THE WATERPROOFING MECHANISM OF ARTHROPODS

III. CUTICULAR PERMEABILITY IN THE FIREBRAT,
THERMOBIA DOMESTICA (PACKARD)

BY J. W. L. BEAMENT

Department of Zoology, University of Cambridge

J. NOBLE-NESBITT

Department of Zoology, University of Cambridge and Department of Zoology,
University of Toronto

AND J. A. L. WATSON

Department of Zoology, University of Cambridge and Developmental Biology Center,
Western Reserve University, Cleveland, Ohio

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INTRODUCTION

The few quantitative studies on transpiration in lepismatid Thysanura have dealt principally with the relationships between temperature and relative humidity on the one hand and survival on the other (e.g. Sweetman, 1938; Lindsay, 1940). Such studies have shown that some of these primitive insects can tolerate surprisingly dry environments. Thus the silverfish Ctenolepisma longicaudata survives at a saturation deficit of 8.7 mm. Hg (cf. Lindsay, 1940). Even under optimal conditions, the firebrat experiences a saturation deficit of 8.0 mm. Hg and the insects can tolerate drier situations (cf. Sweetman, 1938; Sahrhage, 1953). Furthermore, Lindsay (1940) has shown that desiccated Ctenolepisma can resorb water from moist air.

The interpretation of these data is rendered more difficult by the lack of information on the cuticular structure of silverfish. Lower (1958) has provided a preliminary account of the integument in Ctenolepisma longicaudata but, apart from demonstrating that the epicuticle contains an outer layer of lipid, his work casts no light on the problem of cuticular permeability.

The present paper therefore considers the movements of water through the integument of the firebrat; and a paper to be published elsewhere (Noble-Nesbitt, 1964) discusses the structural basis of the observed permeabilities.

TRANSPERSION AND ITS CONTROL

The loss of water from dead firebrats

Adult female firebrats weighing between 20 and 50 mg. were isolated from the stock cultures and placed under optimal conditions, 37°C. and 84% relative humidity, as described in Watson (1964). On the day after ecdysis, when the cuticle was hard but
should have sustained minimal damage, the insects were anaesthetized with carbon
dioxide for 1 min., killed by exposure to hydrogen sulphide for a further minute, and
weighed on a torsion balance. The rates of transpiration were then determined by the
method of Beament (1959), but in an atmosphere of dry nitrogen. The resulting rates
of water loss were expressed as mg. of water lost/mm. Hg saturation deficit/hr./stand-
dard firebrat of 30 mg. body weight, the multiplicand (30 mg./observed weight) being used to correct for differences among the weights of the insects.

The intact *Thermobia* is very waterproof in comparison with other insects, losing on
the average only 0.0129 mg. of water/mm. Hg saturation deficit/hr./standard insect at
room temperature, i.e. between 19 and 26°C. The values in eight insects ranged from
0.004 to 0.026 mg., permeabilities which are comparable to those of unfed larval
*Rhodnius* (cf. Beament, 1959) (Table 1).

<table>
<thead>
<tr>
<th>Days after Ecdysis</th>
<th>Temperature (°C.)</th>
<th>Control insects</th>
<th>Abraded insects</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>34</td>
<td>0.0129, 0.004-0.026</td>
<td>0.0530, 0.044-0.064</td>
</tr>
<tr>
<td>2</td>
<td>34</td>
<td>0.0073, 0.007-0.008</td>
<td>0.0250, 0.021-0.030</td>
</tr>
<tr>
<td>3</td>
<td>34</td>
<td>—</td>
<td>0.0255, 0.021-0.033</td>
</tr>
<tr>
<td>5</td>
<td>34</td>
<td>0.0188, 0.007-0.041</td>
<td>0.0681, 0.007-0.010</td>
</tr>
<tr>
<td>7</td>
<td>34</td>
<td>0.0206, 0.008-0.034</td>
<td>0.0308, 0.008-0.057</td>
</tr>
<tr>
<td>1</td>
<td>34</td>
<td>—</td>
<td>0.0065, 0.006-0.007</td>
</tr>
</tbody>
</table>

Furthermore, the permeability rises with increasing temperature, there being a
sharp transition point at about 28–30°C. (Fig. 1). The curve is of the type shown by
insects with an epicuticular grease or, alternatively, by insects with two wax layers at
temperatures below the upper transition temperature. However, as the transition
point lies below the temperature at which the insects are cultured, the configuration
responsible for the transition phenomenon must reform rapidly on cooling, indicating

*The effect of abrasion*

Were a grease rather than a wax responsible for the impermeability, it should be
possible for a firebrat to repair the damage from abrasion without having to moult, for
a grease can spread across and waterproof a superficial lesion (Beament, 1955). Fire-
brats, isolated as before, were therefore abraded on the day after ecdysis, the scales on
the mesothoracic tergum being removed with a fine brush after 20–30 sec. of carbon
dioxide anaesthesia. Following the abrasion, the insects were returned to 37°C. and
84% relative humidity. The permeability was determined at various times after
abrasion, and at two different temperatures, 34°C. and room temperature; as the
The waterproofing mechanism of arthropods. III

latter ranged from 19 to 26° C., the two series lay on either side of the transition point. Unabraded firebrats at the same point in the stadium served as controls. The results were essentially the same at the two temperatures (Table 1).

![Permeability/temperature curve for Thermobia](image)

Fig. 1. Permeability/temperature curve for *Thermobia*

(1) The removal of scales causes an immediate increase in the permeability of the cuticle; the values immediately after abrasion are significantly higher than those for the intact animals, $P = 0.002$ (room temperature) and $P = 0.008$ (34° C.) (Mann-Whitney ‘U’ test; Siegel, 1956).

(2) The damage caused by the removal of scales is repaired within the stadium; indeed, the permeability may return to that of the controls within 48 hr. of abrasion (Table 1). A further set of data confirms the rapidity of the repair; a series of freshly abraded firebrats lost no more water during 3 days of dehydration than did intact animals (see below).

(3) Although the intact firebrats tested at the lower temperatures showed no significant increase in permeability as the stadium progressed, those tested at higher temperature were more permeable on the 5th and 7th days than they were on the 1st day ($P = 0.05$ for the 1st and 5th days, and $P < 0.027 > 0.028$ for the 1st and 7th days, as measured by the Mann-Whitney ‘U’ test; Siegel, 1956). These probabilities are near the limits of significance but they provide some support for the observation that *Ctenolepisma longicaudata* succumbs more readily to desiccation late in a stadium, perhaps due to the loss of scales (Lindsay, 1940).

The evidence therefore favours the hypothesis that a grease is responsible for the impermeability. It also indicates that the scales, which have a form similar to that of lepidopteran scales (Lower, 1958), do not play a major role in the waterproofing mechanism.
The location of the impermeable layer

As mentioned above, Lower (1958) demonstrated that the epicuticle of *Ctenolepisma longicaudata* contains a superficial layer of lipid, overlying an inner layer of protein. Could the homologous lipoidal layer be the impermeable grease of the firebrat?

If the waterproofing grease were superficial, one might expect it to spread over water (cf. Beament, 1955). This, however, is not the case; the grease shows no tendency to spread from intact or abraded firebrats on to clean water in the range 15–65° C., as judged by the lycopodium test of Beament (1945).

Preliminary attempts to extract lipids from the superficial layers of the cuticle also gave negative results; but they showed that in addition to repelling water, the cuticle repels, to a lesser degree, chloroform and benzene at room temperature. In this it differs from the wax- or grease-covered cuticles of *Rhodnius*, *Calliphora* and *Periplaneta*. Further tests showed that it also repels *n*-octane, methanol, *ethanol*, *n*-decanol, acetone, acetic acid, *n*-octanoic acid, amyl acetate and carbon tetrachloride. It wets, however, with diethyl ether and with methanol or ethanol near their boiling points; and after these treatments, it wets more or less readily with the other solvents in the cold.

The cuticles of approximately 350 firebrats were therefore extracted by plunging the insects into boiling methanol for 10 sec., and the resulting extract was dried down by warming under reduced pressure. The unctuous residue was then extracted several times with cold chloroform. After filtration and evaporation, the chloroform yielded a soft, brownish grease, which spread slowly on to water at room temperature but more rapidly on to water at about 40° C. This grease probably corresponds both to the waterproofing material and to the brown lipoid layer in the tergal and sternal epicuticles of *Ctenolepisma* (Lower, 1958).

The portions of the residue which did not dissolve in chloroform were redissolved in hot methanol, filtered and dried, leaving a hard, whitish precipitate. This material was insoluble at room temperature in the various non-wetting solvents mentioned above, formed a cloudy dispersion when water was added to a methanolic solution, and reduced ammoniacal silver nitrate. Unfortunately, insufficient was obtained for extensive tests; but it could well be the carbohydrate fraction of a shellac (cf. Beament, 1955) and form part of a cement layer (Noble-Nesbitt, 1964).

One may therefore suppose that the waterproofing layer in the integument of *Thermobia* is a stiff, epicuticular grease overlain by a resistant, imperforate lamella. The lamella may be a cement, but its wetting properties are unlike those of a true shellac.

THE UPTAKE OF WATER

Although it is clear from the above discussion that the dead firebrat is highly impervious to the outward flow of water, it is dangerous to extrapolate directly from this to the water balance of the living insect. Many pterygote insects can absorb water from saturated or subsaturated air; but this is an active process, ceasing when the insect dies. Thus the water balance of a living insect, disregarding metabolic water and the water taken in with the food, depends on the point of equilibrium between transpiration and absorption. In most of the pterygotes so far examined, this point lies at or above
relative humidities of 80–90%, but in a few insects net absorption is maintained down to a relative humidity of 50% (Edney, 1956; Beament, 1961). In at least some arthropods, moreover, abrasion disturbs the equilibrium, apparently involving some decrease in the absorptive capacity (Lees, 1947).

Lindsay (1940) has shown that the silverfish *Ctenolepisma longicaudata* can absorb water from air near the point of saturation. The point then arises as to whether absorption plays any part in maintaining the water balance of *Thermobia* and, if so, how the absorptive capacity is affected by abrasion.

### Absorption of water by intact firebrats

Adult firebrats showing the minimum of abrasion were selected from the stock cultures and weighed. They were then placed without food in clean glass vials, and were kept over CaCl₂ at 37°C for 3 days. The two sets, one of eleven and the other of twenty-two animals, lost an average of 25.5 and 24.6% of their initial weights during this period of dehydration, with standard errors of 1.51 and 0.72% respectively. The firebrats were then transferred to chambers maintained at various constant relative humidities (95, 85, 75, 66, 58, 52, 45, 33, 15 and 1%) by saturated solutions of appropriate salts, and were kept there, still without food, at 21°C for 24 hr. At the end of this period, they were reweighed and the changes in weight were calculated.

The results appear in Fig. 2, expressed as percentages of the original weight regained at the various humidities. The graph shows that the point of equilibrium lies at approximately 45% relative humidity, equivalent to a saturation deficit of 10.2 mm. Hg; all animals above 45% increased their weight during the 24 hr. whereas all those below 45% lost weight.

### The effect of abrasion on the absorptive capacity

To test the effects of abrasion, a series of firebrats was selected and dehydrated as above, except that the scales of the mesothoracic tergum were removed under brief anaesthesia immediately after the insects were removed from the stock culture and before they were weighed. The abraded firebrats lost an average of 25.1% of their initial weight during the 3 days of desiccation, with a standard error of 1.10%. This loss is not significantly different from the losses sustained by the intact firebrats, 25.5 and 24.6%, confirming the rapid restoration of waterproofing (cf. p. 325). The insects were then transferred, as before, to chambers at various relative humidities and were left there for 24 hr. before reweighing.

As Fig. 2 shows, the abrasion had little or no effect on the net absorption, at least down to a relative humidity of 58%; but the numbers are too small to assess the significance of the findings. Three animals, however, became moribund or died during the exposure to constant humidity, one each at 95, 84 and 66%. All three lost weight over the 24 hr., amounting to 2, 15 and 19% of the original loss during dehydration. Thus as in the pterygote insects and ticks, the absorption of water is an active process.

A further series of insects was set up to test the effect of abrasion immediately before exposure to constant relative humidity. The procedure was as before, except that the sequence of abrasion and dehydration was reversed; thus the animals were weighed, dehydrated, abraded and then reweighed. The twenty firebrats in this series lost an average of 33.1% of their original weight during the course of dehydration and
abrasion, with a standard error of 0.73%. These losses are significantly higher than those incurred by the unabraded insects or by insects abraded before the initial weighing and dehydration; in the latter case, $t = 6.02$ with 27 degrees of freedom so that $P < 0.001$. This greater loss is not due to the weight of the lost scales; in six control insects, similar abrasion caused an immediate decrease in weight of only 0.02–0.26 mg, with an average of 0.10 mg., equivalent to 0.45% of the initial weight.

![Diagram](image)

Fig. 2. The uptake of water by *Thermobia* exposed to different relative humidities after various treatments. A, Unabraded animals and animals abraded before dehydration; B, animals abraded after dehydration.

The absorptive capacity was also different from that of unabraded firebrats, when the absorption was expressed either as a percentage of the initial weight (Fig. 2) or as a percentage of the weight lost during desiccation. In the former case, the uptake is significantly lower at relative humidities of 58% ($P = 0.028$), 52% ($P = 0.032$) and 45% ($P = 0.014$) (Mann–Whitney ‘U’ test; Siegel, 1956); in the latter, the difference is heightened by the greater loss of weight during dehydration. The point of equilibrium thus lies at a slightly lower saturation deficit, about 9 mm. Hg. Although these
values are significantly lower than those of the unabraded animals, at least at the lower humidities, the difference is small; and it does not seem necessary to postulate anything more than a transitory increase in the rate of transpiration to explain it.

In these experiments on absorption metabolic effects were ignored. On the one hand, respiration could cause losses in weight; the overall loss of weight in all animals during the dehydration and rehydration may be a respiratory effect. On the other hand metabolic production of water by the partial or complete degradation of fat could give some increase in weight (cf. Edney, 1956). Calculation shows, however, that any such increases would be very small compared with the large and rapid changes noted in the experiments, for lipid comprises only 7–9% of the wet weight in adult firebrats (Schneiderman & Watson, unpublished).

CONCLUSION

It appears that Thermobia is an extremely waterproof insect, and that it can take up water through the cuticle with great rapidity, even from very dry air. These properties are not surprising in an insect whose original habitat was almost certainly desert or semi-desert (cf. Alfieri, 1932; Sahrhage, 1953); indeed, the underlying mechanisms may be common to many arthropods (Beament, 1959). Be this as it may, the results emphasize that the physiology of a primitive insect need differ little from that of a morphologically advanced form, a conclusion supported by studies on other systems and other apterygotes (Lasker & Giese, 1956; Lasker, 1959; Noble-Nesbitt, 1963a–c; Watson, 1963, 1964).

SUMMARY

1. The firebrat, Thermobia, is very impermeable to the outward flow of water, losing only 0.013 mg. of water/mm. Hg saturation deficit/hr./standard animal of 30 mg. between 19 and 26°C.
2. The permeability increases to a higher level at a critical temperature just below 30°C and well below the optimal temperature for the insect as a whole.
3. The waterproofing is mobile, for it regains its lower permeability on cooling below the transition temperature and is also restored a short time after abrasion.
4. Extractions support the hypothesis that the impermeable layer is a stiff, epicuticular grease but further suggest that an imperforate lamella, perhaps a cement, lies over the grease.
5. The live firebrat can absorb water from moist air, reaching an equilibrium between absorption and transpiration at a saturation deficit of approximately 10 mm. Hg at a temperature of 21°C.
6. Although abrasion of the integument decreases the net rate of water uptake and lowers the saturation deficit at which equilibrium is possible, it may act only by increasing temporarily the rate of transpiration.

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