a percentage of the weight of a disk when removed from the animal. Upper line, *L. migratoria*; lower line, *P. americana*. Vertical bars, standard errors of means of the number of experiments indicated by each adjacent figure. Values on extreme right for animals killed long before removing cuticle samples from them.

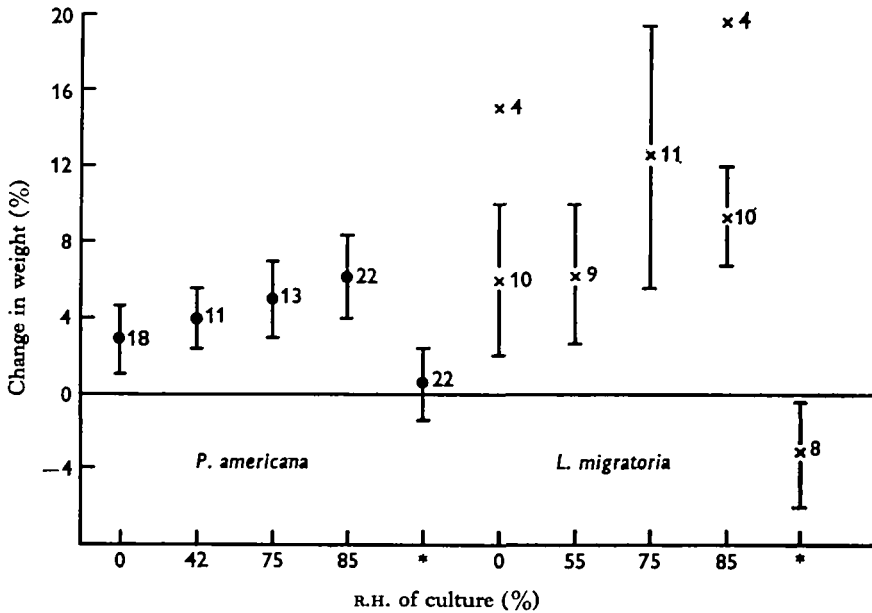


Fig. 2. The change in water content of excised cuticle disks when equilibrated at a humidity representing blood osmotic pressure. The base line represents the weight at excision and uptake or loss of water is shown as a percentage of this weight. *P. americana* equilibrated over 0.175 M-NaCl; *L. migratoria* over 0.35 M-NaCl. *Indicates animals killed before removing disks. Other symbols as in Fig. 1.

subsequent uptake of water, or with the humidity to which the individual animal had been exposed before the experiment.

Finally, Fig. 3 indicates the probable 'osmotic equivalent' of the water in the disks. Disks were suspended over 0.3 M and 0.6 M-NaCl, and higher osmotic equivalents. The figure indicates that the equilibrium value for the cuticle disks of both species

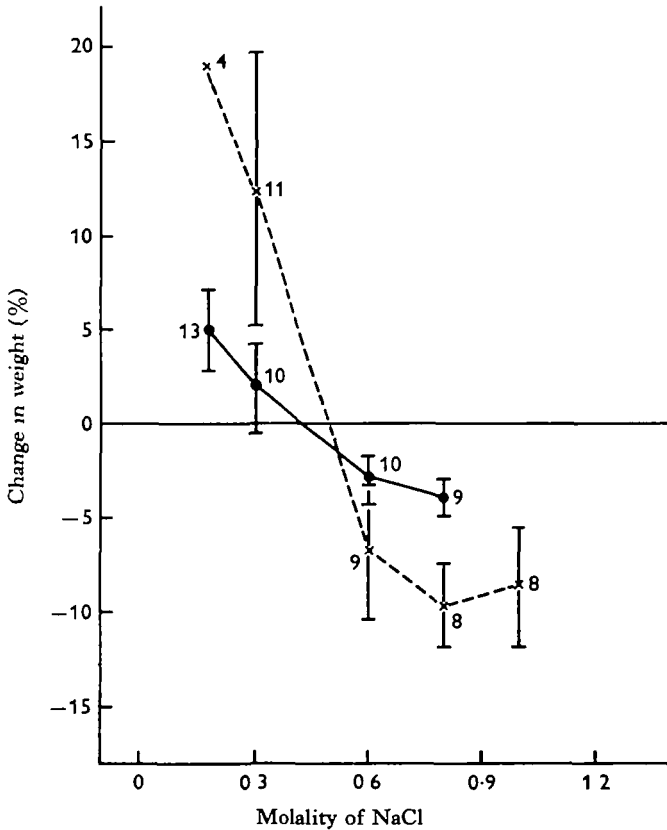


Fig. 3. Relationship between water content of excised cuticle and molality of solution providing the equilibrium humidity. Continuous line, *P. americana*; broken line, *L. migratoria*. Other symbols as in Fig. 1.

Table 1. The freezing-point depression in ° C. of the blood of cockroaches (*P*) and locusts (*L*) after exposure of the animals to various humidities

Dry air		42% R.H.		76% R.H.	85% R.H.	
P.	L.	P.	L.	L.	P.	L.
0.723	0.675	0.670	0.701	0.675	0.601	0.658
0.738	0.701	0.655	0.670	0.634	0.585	0.689
0.795	0.712	0.667	0.651	0.685	0.605	0.654
0.708	0.675	0.645	0.660	0.642	0.621	0.629
0.720	0.690	0.630	0.600	0.620	0.593	0.651
0.653	0.605	0.621	0.695	0.663	0.600	
			0.655	0.638		

would be between 0.45 and 0.5 M-NaCl. This is some three times the osmotic pressure of the average blood sample, and more than twice the highest value obtained from a single blood sample (Table 1).

DISCUSSION

Our demonstration that the cuticular membrane—in all probability we are concerned here with the untanned chitin-protein material—is not in equilibrium with the osmotic pressure of the blood of an insect is a significant one. It is not, however, spectacular. Recent discussions (e.g. Beament, 1965) have cited values of tens and even hundreds of atmospheres as the pressures against which pumps may have to work in the extreme cases of insects which take up water from very dry atmospheres. However, we are not here concerned with insects known actively to take up water, and indeed Edney (1966) has recently been unable to demonstrate any active uptake from water vapour by the cockroach we have used, though this occurs in *Arenivaga*. However, the quantity of water represented by the disequilibrium is more impressive. A cockroach cuticle in equilibrium with its blood would contain 20% more water than it does in life; a locust cuticle would contain 60% more. Without embarking on any lengthy discussion of mechanisms, one is certainly reminded of the analogy of the ram: a large volume at low pressure may be converted into a small volume at a high pressure. From every modern idea on the probable state of water in the cuticle (e.g. Bernal, 1965) the relationship between the water content of the cuticle and osmotic pressure will be very far from linear. But what is quite clear from these experiments is that something keeps the cuticle generally with a lower water activity than the blood. This requires an active mechanism and an expenditure of energy: a maintained difference of some 14 atmospheres. The anatomy of insects is such that the source of this energy can only lie in the epidermal cells themselves. While therefore we are no closer to the detail of the mechanism, we have at last positive evidence for its location within the integument as a whole, and for the hypothesis that water regulation may be achieved by lowering the level of water activity in the chitinous membrane.

SUMMARY

The water in cuticles excised from living cockroaches and locusts is not in osmotic equilibrium with their blood. It represents on average an osmotic pressure 15 atmospheres greater than the blood osmotic pressure. In the locust a cuticle in equilibrium with the blood would contain 60% more water. This is cited as evidence for the continuous expenditure of energy by the epidermal cell in regulating the water balance of the cuticle.

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