

WATER CONTENT AND WATER ACTIVITY IN THE CUTICLE OF TERRESTRIAL ISOPODS

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INTRODUCTION

The terrestrial isopods make an interesting parallel to insects in their adaptations to life on land (cf. Edney, 1968). In general, isopods have a poorer resistance to desiccation, but their behavioural reactions to external humidity contribute strongly to their success in terrestrial conditions, as reviewed by Edney (1968) and Lindqvist (1968). A thorough study of the permeability characteristics of the isopod cuticle is that by Bursell (1955), who dealt with *Oniscus asellus*. Since the effect of environmental humidities on terrestrial isopods is more pronounced than on most insects, we could expect that the water content and water activity of their cuticle also would reflect this difference. The insects *Locusta* and *Periplaneta*, both of which are relatively hardy in dry conditions, have a reduced water activity in the cuticle as compared to that of their blood (Winston, 1967; Winston & Beament, 1969); different environmental humidities do not affect the cuticular water content and only slightly the cuticular water activity in these insects.

The present paper aims at assessing the free water content and the osmotic characteristics of the cuticle of several species of terrestrial isopods, in the hope that it may throw some light on the mode and the site of water regulation in the cuticle.

MATERIAL AND METHODS

Porcellio scaber Latr. and *Cylisticus convexus* De Geer were collected from the south-western coast of Finland and *Armadillidium vulgare* Latr. and *Oniscus asellus* L. from near Visby, Sweden. In the laboratory they were reared in small plastic containers provided with paper towelling which was moistened at times to keep the relative humidity in the containers in excess of 90%. The room temperature was thermostatically controlled at about 24 °C and the experiments were conducted at the same temperature. The animals were fed on fresh carrot, but before each experiment they were starved for 24 h. Only adult intermoult animals of both sexes were used; no difference was observed between the sexes in the cuticular properties studied.

As the water contents of the cuticles from different tergites of the same animal differed slightly but irregularly, the samples were taken from the third tergite only, and in the following way. The animal was decapitated and the third thoracic shield

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was excised as rapidly as possible. The cuticle was wiped with a piece of filter paper to remove any remnants of tissue, and the lateral edges of the tergite were trimmed to a rectangle weighing 1-1.5 mg. This was wrapped in aluminium foil and weighed on a Cahn Electrobalance to the nearest 0.001 mg. The aluminium foils with the cuticle inside were dried *in vacuo* over CaCl_2 at room temperature for 24 h and reweighed to obtain the free water content. The dissection of the animals was conducted in a chamber having a relative humidity of about 98%; the balance was situated in the same chamber. The chamber was provided with a glass window and two holes through which the operator could push his hands to work inside.

The osmotic equilibrium point of the cuticles was determined by wrapping other samples in cups of aluminium foil and weighing them; after this the cups were opened and were left hanging in small glass jars containing different NaCl solutions to control humidity. Humidities were calculated according to the tables of Washburn (1928). Then the jars were put into large well-insulated boxes to minimize and smooth out any changes in temperature. Any errors resulting from possible precipitation were corrected by the use of empty control cups. After 3 days the cups were weighed again and dried for 24 h to obtain the free water content. Before the cuticles were excised for equilibration, the animals were dried over silica gel for 30 min to remove the free water in the cuticle and to reduce variability.

The experiments reported here were conducted during the period from December to March. Blood osmotic pressures were determined by the method of Gross as described in a previous paper (Lindqvist, 1970).

RESULTS

Both *Armadillidium vulgare* and *Porcellio scaber* are relatively hardy in terms of water loss under terrestrial conditions, and for this reason their cuticular water content was measured first. In both species the free water content of the cuticle remained rather stable during desiccation of live animals for various times (Fig. 1). The mean water content was $54.0 \pm 0.78\%$ ($N = 70$) for *P. scaber* and $52.7 \pm 1.11\%$ ($N = 28$) for *A. vulgare*. The difference between the species is not significant. There was no trend as regards the desiccation time, nor did the body weight affect the cuticular water content. The mean weight of *P. scaber* was 51.1 mg (range 34.1-77.6 mg) and that of *A. vulgare* 70.0 mg (range 40.3-109.7 mg).

In those animals which were dehydrated for up to 7 h the cuticular water content was not significantly changed, even when the water loss from the body had amounted to about 25% of the initial body weight. The animals which died, however, after having lost 30% or more of body weight had cuticular water contents distinctly lower than those shown above; values from about 40% to as low as 25% were obtained depending upon the length of time the animals had been dead. This difference between living and dead animals would indicate that in dead animals water was being lost from the cuticle faster than it could enter from the blood, and that an active mechanism therefore maintains the high water content of the cuticle in living animals.

In these tests *P. scaber* showed a slight tendency for lower water contents in the cuticle after some desiccation, as compared to non-desiccated animals. This was tested again in a further experiment at another time; there was a significant drop from

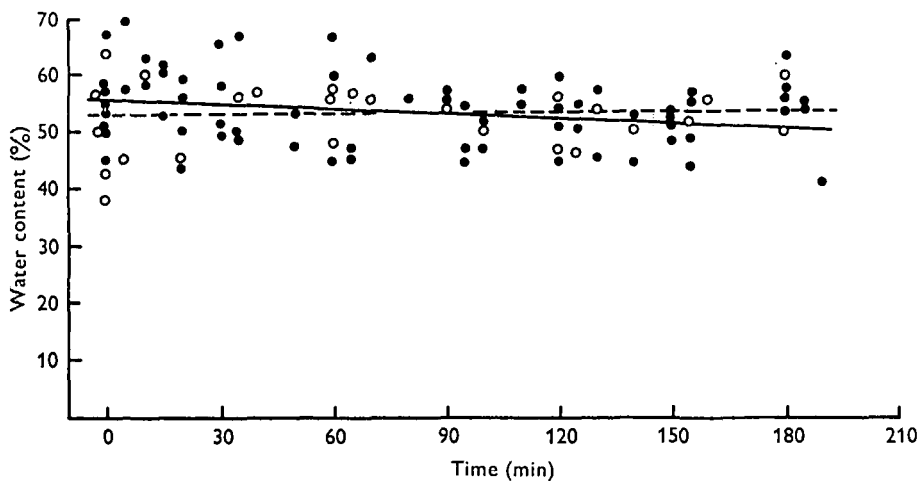


Fig. 1. The water content of the cuticle of *A. vulgare* (open circles) and of *P. scaber* (closed circles) after desiccation of intact animals for various times over silica gel. The unbroken line is the regression curve for *A. vulgare*, the broken one for *P. scaber*.

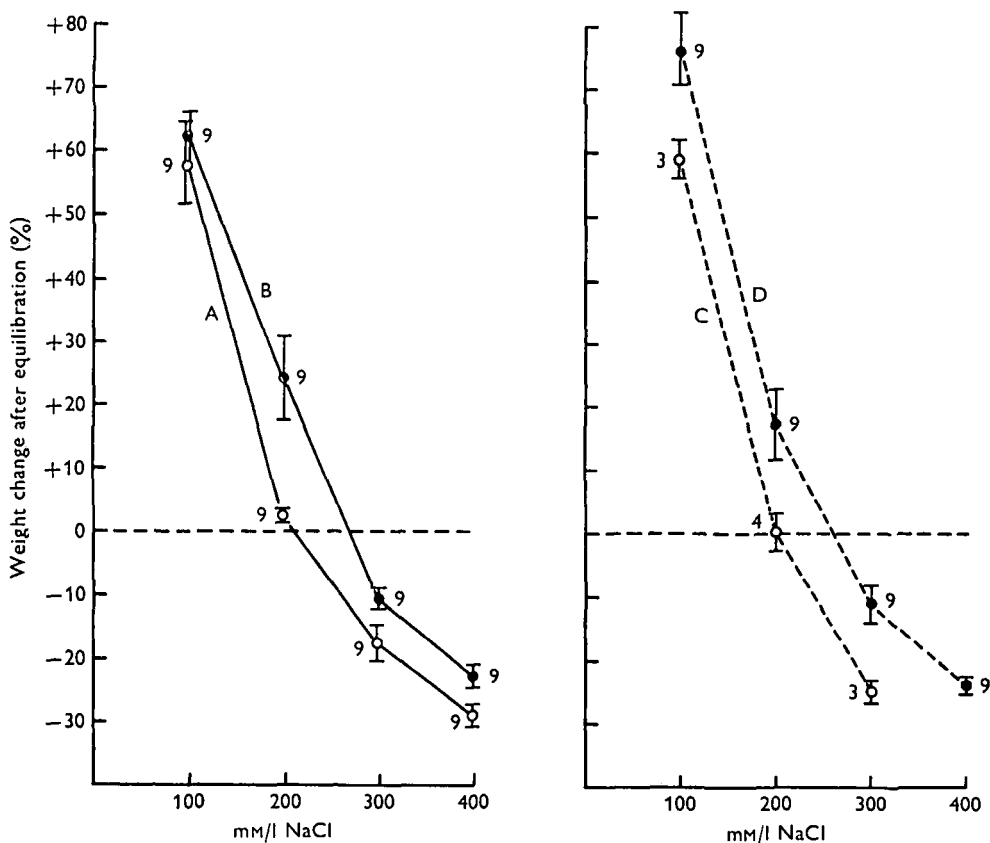


Fig. 2. The change in weight in percentage of the excised cuticle after equilibration at various humidities provided by different molal concentrations of NaCl. A, *P. scaber*; B, *A. vulgare*; C, *O. asellus*; D, *C. convexus*. Vertical bars indicate the standard errors of means of the number of animals shown by the adjacent figures.

$56.8 \pm 1.66\%$ to $50.4 \pm 1.61\%$ after 30 min. dehydration ($N = 7$ in both cases). In *A. vulgare* there was no change in the cuticular water content after this short desiccation period.

For measurements of the water activity of the excised cuticle, two less hardy species, *Oniscus asellus* and *Cylisticus convexus*, were included. Both have been found to have considerably higher rates of loss than either *P. scaber* or *A. vulgare* (cf. Warburg, 1965, and unpublished data). The water activity of cuticle in living, normal animals was in equilibrium with 200–270 mM/l NaCl (Fig. 2, Table 1). This is the concentration of salt over which the excised cuticle neither gained nor lost weight, i.e. it had the same vapour pressure as the solution. (The values are estimations of the point at which a plot crosses the line for no gain in weight, rather than direct measurements.) In contrast, the freezing-point determinations showed the blood pressures to be higher, averaging between 290 and 330 mM-NaCl (equiv.) per litre for the four species (Table 1). (The values for blood osmotic pressure of *P. scaber* and *O. asellus* are approximations from an earlier study (Lindqvist, 1970), while measurements of *A. vulgare* and *C. convexus* are 331.2 and 319.6 mM/l NaCl respectively ($N = 8$ in both cases) under similar conditions.) Thus the water activity of the cuticle is higher than that of the blood.

Table 1. *The osmotic relationship between excised cuticle and blood in four species of terrestrial isopods*

(The equilibrium points are estimations of the points at which plot (Fig. 2) crossed the line for no weight gain or loss.)

	Equilibrium point of the excised cuticle (mM/l NaCl)	Osmotic pressure of the blood (equivalent to mM/l NaCl)	Difference in osmotic pressure between cuticle and blood in atm.	Water content of the excised cuticle after equilibration	
<i>Armadillidium vulgare</i>	270	c. 330	-1.5	$50.8 \pm 0.85\%$	($N = 36$)
<i>Porcellio scaber</i>	213	c. 330	-2.8	$59.2 \pm 1.05\%$	($N = 36$)
<i>Oniscus asellus</i>	202	c. 290	-2.1	$61.0 \pm 3.29\%$	($N = 10$)
<i>Cylisticus convexus</i>	260	c. 320	-1.5	$49.6 \pm 1.25\%$	($N = 36$)

The free water contents of cuticles determined after equilibration (Table 1) were approximately 59% in *P. scaber*, 51% in *A. vulgare*, 61% in *O. asellus*, and 50% in *C. convexus*. These values did not differ significantly from those obtained at the same time from non-desiccated (and non-equilibrated) animals. In all four species, however, there is a statistically significant ($P < 0.05$) negative correlation between the water content and the osmotic equilibrium point of the cuticle ($r = -0.979$; Table 1). It can be seen that the higher the water content of the cuticle, the higher the water activity.

The measurements of cuticular water described here were conducted from December 1969 till late March 1970. There appeared to be a definite pattern in the water contents; the values obtained in December were all higher (especially in non-desiccated animals) than those obtained in the spring for both *P. scaber* and *A. vulgare*. The environmental conditions were kept as stable as possible during this period and they probably did not contribute to this gradual change. It is conceivable that the cuticular water is controlled periodically, linked with seasons or the breeding cycle. Gupta (1963) reported

that both *P. scaber* and *O. asellus* show an annual rhythm in the intensity of their humidity preference in a humidity chamber; whether this behavioural preference shows any relation to the control of cuticular water remains to be seen.

DISCUSSION

The transpiration rates of terrestrial isopods show a characteristic curve in dry air: at first the rate of water loss is high and only after some time does it reach a steady level (Edney, 1951; Bursell, 1955; Lindqvist, 1968). In *Armadillidium vulgare*, for instance, the initial rate $60 \mu\text{g}/\text{mm}^2/\text{h}$ was about six times higher than the plateau level (about $10 \mu\text{g}/\text{mm}^2/\text{h}$) (Lindqvist, 1968), which was reached only after 120–150 min desiccation. This is reflected in no way in the cuticular water content, indicating a strong degree of regulation of water levels in the face of high evaporation rates. *Porcellio scaber* showed nearly as good regulation, but there was always a difference of 1–5% in cuticular water content between desiccated and non-desiccated specimens.

The water content and the water activity of the cuticle are not necessarily comparable (Winston & Beament, 1969), but in these isopods they appear to be so. It is apparent in Table 1 that when the water content is high the water activity is also high (the equilibrium point is low), and vice versa. Thus, such a relationship would be expected to hold in other situations and, in general, when we speak of changes in one, it will mean changes in the same direction in the other.

The fact that the free water content of the cuticle remained almost unchanged during desiccation most probably means that there are some mechanisms, working in the living animal only, that drive water into the cuticle at rates which make up the evaporative losses. This is the more remarkable as the animal may lose 25% of its body weight without a significant drop in the free water content of the cuticle. As the body water content of normal *P. scaber* is between 65 and 72% (unpublished data), such losses could mean an increase of up to 45% in osmotic pressure of body fluids, provided that water levels were reduced by the same amount in all compartments. To explain the high sustained water activity in the cuticle, there may exist at least the following three alternatives. First, there may be special mechanisms in the epidermis or in the cuticle itself which tend to maintain the stability of the cuticular water activity (though not in osmotic equilibrium with the haemolymph) in spite of osmotic changes. Winston & Beament (1969) showed this pattern in *Periplaneta americana* and *Locusta migratoria*. Second, the blood osmotic pressure may not appreciably change with desiccation, but the blood volume would instead decrease with concomitant withdrawal of solutes from the haemolymph. Hence, the blood bathing the cuticle would be osmotically stable, reducing the energy needed to maintain the differential between the haemolymph and the cuticle. Quite recently Horowitz (1970) reports that during desiccation of *P. scaber* its blood osmotic values remained relatively constant for some period of time; this result definitely refers to this kind of regulation. Among the insects some cases are known where the blood osmotic pressure is regulated against the effects of hydration and dehydration (Edney, 1966; Djajakusumah & Miles, 1966; Wall, 1970), and the best example of such regulation known is in *Leucophaea maderae* where the water content of the cuticle (Winston & Hoffmeier, 1968) and the blood osmotic pressure

(Laird, 1970) are unaffected by long periods of desiccation and starvation. The potamonid crab *Sudanonautes africanus* shows increased concentrations of several plasma ions except sodium at mild desiccation (Lutz, 1969). It may be of some interest to note here that the fresh-water isopod *Asellus aquaticus* has about 20–30% of its total body sodium outside the haemolymph and most of it is concentrated in the Zenker's organ (Lockwood, 1959). The role of this sodium is not known, but it might be used in regulating the blood sodium levels. A third alternative may be that the blood osmotic pressures change somewhat according to hydration and dehydration, but all tissues are regulated osmotically and kept stable and the cuticle is just another tissue. However, this is not very probable, as Horowitz (1970) found that in *P. scaber* muscles at least lose water into the haemolymph during desiccation and some water may also be drawn in from the gut. Work has been started to investigate this problem more closely.

Further evidence for an active cuticular regulatory principle is the drop in water content at death. This would indicate that a barrier, possibly at the base of the cuticle, breaks down when the animal dies, allowing the rest of the cuticle to equilibrate with the blood. More water would be drawn out of the cuticle if the blood osmotic pressure also rises at this time. Thus these animals somehow possess a mechanism akin to the 'water pump' of some insects (Winston, 1967; Winston & Beament, 1969) which maintains cuticular water content and activity in the face of changing rates of water loss.

The adaptive advantage to keeping the water activity above that of blood even during desiccation is not clear. The few insects in which a 'pump' is known (Winston, 1967; Winston & Beament, 1969) show just the opposite effect; water activity is kept lower in the cuticle than in the blood, a condition which would seem to aid in the reduction of transpiration. In the terrestrial isopods the relatively poor waterproofing of the cuticle allows rapid water loss and, apparently, water must be 'pumped' in to maintain the level in the cuticle. If it were not, the cuticle would become dry and brittle, an obvious disadvantage, and the many sense organs of the cuticle possibly could not function properly in such a milieu. Also, a high cuticular water content may be advantageous in view of the fact that the terrestrial isopods seem to excrete most of their nitrogen as ammonia in gaseous form through body surfaces (Wieser & Schweizer, 1970).

SUMMARY

1. The water content of the cuticle of both desiccated and non-desiccated terrestrial isopods *Porcellio scaber* and *Armadillidium vulgare* was measured. The animals were desiccated for various times (up to 3 h) over silica gel and the mean water content of the cuticle was $54.0 \pm 0.78\%$ for *P. scaber* and $52.7 \pm 1.11\%$ for *A. vulgare*. There was no trend as regards the desiccation time, nor did the body weight affect the water content.

2. The water content of the cuticle remained virtually unchanged as long as the animal was alive in the desiccator. It dropped significantly after the animal had died after having lost some 30% of its body weight.

3. The cuticular water content of non-desiccated *P. scaber* tended to be slightly higher than that of desiccated ones. In *A. vulgare* no significant difference was observed between non-desiccated and desiccated specimens.

4. The water activity of the excised cuticle of the above two species and of *Oniscus asellus* and *Cylisticus convexus* was above that of the haemolymph and therefore not in osmotic equilibrium with it. The osmotic equilibrium points were below the osmotic pressures of the blood; the difference amounted from 1.5 to 2.8 atm. in different species.

5. The difference in water activity between blood and cuticle, the maintenance of water content with desiccation, and the drop in water level at death, all indicate the presence of an active mechanism regulating the cuticular water in terrestrial isopods.

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REFERENCES

- BURSELL, E. (1955). The transpiration of terrestrial isopods. *J. exp. Biol.* **32**, 238-55.
- DJAJAKUSUMAH, T. & MILES, P. W. (1966). Changes in the relative amounts of soluble protein and amino acid in the haemolymph of the locust, *Chortoicetes terminifera* Walker (Orthoptera: Acrididae), in relation to dehydration and subsequent hydration. *Austr. J. Biol. Sci.* **19**, 1081-94.
- EDNEY, E. B. (1951). The evaporation of water from woodlice and the milliped *Glomeris*. *J. exp. Biol.* **28**, 91-115.
- EDNEY, E. B. (1966). Absorption of water vapour from unsaturated air by *Arenivaga* sp. (Polyphagidae, Dictyoptera). *Comp. Biochem. Physiol.* **19**, 387-408.
- EDNEY, E. B. (1968). Transition from water to land in isopod crustaceans. *Am. Zool.* **8**, 309-26.
- GUPTA, M. (1963). Seasonal variation in the humidity reaction of woodlice, *Oniscus asellus* L. and *Porcellio scaber* Latr. (Crustacea: Isopoda). *Proc. natn Inst. Sci. India* **29**, 203-6.
- HOROWITZ, M. (1970). The water balance of the terrestrial isopod *Porcellio scaber*. *Ent. exp. & appl.* **13**, 173-8.
- LAIRD, T. B. (1970). Humidity effects on hemolymph in *Leucophaea maderae* F. *Am. Zool.* **10**, 233.
- LINDQVIST, O. V. (1968). Water regulation in terrestrial isopods, with comments on their behavior in a stimulus gradient. *Ann. zool. Fenn.* **5**, 279-311.
- LINDQVIST, O. V. (1970). The blood osmotic pressure of the terrestrial isopods *Porcellio scaber* Latr. and *Oniscus asellus* L., with reference to the effect of temperature and body size. *Comp. Biochem. Physiol.* **37**, 503-10.
- LOCKWOOD, A. P. M. (1959). The extra-haemolymph sodium of *Asellus aquaticus* (L.). *J. exp. Biol.* **36**, 562-5.
- LUTZ, P. L. (1969). Salt and water balance in the West African fresh-water/land crab *Sudanonautes africanus africanus* and the effects of desiccation. *Comp. Biochem. Physiol.* **30**, 469-80.
- WALL, B. J. (1970). Effects of dehydration and rehydration on *Periplaneta americana*. *J. Insect Physiol.* **16**, 1027-42.
- WARBURG, M. R. (1965). Water relation and internal body temperature of isopods from mesic and xeric habitats. *Physiol. Zoöl.* **38**, 99-109.
- WASHBURN, E. W. (1928). *International Critical Tables*, vol. 3. New York: McGraw-Hill.
- WIESER, W. & SCHWEIZER, G. (1970). A re-examination of the excretion of nitrogen by terrestrial isopods. *J. exp. Biol.* **52**, 267-74.
- WINSTON, P. W. (1967). Cuticular water pump in insects. *Nature, Lond.* **214**, 383-4.
- WINSTON, P. W. & BEAMENT, J. W. L. (1969). An active reduction of water level in insect cuticle. *J. exp. Biol.* **50**, 541-6.
- WINSTON, P. W. & HOFFMEIER, P. (1968). Influence of ambient humidity on water content of cockroach cuticle. *Proc. Int. Union Physiol. Sci., Washington* **7**, 471.