CUTICULAR WATER CONTENT
AND THE RATE OF EVAPORATION IN THE TERRESTRIAL
ISOPOD *PORCELLIO SCABER* LATR.

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INTRODUCTION

The water content of the cuticle of normal and desiccated *Porcellio scaber* Latr. and *Armadillidium vulgare* (Latr.) has been measured earlier (Lindqvist, Salminen & Winston, 1972). The cuticular water content remains unchanged during desiccation as long as the animal is alive, suggesting the presence of an active mechanism for the maintenance of this water content. Furthermore, the osmotic activity of the cuticular water was found to be higher than that of the haemolymph. It is known that various treatments can alter the total rate of water loss from terrestrial isopods; among these are hydration and amputation of various appendages (Lindqvist, 1968; Lindqvist & Mäkilä, 1972). Hence, in order to obtain more information on how the cuticular water is regulated we have measured the water content of the cuticle after the above treatments. The objective of this study was to get some insight on the presence of possible water barriers and on the permeability characteristics of the isopod cuticle.

MATERIALS AND METHODS

The methods used in measurements of the amount of free cuticular water were essentially the same as described before (Lindqvist *et al.* 1972). *Porcellio scaber* Latr. were collected from the south-western coast of Finland, reared in plastic containers in the laboratory and fed on fresh carrot. All experiments were carried out at room temperature (24 °C). Adult animals of both sexes were used. For hydration, the animals were kept for 3 days in small boxes containing wet paper (and hence in water-vapour-saturated atmosphere) without food. Amputation of the fourth pair of legs was carried out with fine scissors on unanesthetized animals. This could be also done by pulling the legs out with small forceps; in both cases there was little if any bleeding. The antennae were cut off with fine scissors at the first segment. Prior to each experiment the animals had been starved for 3 days (including the 3 days for hydration). The free-water content of the cuticle was determined for the third thoracic shield.

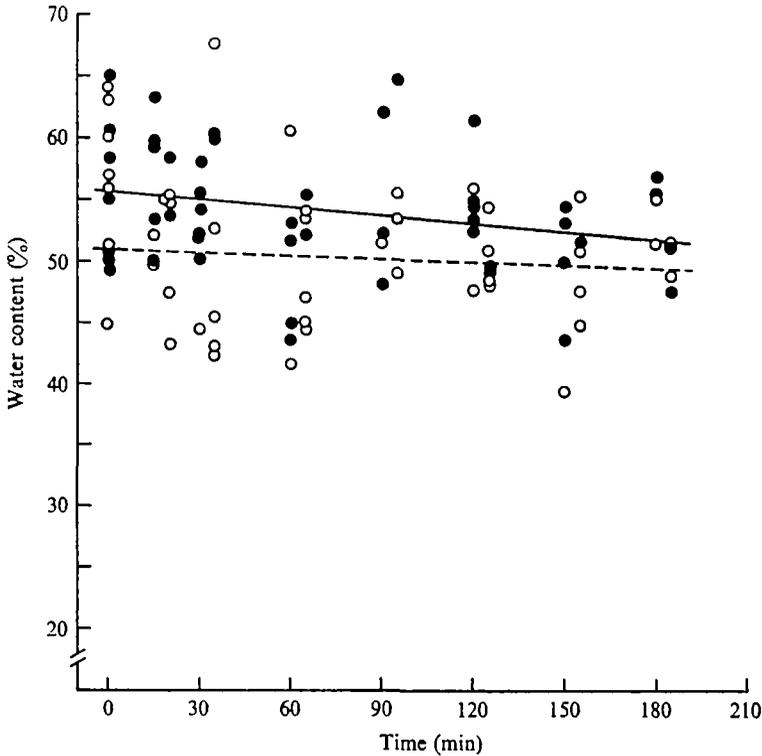* Present address: University of Kuopio, 70101 Kuopio, Finland. (Reprints.)

Fig. 1. The water content of the cuticle of *Porcellio scaber* after various periods of desiccation in dry air. Closed circles, antennectomized 24 h earlier; open circles, controls. The solid line is the regression curve for the antennectomized, the broken line for the control (unoperated) animals.

RESULTS

For a still unknown reason, amputation of both antennae of *P. scaber* reduces the rate of evaporation in dry air (Lindqvist, 1968) and this effect persists at least for 7 days (Lindqvist & Mäkilä, 1972). For the present experiment the animals were desiccated over silica gel for various periods of time (up to 185 min), after which the cuticle was excised for water measurement. If the antennae were amputated immediately before desiccation, the operated animals had higher water contents in the cuticle compared to the controls (unoperated): $56.5 \pm 0.81\%$ ($n = 25$) and $53.8 \pm 0.92\%$ ($n = 16$), respectively. This difference is significant at the level $P < 0.05$. A similar difference is observed if the water content is measured 24 h after antennectomy (Fig. 1). The mean cuticular water content of the operated animals was $54.2 \pm 0.76\%$ ($n = 47$) and that of the controls $51.5 \pm 0.89\%$ ($n = 47$). The mean values differ at the level $P < 0.05$. However, the animals that were not desiccated at all had about the same cuticular water content regardless of the treatment: $55.6 \pm 2.26\%$ ($n = 7$) for the operated, and $56.8 \pm 2.58\%$ ($n = 7$) for the controls. If these animals are excluded, the difference in cuticular water content between the operated and control animals (24 h after antennectomy) is still more significant ($54.0 \pm 0.80\%$ ($n = 40$) and $50.6 \pm 0.88\%$ ($n = 40$), respectively ($P < 0.01$)). In this experiment (as in all subsequent experiments) each control animal was matched with an operated animal
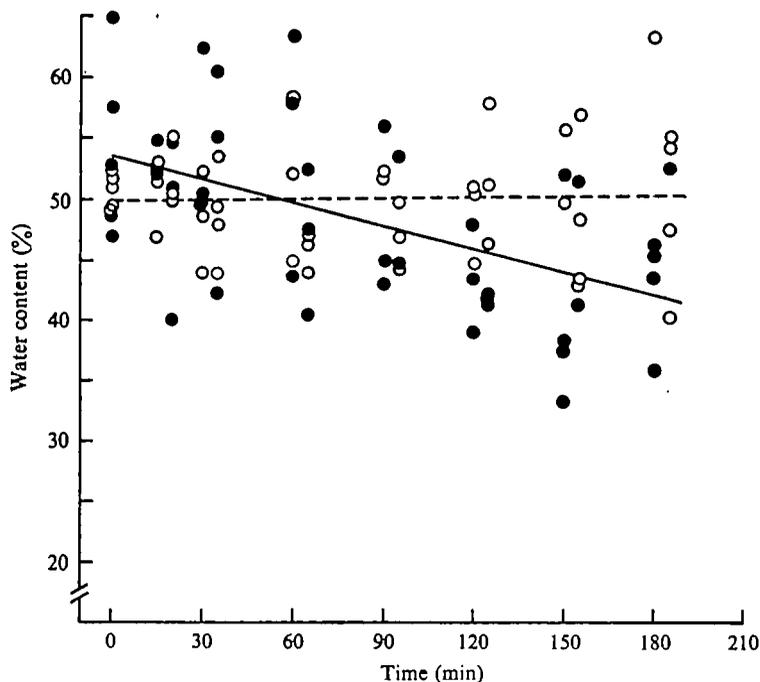
Fig. 2. The water content of the cuticle of *Porcellio scaber* after various periods of desiccation in dry air. Closed circles, the fourth pair of legs removed 24 h earlier; open circles, controls. The solid line is the regression curve for the leg-amputated, the broken line for the control (unoperated) animals.

of about equal size and same sex, and the cuticular samples were taken from the two only 5 min apart (as shown in Figs. 1 and 2). These experiments show that when the rate of water loss is reduced (after antennectomy) the cuticular water content is elevated, though this effect shows up only after desiccation.

The total evaporation rate is increased after removal of a pair of walking legs in *Porcellio* (Lindqvist & Mäkilä, 1972). The fourth pair of legs was removed 24 h before desiccation; the animals were desiccated for various periods of time (up to 185 min) over silica gel before the cuticular samples were taken. The mean water content of the cuticle was $48 \cdot 5 \pm 1 \cdot 10 \%$ ($n = 46$) for the operated animals, and $50 \cdot 0 \pm 0 \cdot 60 \%$ ($n = 46$) for the controls (Fig. 2). The mean values do not differ significantly. However, the water content of the cuticle of leg-amputated animals decreases with increasing desiccation. Linear regression analyses (water content *vs.* time) gave the following formula:

$$Y_{\text{(operated)}} = -0 \cdot 064X + 53 \cdot 7,$$
$$Y_{\text{(control)}} = 0 \cdot 005X + 49 \cdot 6.$$

The slope of the regression curve for the operated animals differs significantly ($P < 0 \cdot 001$) from zero as well as from the control curve (the significancies were calculated according to Hald, 1952). The undesiccated leg-amputated animals may have slightly higher water contents in their cuticle compared to undesiccated control animals.

The evaporation rate of *Porcellio* will be increased by hydration (Lindqvist, 1968) in the same way as by the removal of a pair of legs. The cuticle of hydrated *Porcellio* seems to be unable to hold water in dry air, and so we get decreasing water contents with increasing desiccation. The mean water content of the cuticle was $46 \cdot 5 \pm 1 \cdot 41 \%$ ($n = 32$) for the hydrated animals, and $46 \cdot 6 \pm 0 \cdot 96 \%$ ($n = 32$) for the normal ('dry') animals. Linear regression analyses gave:

$$Y_{(operated)} = -0 \cdot 077X + 53 \cdot 7,$$
$$Y_{(control)} = -0 \cdot 007X + 47 \cdot 3.$$

Again the slopes differ very significantly ($P < 0 \cdot 001$) from each other. Thus the effect of hydration on the cuticular water is approximately the same as that of the leg removal, i.e. the cuticle seems to be unable to hold water in dry air.

The level of the control values varies somewhat among the different experiments reported herein; the lower values were obtained towards late spring. Some of the reasons for this variation were discussed earlier (Lindqvist, Salminen & Winston, 1972); among them may be season, breeding cycle, starvation, etc. All the experiments were carried out on adult animals of somewhat varying sizes. However, no significant correlation between cuticular water content and body size could be detected, though it is known that the osmotic concentration of the haemolymph is size-related in *Porcellio* (Lindqvist, 1970).

DISCUSSION

The results obtained here on the relationship between cuticular water content and the overall rate of evaporation in *Porcellio* reveal an interesting pattern. On desiccation there seems to exist an inversed relationship between water loss and the cuticular water content. That is, when evaporation is high the amount of free water in the cuticle drops, and when evaporation is low the amount of free water in the cuticle is increased. At first, this prompted us to do some model building to explain the observed phenomena. For example, when there is a reduction in the total water loss the water content of the cuticle is elevated and it remains so despite desiccation. This may be an indication that water moves into the cuticle at a certain rate, but there is a barrier (on the surface?) which slows down evaporation. When this barrier is tight, more water will be contained by the cuticle itself. Similarly, if this barrier is leaking (as in hydration), evaporation is relatively high and the water content of the cuticle drops gradually. Thus it appears as if there were two barriers against water movement in the cuticle of *Porcellio* – one deeper in the cuticle or in the epidermis which allows passage of water at a certain fixed, but unaltered, rate, the other one nearer to the surface whose permeability could be altered by various treatments (possibly indicating the presence of physiological regulatory mechanisms for the cuticular water).

However, though this kind of cuticular model seems very attractive in the light of the present experiments, a few words of caution are also warranted. First, what we have been observing as regards the cuticular water may merely be changes in the water-binding properties of the cuticle. (The cuticular proteins may be involved in this process.) Secondly, the earlier tests to prove the presence of a waterproofing mechanism in the epicuticle of terrestrial isopods have given conflicting results (Auzou, 1953; Bursell, 1955; Warburg, 1965a, b) and the problem has not been settled yet. Thirdly,

recent experiments have shown that the exoskeleton is not the only major route for water loss in terrestrial isopods but regurgitated fluid from the mouth (and possibly also from the anus in hydrated animals) may contribute about one third to the total water loss (Lindqvist, 1971, 1972). The discharge from the alimentary canal spreads rapidly over the cuticle and especially over the articular membranes. How much this source of water contributes to the cuticular water is unknown yet. The high initial rate of evaporation during desiccation results almost exclusively from regurgitated fluid (Lindqvist, 1972), and yet the cuticular water content keeps the same in normal *Porcellio* and *Armadillidium* as long as the animal is alive (Lindqvist *et al.* 1972), indicating that the role of the regurgitated fluid may not be great. Only the slight drop observed in the cuticular water content between non-desiccated and desiccated *Porcellio* (Lindqvist *et al.* 1972) may result from the evaporation of this water from the cuticle. Also, the regurgitated fluid may contribute somewhat to the cuticular water in non-desiccated leg-amputated animals. Fourthly, the isopod exoskeleton is not homogenous but the articular membranes make up a relatively large part of the total area. The cuticular water content was measured for the (third) tergite only, and nothing is known of the water relations of the articular membranes.

The way in which the total water loss is affected by the removal of antennae or a pair of legs is still very much a puzzle. We suppose that volume (stretch) receptors might be involved, and it is at least conceivable that information from the stretch receptors (wherever they are) might be used to produce alterations in the evaporation rate or in the cuticular permeability. This aspect of the sensory anatomy of terrestrial isopods is poorly known. Only the pereiopodal mechanoreceptors of *Ligia* have been described (Alexander, 1969, 1970). On the other hand, amputation of appendages may stimulate moulting as it does in the land crab *Gecarcinus* (Skinner & Graham, 1970), with concomitant changes taking place in the cuticle and in the epidermal layer in preparation for moulting. Our observations showed that removal of either antennae or a pair of legs will generally lead to a moult in about 3 weeks, though no detailed measurements were made of the incidence of moulting. However, it is difficult to see in this connexion why the effect of antennectomy is opposite to leg removal as far as the changes in evaporation rates are concerned. There is also the possibility that the changes observed in cuticular water after various treatments may not necessarily be caused by the same mechanisms.

SUMMARY

1. The free-water content of the cuticle of the isopod *Porcellio scaber* Latr. was measured after treatments which are known to affect the total rates of water loss from the animal. The animals were desiccated for various periods of time in dry air, after which the cuticular samples were taken.

2. After antennectomy, which is known to reduce the evaporation rate in dry air, the water content of the cuticle remains steady but at a higher level than that of normal animals. The mean cuticular water contents 24 h after operation were $54.0 \pm 0.80\%$ and $50.6 \pm 0.88\%$ ($P < 0.01$) respectively for desiccated animals. The water content of the cuticle of non-desiccated animals was not altered by this treatment; the differences show up only after desiccation.

3. Both hydration and removal of a pair of legs can result in an increased rate of

evaporation; during desiccation the cuticular water content decreases with increasing(
desiccation. The slope of the regression curve for both hydrated and leg-amputated
animals differs significantly from both the zero slope as well as from the control curve.

4. A tentative model for the water permeability characteristics of the cuticle of
Porcellio is presented to explain the above data.

REFERENCES

ALEXANDER, C. G. (1969). Structure and properties of mechanoreceptors in the pereiopods of *Ligia oceanica* Linn. (Crustacea, Isopoda). *Comp. Biochem. Physiol.* **29**, 1197–205.

ALEXANDER, C. G. (1970). Studies on the nervous system of an isopod crustacean, *Ligia oceanica*. *Comp. Biochem. Physiol.* **33**, 323–32.

AUZOU, M.-L. (1953). Recherches biologiques et physiologiques sur deux Isopodes Onisciens: *Porcellio scaber* Latr. et *Oniscus asellus* L. *Annls Sci. nat. (Zool.)* **15**, 71–98.

BURSELL, E. (1955). The transpiration of terrestrial isopods. *J. exp. Biol.* **32**, 238–55.

HALD, A. (1952). *Statistical Theory with Engineering Applications*. New York and London: John Wiley and Sons.

LINDQVIST, O. V. (1968). Water regulation in terrestrial isopods, with comments on their behavior in a stimulus gradient. *Annls 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.

LINDQVIST, O. V. (1971). Evaporation in terrestrial isopods is determined by oral and anal discharge. *Experientia* **27**, 1496–8.

LINDQVIST, O. V. (1972). Components of water loss in terrestrial isopods. *Physiol. Zoöl.* (in the Press).

LINDQVIST, O. V. & MÄKILÄ, H. (1972). (In preparation.)

LINDQVIST, O. V., SALMINEN, I. & WINSTON, P. W. (1972). Water content and water activity in the cuticle of terrestrial isopods. *J. exp. Biol.* **56**, 49–55.

SKINNER, D. M. & GRAHAM, D. E. (1970). Molting in land crabs: stimulation by leg removal. *Science, N.Y.* **169**, 383–5.

WARBURG, M. R. (1965a). Water relation and internal body temperature of isopods from mesic and xeric habitats. *Physiol. Zöol.* **38**, 99–109.

WARBURG, M. R. (1965b). The evaporative water loss of three isopods from semi-arid habitats in South Australia. *Crustaceana* **9**, 302–8.