

TRANSPIRATION IN *PODURA AQUATICA* L. (COLLEMBOLA, ISOTOMIDAE) AND THE WETTING PROPERTIES OF ITS CUTICLE

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INTRODUCTION

The unwettable nature of the cuticle of Collembola has long been known (Imms, 1906; Brocher, 1910). The cast skins of *Anurida maritima* on shores are not wetted by waves, and the living insect, when submerged for long periods by the tide, remains glistening because it is surrounded by a thin air-film. A similar air-film can be observed when *Podura aquatica* is forcibly submerged. Areas of cuticle which are extremely hydrofuge have been demonstrated on many pterygote insects (e.g. *Gyrinus*) and on *Peripatopsis*, which is regarded as a primitive arthropod (Manton, 1937; Manton & Ramsay, 1937). Further information about the surface properties of apterygote cuticle is of interest in relation to problems of phylogeny (see Beament, 1962), as well as in interpreting the role played by the cuticle in the surface phenomena which are so important in the life of the insect.

The habitats of the Collembola vary considerably; thus the litter- and bark-dwelling species have little free water to contend with (Ghilarov, 1959). But *Podura*, which lives on a free water surface, is constantly exposed to the dangers of submersion; it has been specially considered in relation to the movement by insects over water surfaces (Brocher, 1910; Wigglesworth, 1953; Paclt, 1956) and surface properties of the cuticle evidently play an important part in such locomotion. More recently, Baudoin (1955) has re-investigated this relationship, principally in *Anurida*.

The hydrofuge nature of the cuticle is associated with an exposed monolayer of lipid, and such an oriented monolayer is also considered to be extremely impermeable to water (Beament, 1960). But the Collembola are very sensitive to desiccation (Davies, 1928); many authors (see Richards, 1951) have accordingly assumed that a very permeable cuticle must be present, though the evidence is based on the survival and appearance of animals, rather than on actual measurements of permeability. Experiments of this kind can be misinterpreted; the appearance of shrivelling depends on the mechanical properties of a cuticle or egg-shell, and survival is an indication of tolerance of water-loss rather than a specific measure of desiccation. Small objects have a high ratio of surface area to volume, so that small animals dry up more rapidly than large ones with similar coverings. It has indeed been demonstrated that small insects, mites and their eggs can be very resistant to desiccation (Beament, 1946; Lees & Beament, 1948).

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The work described in this paper sets out to investigate the permeability (quantitatively) and wettability of the cuticle, and to correlate these with the fine structure and physiology of the cuticle. The implications of these findings on the life of the insects will also be discussed.

MATERIALS AND METHODS

Podura aquatica L. was collected from the surfaces of ponds and ditches in the Cambridgeshire area and kept in small aquaria in the laboratory. These aquaria formed the stock cultures. The method used to isolate individual specimens has been outlined elsewhere (Noble-Nesbitt, 1963*b*).

The relative humidity chamber

To follow the loss in weight of specimens of *Podura aquatica* hung in atmospheres of varying humidities the requirements of a balance were: (i) that the balance should be linear over the total weight range of the insect used, *Podura aquatica*, i.e. over the range of 0.1–0.3 mg., (ii) that the balance should read accurately to 1% of the total body weight of the insect, i.e. to 0.001 mg. (= 1 μ g.). A glass fibre balance was made which satisfied these requirements. The tip of the balance was viewed through a binocular microscope held horizontally and having a $\times 17$ calibrated eyepiece reading directly to one-hundredth of the scale, and which could be used to distinguish points half way between the scale markings. This gave effectively 200 scale divisions. Deflexions of the balance tip were noted when wire hooks, calibrated with a 10 mg. torsion balance, were added to the fibre balance. The fibre used in the experiments gave a deflexion of 84 for 0.10 mg. so that each division was equal to 0.0012 mg. (= 1.2 μ g.).

The fibre balance was firmly held in a cork, which also held a carrier fibre. The hole in the cork allowed the introduction of a manipulator when required. When not being used, this hole was plugged with a glass rod. The cork fitted firmly into a Carrell flask with a straightened neck. The saturated salt solutions used to maintain constant relative humidity (see below) were contained in the flask. The flask was firmly held by stout elastic bands to a horizontal stand which was attached to a micromanipulator, so that the whole apparatus could be finely adjusted. Fig. 1 shows the arrangement of the apparatus.

The tip of the balance was viewed through the vertical face of the flask, using the binocular microscope held horizontally. Hooks bearing the specimen were transferred between the balance and the carrier by means of the manipulator, and the weight of the hook unit was arrived at by noting the deflexions of the tip of the balance. The fine adjustment afforded by the micromanipulator, to which the apparatus was attached, allowed the setting of an accurate zero before each weighing.

The specimen which was to be desiccated was first anaesthetized with carbon dioxide and then attached to a pre-weighed glass hook with 'Newskin'. In a pilot series of experiments, in which *Pieris brassicae* larvae were copiously coated with 'Newskin', only very small differences in transpiration rate were obtained. Accordingly, it is thought that the very small amounts of 'Newskin' used to attach *Podura aquatica* to the glass hook in these experiments would have little or no effect on the transpiration rate. The amount of 'Newskin' used was less than 1 μ g., and its weight was

therefore ignored. The glass hook was then suspended from the carrier fibre and the cork, bearing the balance and the carrier, was carefully introduced into the flask, a stopper being removed from its neck just prior to this so that the relative humidity in the flask would not be altered appreciably. The flask was then quickly tied down in its stand with the elastic bands and the balance tip was set to zero. The manipulator was then used to transfer the hook unit to the balance and the weight of the hook plus the insect was measured. From this, the weight of the insect was arrived at by subtracting the weight of the hook. The loss in weight due to transpiration was then followed by taking readings of the balance at suitable intervals of time. These experiments were conducted at room temperature ($17-23^{\circ}\text{C}$., but usually $19-21^{\circ}\text{C}$.) and the relative humidity was varied between 0 and 95 % by using suitable salt solutions (O'Brien, 1948; see Table 1).

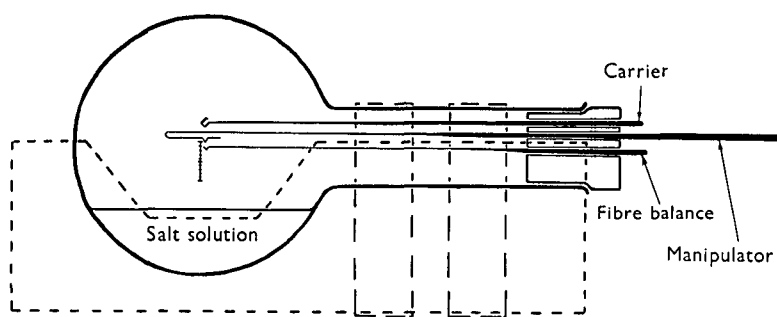


Fig. 1. The relative humidity chamber. The broken lines show the 'shoe' and elastic bands used to attach the apparatus firmly to the micromanipulator. For explanation see text.

Table 1. Saturated salt solutions used to maintain relative humidities of different values at 20°C . (from O'Brien, 1948)

Salt	% R.H.
P_2O_5	0
ZnCl_2	10
CaCl_2	32
$\text{C}_6\text{H}_{12}\text{O}_6 \cdot \frac{1}{2}\text{H}_2\text{O}$	55
$\text{CO}(\text{NH}_2)_2$	81
$\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$	90
$\text{Na}_2\text{SO}_4 \cdot 7\text{H}_2\text{O}$	95

The temperature/humidity chamber

Fig. 2 illustrates the apparatus used for experiments on the effect of temperature on cuticular permeability in *P. aquatica*. It was also used in all subsequent experiments, including the effect of dusts on the transpiration rate. This apparatus was a modification of the apparatus described in the previous section. Most noteworthy was the incorporation of a magnetically driven fan to stir the air and a temperature control unit to control temperature to within $\pm 0.02^{\circ}\text{C}$. of the required temperature. The temperature control unit was of the type devised by Beament & Machin (1959). The temperature of the air was measured with a 'gold-line' thermometer, which read to 0.1°C .

The balance was clamped in a holder which also held the carrier and the manipu-

lator. The holder was made from a Perspex rod and the balance, carrier and manipulator passed through rubber diaphragms which provided an air-tight seal. The holder was clamped in a side tube attached to the end of the box which formed the main body of the apparatus and the rubber diaphragms formed an air-tight seal at the junction of the holder and the box. The balance-holder was a sliding fit in the side tube. A cork was used to seal the side tube when the balance-holder was not in place.

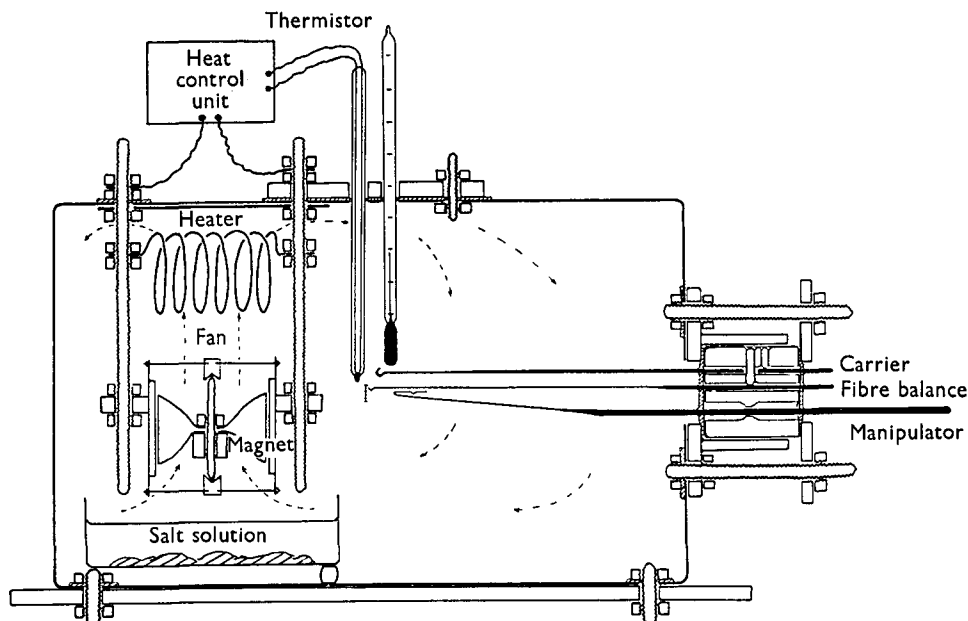


Fig. 2. The temperature/humidity chamber. The dotted arrows show the direction of the air-flow. For explanation see text.

The balance tip was viewed through one side of the Perspex box, and the microscope was mounted horizontally in a vertical-movement travelling stand so that the zero could be accurately adjusted.

The method of attaching the specimen to the glass hook was modified from that used in the apparatus described in the previous section. A small piece of fine nylon thread was attached to the glass hook with 'Newskin' and a noose was fashioned in the thread. This array was weighed on the fibre balance; only arrays of approximately $50\text{ }\mu\text{g}$. weight or less were used. One such array could be used for several determinations, with no inaccuracy due to an unknown weight of adhesive. The anaesthetized insect was attached to the glass hook by having the nylon noose tightened round the body just behind the head. In this region the body is very thin and the insect could not struggle free from the noose, although this was not tight enough to cause damage.

The hook with the insect held in the nylon noose was suspended from the carrier whilst being introduced into the temperature-humidity chamber, which was equilibrated for at least $\frac{1}{2}$ hr. beforehand. After the balance-holder had been firmly clamped, the fibre balance was set to zero on the scale and the hook-array was transferred to the fibre balance.

Experiments were conducted at 1° C. intervals over the temperature range 17–29° C. and at a relative humidity of 31–32 %, maintained by saturated calcium chloride solution held in a flat dish below the fan assembly. An 'Edney' paper hygrometer served to indicate when equilibrium had been attained after the apparatus had been opened up for overhauling, etc. Otherwise the relative humidity was maintained at 31–32 % so that long equilibration before each experiment was not necessary.

Estimation of area

In the experiments to be described the area of each insect was estimated from its body weight as determined at the beginning of the experiment, using the relationship

$$A = KW^{2/3} \quad (1)$$

where A = area in cm², W = weight in mg., K = a constant. The value of the constant, K , was estimated by measuring the weights and areas of six individuals and substituting the values in equation (1). The area was estimated by taking measurements using a calibrated eyepiece micrometer, of head and body lengths, widths and depths. The area of the mean equivalent cylinder calculated from these measurements was taken to be the area of the insect. At best this is only an approximation, but since it is derived from data similar to those of Holdgate (1956), it should provide results that are readily comparable with those for the insects considered by Holdgate. In any case the error will be more or less the same for each individual so that the accuracy of comparisons within species should not be impaired (Holdgate, 1956). The value obtained for K was 6.0 and this was used in all subsequent experiments to obtain surface areas from initial weights.

EXPERIMENTS AND RESULTS

The effect of relative humidity on the transpiration rate

Transpiration rates obtained by using the relative humidity chamber (Fig. 1) were calculated as mg./cm.²/hr., (a) at the relative humidity (R.H.) of the experiment (Fig. 3), and (b) corrected to 0 % R.H. (Fig. 4), and as mg./cm.²/mm. Hg/hr., to correct for differences in the saturation deficit (cf. Beament, 1958) due to variations of temperature (Fig. 5). In calculating these rates the steady rate of water-loss during the first 30–40 % loss in body weight was used. Above 40 % loss in body weight the rate of loss of water tailed off more or less sharply to a maximum at 70–85 % loss in body weight. 'Death', i.e. no further movements visible, usually occurred when 30–35 % loss in body weight had occurred (i.e. when approximately half of the body water had been lost).

Figure 3 shows a fairly linear relationship between % R.H. and transpiration rate, the transpiration rate being greatest at 0 % R.H. However, when 'corrected' rates are plotted (Figs. 4, 5), instead of a uniform transpiration rate becoming evident as expected, abnormally high rates at 90 and 95 % R.H. appear. No adequate explanation of this can be advanced.

The rates found are high and comparable with those reported for aquatic insects by Holdgate (1956). In an experiment carried out on the litter-dweller *Orchesella villosa* a slightly lower rate was found (3.0 mg./cm.²/hr. against 9.5 mg./cm.²/hr. for *Podura aquatica*, both at 21° C. and corrected to 0 % R.H.)

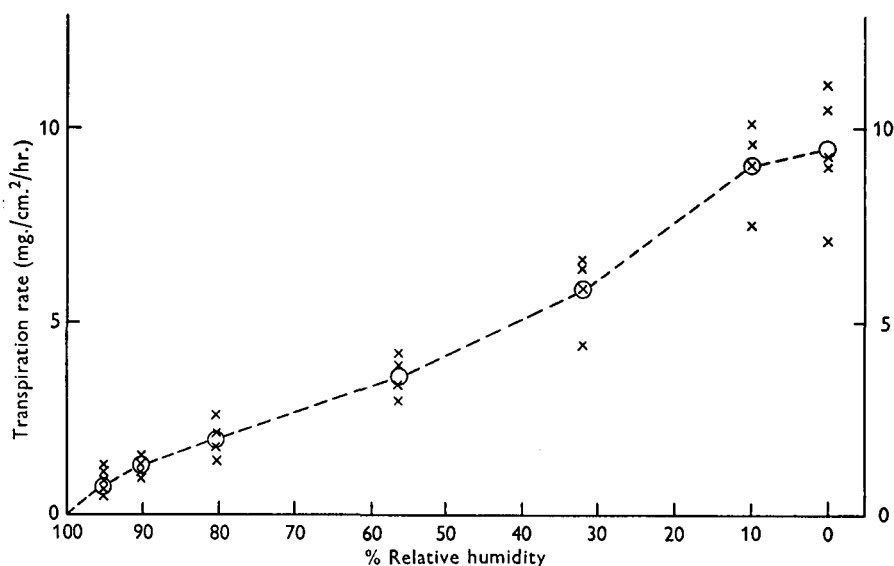


Fig. 3. The effect of relative humidity on the transpiration rate in *Podura aquatica*. Crosses show individual readings and circles the average readings at the different values for relative humidity. The broken line joins the average readings.

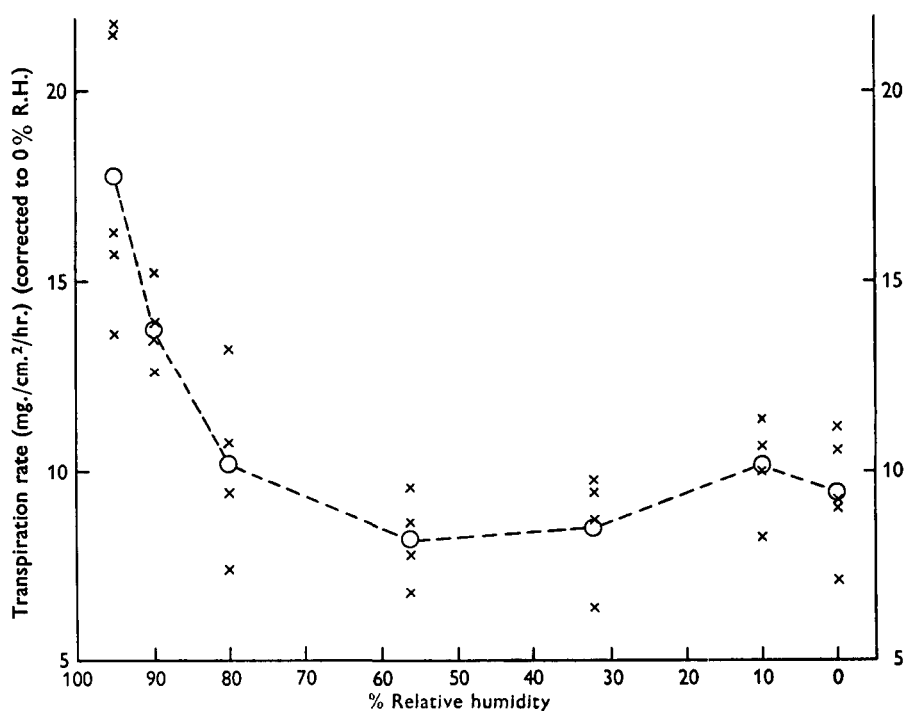


Fig. 4. The effect of relative humidity on the transpiration rate in *Podura aquatica*. The data shown in Fig. 3 are here shown corrected to 0% R.H. Symbols as for Fig. 3.

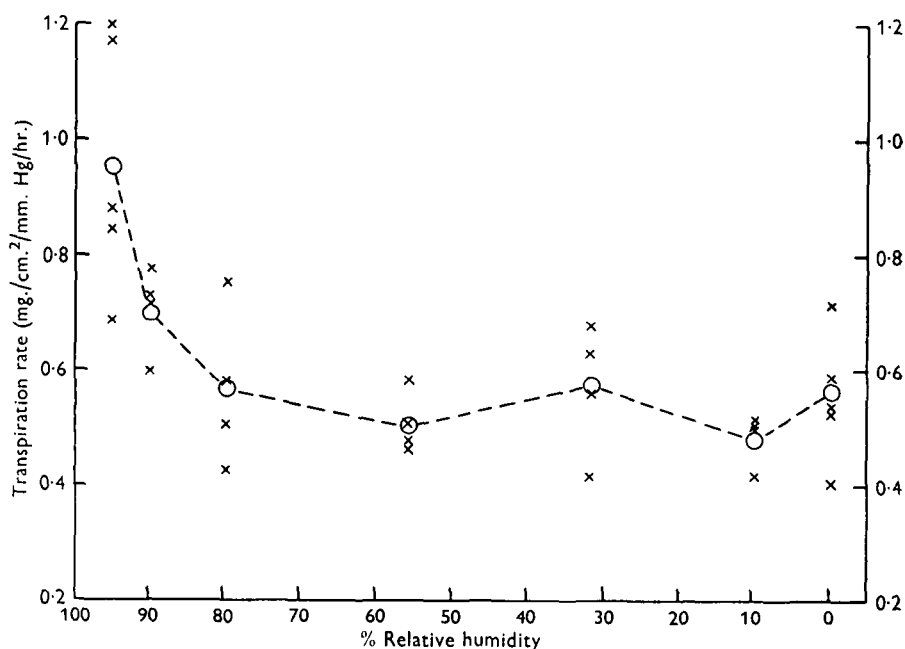


Fig. 5. The effect of relative humidity on the transpiration rate in *Podura aquatica*. The data shown in Figs. 3 and 4 are here shown corrected for differences in saturation deficits. Symbols as for Fig. 3.

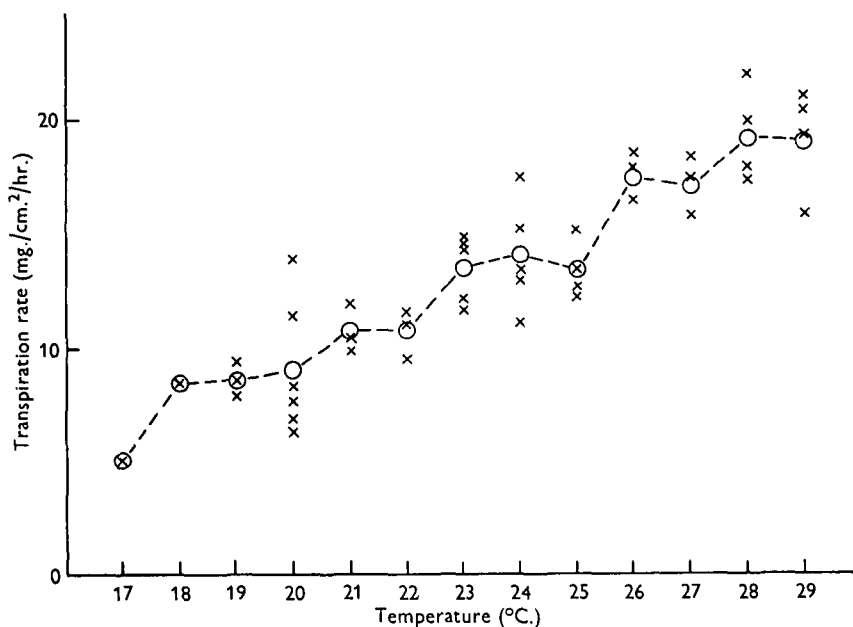


Fig. 6. The effect of temperature on the transpiration rate in *Podura aquatica*. Crosses show individual readings and circles the average readings at different temperatures. The broken line joins the average readings.

The effect of temperature on the transpiration rate

The results obtained using the temperature/humidity chamber are shown in Figs. 6 and 7. The rates at each temperature are expressed (i) as $\text{mg./cm.}^2/\text{hr.}$ corrected to 0% R.H. to be directly comparable with the results obtained when R.H. was varied (Figs. 6, 4), and (ii) as $\text{mg./cm.}^2/\text{mm. Hg/hr.}$ to correct for varying saturation deficits at the different temperatures.

Figure 6 shows a slight, more or less linear, increase in rate with temperature, the values at 20° C. being similar to those found during the experiments with varying R.H.

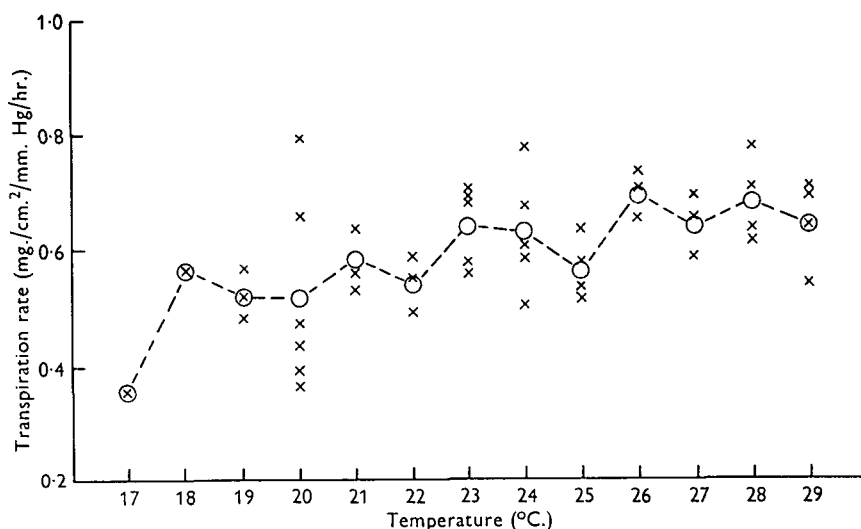


Fig. 7. The effect of temperature on the transpiration rate in *Podura aquatica*. The data shown in Fig. 6 are here shown corrected for differences in saturation deficits. Symbols as for Fig. 6.

(compare with Fig. 4). No evidence of a discontinuity in the temperature/transpiration rate curve is seen over this temperature range, which covers the range in which a critical temperature may be expected to occur in aquatic insects (Beament, 1961*a*). The transpiration rate is high at all temperatures.

Figure 7 shows the same data corrected for differences in saturation deficit at the different temperatures. The points lie on a line more or less parallel to the abscissa, with perhaps a slight positive slope. There is little or no direct effect of temperature on the permeability of the cuticle over this temperature range. This agrees with temperature curves of other insects remote from the critical temperature (Beament, 1959, 1961*a, b*).

An experiment conducted on *Folsomia candida* (Willem) at 27° C. showed a similar transpiration rate (13.8 $\text{mg./cm.}^2/\text{hr.}$ against 17.2 $\text{mg./cm.}^2/\text{hr.}$) for *Podura aquatica*. This agrees with the finding that the survival times observed for *P. aquatica* during these experiments were similar to those reported by Ögel (1958) for *Folsomia candida* (Willem).

*The effect of dusts on the transpiration rate**Immediate effects*

Ögel (1958) has reported decreased survival times in *F. candida* (Willem) after treatment with dusts. The effect of rolling *Podura aquatica* ten times in a tube containing abrasive dust (Almicide) on its transpiration rate at 25° C. and 31% R.H. was investigated. The insects after treatment with the dust were allowed to remain on a water surface for at least 10 min. to ensure they were fully hydrated and not suffering from side-effects due to the treatment.

The results are given in Table 2. It will be seen that in all cases the transpiration rate was increased above the level attained by normal insects, with an average increase of 1½–2 times over the normal values.

Table 2. *The effects of abrasive dust on the transpiration rate of Podura aquatica expressed as mg./cm.²/mm. Hg/hr.*

Controls	Hours after treatment				Moulted
	0–1	15–22	40–45	64	
0.52	0.96	0.67	0.62	0.75	0.49
0.53	1.13	0.75	0.76	—	—
0.58	1.17	—	0.80	—	—
0.64	—	—	—	—	—

These findings agree with those of Ögel that the survival time of *Folsomia candida* (Willem) at 10 and 50% R.H. and 28° C., is lowered by abrasion to about half the normal value, and lend support to the view that a wax layer of some sort is present on the cuticle surface.

Recovery after abrasion

Ögel (1958) reported that in *F. candida* (Willem) the waterproofing was restored to the normal value at the next moult. This has been confirmed in *Podura aquatica*, but the investigation has been extended to include a consideration of repair before the subsequent moult. Insects treated with dust soon after moulting were desiccated at varying times subsequent to the treatment, and the transpiration rates were compared. The results of this investigation are summarized in Table 2. After its immediate, approximately twofold, increase the transpiration rate falls to approximately mid-way between this value and the control value. This occurs in the first 12 hr., and subsequently there is no detectable decline in that instar. After moulting, however, the control level is re-attained.

This partial recovery from the effects of abrasion is paralleled in the pterygote insects where imperfect secretion of new wax occurs (Wigglesworth, 1945). This suggests that in *P. aquatica*, too, a comparable 'wound-healing' reaction probably occurs. Since the tubercle-tips are implicated as the sites of the wax layer, and since the pore canals terminate in these tubercles (Noble-Nesbitt, 1963*a*), it is possible that the cytoplasmic filaments are concerned in this repair.

The differential wettability of the cuticle of different regions of the body

The cuticle is not uniform in its wetting properties over the whole of the body. This feature is of common occurrence in insects. The hydrofuge tips of the respiratory horns of mosquito larvae provide a good example of localized differences in wetting properties (see Wigglesworth, 1953, for review of this).

The general body surface

If a small drop of water is dropped on to the surface of *P. aquatica* it will not remain there, but rolls off; the surface is unwettable. If a specimen is forcibly submerged it quickly returns to the surface and is unwetted. This happens whether the insect is living or whether it is fixed in an aqueous fixative. Indeed, specimens fixed with

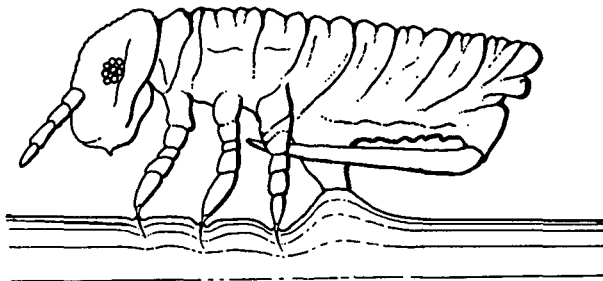


Fig. 8. *Podura aquatica* on a water surface. Note that the unguis penetrate the surface film, and that the ventral tube vesicles are wetted. (Redrawn after Wigglesworth, 1953.)

Baker's formaldehyde-calcium fixative have been kept over a period of many months on the surface of Baker's formaldehyde-calcium-cadmium storage solution (Pantin, 1948), without loss of their unwettable. Such prolonged retention of this property whilst in contact with a water surface is surprising (Beament, 1960).

On close examination of a submerged specimen it is evident, because of the silvery appearance of the surface, that a thin film of air is taken down below the water surface with the insect. This shows that the general surface of the body is unwettable.

The ventral tube vesicles

The cuticle overlying the ventral tube vesicles is readily wettable (cf. Wigglesworth, 1953; Brocher, 1910; Baudoin, 1955). When a water surface is brought against the vesicles the meniscus rises, showing that the contact angle is less than 90° , i.e. that the vesicles are wettable (Fig. 8). When the surface water is pulled away the surface in contact with the vesicles is pulled up above the general surface level until the column of water so formed breaks. The vesicles thus cling to the water surface, a property of wettable surfaces.

The unguis

These 'claws' on the tips of the limbs are also wettable and penetrate water surfaces (cf. Nutman, 1941; Baudoin, 1955). When a water surface is brought against a claw

the water surface rises in a meniscus as the water wets the claw (Figs. 8, 9a). As the limb is pressed further into the water surface the hydrofuge tibiotarsal cuticle causes the water surface to be depressed (Fig. 9b) because its contact angle is greater than 90° . Superficially, therefore, the limb appears to be completely hydrofuge, but the penetration of the surface by the unguis is a very important factor in locomotion.

When the limb is raised out of the water surface the surface in contact with the unguis is pulled well above the general surface level, as described above for the ventral tube vesicles. The unguis therefore also cling to the water surface.

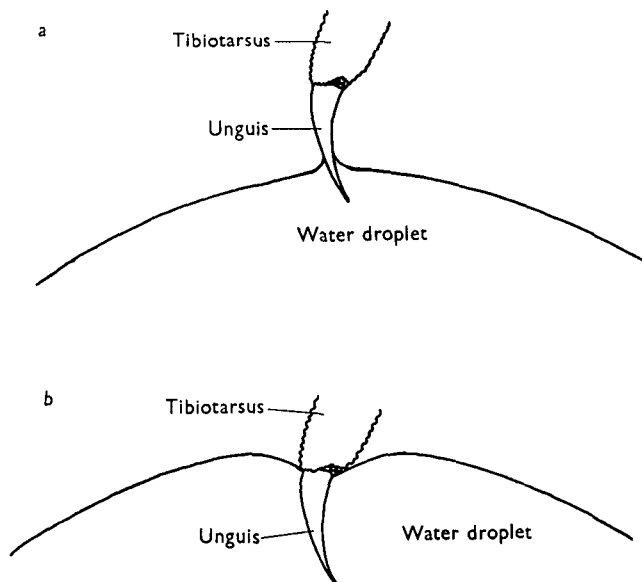


Fig. 9. Drawings of a limb of *Podura aquatica* brought into contact with a water droplet. (a) The droplet impinging against the unguis, which is wetted; (b) further penetration of the limb into the droplet, showing the unwettable nature of the tibiotarsus, which causes the surface of the water droplet to be depressed.

The nature of the hydrofuge surface agent

Previous work on the wetting properties of insect cuticles has implicated lipids as the surface agents (Holdgate, 1955; Beament, 1960). In some instances the tips of specialized hairs form an effective hydrofuge surface above the general level of the cuticle surface. This is the situation found in plastrons (Crisp & Thorpe, 1948; Thorpe, 1950). Imms (1906) suggested that in *Anurida maritima* (Guérin) hydrofuge hairs were responsible for the unwettable nature of the cuticle, and Brocher (1910) suggested that this was the situation in *Podura aquatica* too. In view of the sparse covering of hairs, this is unlikely, and further investigations have therefore been carried out.

The effect of reduced atmospheric pressure

Though plastrons effectively produce a hydrofuge surface above the general level of the cuticle and can withstand greatly increased pressures, once water penetrates the interstices between the hydrofuge hairs the plastron collapses and the effective

hydrofuge surface is lost (Crisp & Thorpe, 1948; Thorpe, 1950). Further it is usual for materials to be much more readily wetted after being in previous contact with water (Beament, 1960). In order to achieve this contact between the general cuticular surface and water it is essential that the surface air film should be removed. This can be accomplished under vacuum and water then reaches the cuticle. By this method, too, micropyles can be filled with water (Beament, 1948). Experiments with *P. aquatica* were therefore designed to ensure that water made actual contact with the cuticle, the air film being removed under vacuum.

The apparatus used is shown in Fig. 10 (see Beament, 1948). During an experimental run the living specimen was introduced through the neck *N* into the chamber *C*. The reservoir *R* was then attached to the neck *N* and the connexion ground in to

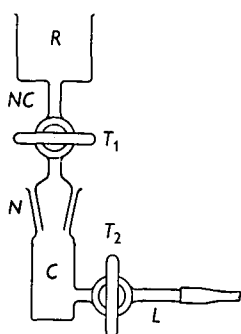


Fig. 10. The vacuum apparatus used in experiments on the wettability of the cuticle. For explanation see text.

ensure an air-tight joint. Silicone vacuum grease was used over the joint surfaces. Boiled, air-free distilled water was run into reservoir *R* and stop-taps *T*₁ and *T*₂ were opened to allow water to run down the capillary *NC* to just below the stop-tap *T*₁, which was then closed. The filter-pump was then connected to the side-limb, *L*, and when the insect was remote from the point of origin of the side-limb *L* from the chamber *C* (to ensure that it would not be sucked out of the chamber), the chamber was evacuated. Stop-tap *T*₂ was then closed and stop-tap *T*₁ slowly opened to allow water to enter and fill chamber *C* completely. In this way, it was ensured that the whole surface of the specimen came into contact with water. No silvery air film surrounded the specimen, which sank under gravity.

After a short period of submersion air was admitted by opening stop-tap *T*₁ and allowing some water to run out through stop-tap *T*₂. The insect was then brought to the surface of the water, and it was unwetted, apart from its claws, indicating that the retreating contact angle was still above 90°. This suggests that the unwettability of the cuticle does not wholly depend upon maintaining an air-film above the general surface of the cuticle. Further, all polar groups of any wax that may be on the surface of the cuticle must be already firmly attached and not free to come quickly to the outer surface (see Holdgate, 1955; Beament, 1960). This degree of orientation of a wax layer is usually associated with great impermeability to water, though this is not found in *P. aquatica*. A consideration of the fine structure of the surface layers of the cuticle helps in reconciling these apparently contradictory findings.

The Lycopodium test

Greases such as the cuticular grease of a cockroach, when touched on to a clean water surface, spread out over it. This can be followed visually by spreading *Lycopodium* powder on the clean water surface before touching it with the grease (Beament, 1945). When intact specimens of *Podura aquatica* were touched on to such *Lycopodium*-covered water surfaces no dispersal of the *Lycopodium* occurred, suggesting that the surface was not a surface-active grease such as that found in the cockroach.

The effects of organic solvents

Beament (1946, 1948) described the effects of methyl and ethyl alcohols on cuticular waxes. The effects of these alcohols on *Podura aquatica* were noted for comparison. The insect was held on a glass rod in one arm of a micromanipulator, either by a nylon noose, or by the adhesive 'Newskin'. Results were similar with both methods of attachment, tests being conducted remote from the area treated with 'Newskin'. The other arm of the micromanipulator held a fine-bore pipette containing the test substance which could be extruded at the tip and brought against the cuticle surface. Successive treatments with test substances and water were achieved by changing the pipettes in the micromanipulator arm. Alternatively, the whole insect was shaken up in a tube containing the test substance and the effect was noted.

Table 3. *The effect of treatment with chloroform on the wetting properties of the cuticle of Podura aquatica*

(a) Using small drops of cold chloroform impinged on to the cuticle

Treatment (mins.)	Subsequent wetting properties
3	Non-wettable
4	Slight wetting
7	Wetting with difficulty
10	Readily wettable
20	Readily wettable

(b) Using whole animals dropped into hot chloroform

Treatment (sec.)	Subsequent wetting properties	Comments
10	Non-wettable	Whitish deposit over surface
15	Wettable	Whitish deposit on furcula non-wettable
30	Wettable	No deposit
60	Wettable	Traces of whitish deposit

Aqueous ethanol above 70% and aqueous methanol above 90% wet the cuticle surface. If the treated cuticle is then allowed to dry out (2 min. was found to be a sufficient interval) it is not subsequently wetted by water. Even after fairly long treatment (30 min.) with the absolute alcohols, no subsequent wetting with water is possible, showing that any extraction of the surface material by these solvents must occur slowly. This agrees with the findings of Beament (1949) for egg-shells and suggests that a wax may be the surface agent in *P. aquatica*.

Further evidence supporting this suggestion is obtained by treating the cuticle with

chloroform. Very short treatment with hot chloroform (20 sec. or longer) produces a surface which, after drying out, is readily wetted by water. A whitish deposit, which may be a precipitated wax, is also occasionally seen on areas of the cuticle after this treatment. Similarly, exposure to cold chloroform for 10 min. produces a surface readily wetted by water. Partial wetting, or no wetting at all, follows shorter treatments. The results are summarized in Table 3. They suggest that a chloroform-soluble substance (possibly a wax, therefore) is responsible for the hydrofuge properties of the cuticular surface. Ögel (1958) showed that chloroform also destroyed the hydrofuge property of the cuticle of *Folsomia candida* (Willem).

Though no critical temperature has been found in the temperature range 17–29° C., treatment with abrasive dust suggests that a wax layer is present. Taken with the other evidence presented here this points to the presence of a wax on the surface of the cuticle, imparting a slight restraint on transpiration but strongly hydrofuge properties.

The structural basis for the wetting properties of the cuticle

Surface roughness

The hydrofuge cuticle of *Podura aquatica* is tuberculate and therefore very rough (Noble-Nesbitt, 1963a). Increasing roughness makes contact angles diverge further from 90° (Holdgate, 1955). Thus, provided that the contact angle of the surface agent is above 90°, greater roughness will result in an even higher contact angle to the cuticle surface as a whole, therefore making it strongly hydrofuge. Wax has a contact angle to water of above 90° (100–110°; Holdgate, 1955) and the rough surface, if covered by a wax as seems likely (see above), would be extremely hydrofuge, as found in this insect.

Both the light microscope and the electron microscope show that the cuticle is smooth over the ventral tube vesicles (Noble-Nesbitt, 1963a). The light microscope also reveals a smooth cuticle over the unguis. Therefore, even if the same wax that covers the general body surface cuticle also occurs on these areas, the contact angle will be very close to 90° before contact with water. Contact with water tends to lower contact angles (Holdgate, 1955). Thus, the contact angle, already near to 90°, could easily fall to below 90° and the cuticle would then be wettable, as the ventral tube vesicles are found to be. Being in more or less constant contact with water, the contact angle would be maintained at the lower value of retreating contact angles (Holdgate, 1955), which can be as low as 10° for insect waxes (Crisp & Thorpe, 1948).

The possible function of the secretion of the cephalic gland which passes to the ventral tube vesicles via the ventral groove (cf. Imms, 1906) presents an interesting problem. This secretion has been supposed to aid in the adhesive properties of the ventral tube vesicles (Imms, 1906). Being an aqueous fluid, it would be expected to lower the contact angle of the cuticular surfaces with which it comes into contact. It is possible, therefore, that it maintains a low contact angle in the cuticle of the ventral tube vesicles with the result that the vesicles are always ready to cling to water surfaces.

The boundary between the rough hydrofuge cuticle and the smooth hydrophil cuticle is clearly defined, and in the ventral tube is marked by a cuticular flap (Noble-Nesbitt, 1963a). This flap, when the ventral tube is extruded and the vesicles are in

contact with water, will have the effect of increasing the angle between the cuticle surface and the water surface at the point where the hydrofuge cuticle begins. This, arrangement makes it less likely that the hydrofuge cuticle will be wetted, and the flap thus acts as a levelling device. A similar situation is found in *Gyrinus*, which has structural modifications of the smooth cuticle at the water-line (Holdgate, 1955).

The distribution of the surface agent over the hydrofuge cuticle

Normal procedures followed in the preparation and examination of material for electron microscopical investigations result in the removal of unbound wax layers. However, when wax layers are bounded on both sides by materials unaffected by these procedures it is possible to distinguish wax layers as the space left between the unaffected materials (Locke, 1957).

Where wax layers are overlain by cement there is often a close association of cement and wax, such that the surface of the cement layer has lipoid characteristics (Way, 1950; Holdgate & Seal, 1956); or a further wax layer occurs above the cement (e.g. *Tenebrio*; Holdgate, Menter & Seal, 1954; Holdgate & Seal, 1956; Beament, 1959).

The distribution of these layers over the cuticle is therefore of great interest in the present study. Modifications of the epicuticle of the tuberculate cuticle occur over the minor tubercles (Noble-Nesbitt, 1963*a*). The tubercle tips have a different epicuticular structure from the rest of the cuticle and this suggests a discontinuous layer at the outermost surface of the cuticle. If this is a wax layer, or a wax-impregnated layer, which contains highly-orientated lipids, then the tubercle tips may well be both hydrofuge and impermeable (Beament, 1960). As these tips are only about 0.4μ apart, a plastron-like effect could operate, the water being held away from the surface and air trapped in the trough between the tubercles. This would provide the silvery appearance under water. The non-collapsible nature of the tubercles (Noble-Nesbitt, 1963*a, b*) will account for the retention of the hydrofuge properties after submersion under vacuum.

Between the tubercles, the cement and wax layers are extremely thin and do not appear to be continuous over the bases of the troughs (Noble-Nesbitt, 1963*a*). This is as though the materials of these layers spill over from the tubercles. It is of interest to note in this connexion that the pore canals terminate in the tubercles (Noble-Nesbitt, 1963*a*), and Ögel (1958) has suggested that they pour out cement in *Folsomia candida* (Willem). The result of the absence of cement and wax layers in the troughs will be to produce a 'pin-hole' effect on the transpiration rate, giving an overall high cuticular permeability. High transpiration rates are therefore to be expected.

The effects of dusts are readily explained on the basis of this structure. Removal of the hydrofuge, impermeable coverings of the tubercle tips will break down the hydrofuge nature of the cuticle, thereby allowing aqueous solutions (e.g. as used in the argentaffin test, Ögel, 1958) to make contact with the cuticle. The removal of these covering layers will increase transpiration by increasing the effective transpiring area.

In conclusion, the hydrofuge properties of the cuticle appear to depend upon tubercles having specialized outer epicuticular layers, which may be a highly orientated wax layer and a cement layer, over their tips. The tubercles provide a rough surface, which will help to make the surface more hydrofuge. Ögel (1958) suggested

that the tubercles may play a part in the hydrofuge properties of the cuticle in *F. candida* (Willem), by increasing the surface roughness. The hydrophil areas of the cuticle are characterized by a smooth cuticle devoid of tubercles, and it is suggested that their contact angles are maintained near to retreating contact angle values. They are demarcated from the hydrofuge areas by cuticular flaps, at least in the case of the ventral tube vesicles.

The biological effects of cuticular wettability

We have seen that, when submerged, *Podura aquatica* is surrounded with an air film. It is possible that this then acts as a plastron in this non-tracheate, skin-breathing insect, as shown for *Anurida maritima* (Guérin) by Imms (1906). If the epicuticle between the minor tubercles of the cuticle is highly permeable, as suggested above, then respiratory exchange will be expected to occur rapidly in these areas (Richards, 1951). The significance of these troughs may therefore lie in relation to the cuticular respiration which occurs in these insects. Tracheate insects do not have to depend upon gaseous diffusion over the cuticle of the general body surface, which therefore can be covered with impermeable (and hydrofuge) waxes. A similar continuous wax covering in skin-breathing insects would cut down respiratory exchange. By leaving holes in the hydrofuge layer, however, a freely permeable, yet extremely hydrofuge, cuticle is possible, and on submersion, the pockets of air are retained exactly where they are most required. A further effect of the air film is to increase buoyancy and so to aid the return of the insect to the surface after submersion by waves, by floods, or experimentally. In the natural habitat submersion may occur whilst the insect is clinging to plants.

An observation of interest made whilst observing the effects of submersion on *Podura aquatica* is the manner in which the insect 'swims' underwater. If an insect is forcibly submerged in a water-filled, narrow-bore tube held horizontally, it swims actively horizontally, in its air film, and reaches the meniscus. In this case, the insect attains the surface in spite of the effects of bouyancy, though the manner in which it determines which way to swim is not obvious. Equally striking is the method of propulsion. The furcula is rapidly extended repeatedly, and the insect 'springs' through the water.

The wettable areas of the cuticle also have distinct biological implications. The ventral tube serves to anchor the insect in the surface water film (Brocher, 1910; Wigglesworth, 1953; Baudoin, 1955, Paclt, 1956); though the ungues also assist in this. Contact of the ventral tube vesicles with the aqueous medium is also important in exchange of substances between the insect and the medium (Noble-Nesbitt, 1963c).

The effect of the ungues penetrating the water surface whilst the tibia depresses the water surface is like a spiked shoe in grass, or ice, in which the spike penetrates the surface and provides lateral purchase, whilst the sole prevents further sinking into the surface, giving 'buoyancy'. The ungues therefore provide lateral purchase in the water, and the unwettable tibia provides the resistance to sinking. The insect, therefore, is able to walk easily over the surface of calm water, using its limbs only. If the limb were totally hydrofuge (Brocher, 1910; Wigglesworth, 1953; Paclt, 1956), such walking would not be easy; it would be like having smooth-soled shoes with which

to walk over ice. Baudoin (1955) comes to the same conclusion in considering *Podura* and *Anurida*.

The wettability of the unguis explains another observation. Nutman (1941) has described Collembola (e.g. *Onychiurus armatus* (Tullb.)) walking up vertical surfaces without using the ventral tube, whereas Ruppel (1953) showed that the ventral tube was necessary for adhesion to smooth clean glass at angles greater than 55° . I have noticed that *Podura aquatica* readily climbs up the sides of aquaria. When enclosed in a tube with moisture on the walls a specimen can be seen to walk upside down, using only its unguis, gaining purchase in the very thin surface layer of moisture. When larger drops of water are reached, and its claws come into contact with the water drop, the ventral tube is everted and holds on to the water surface, though this is not invariable by any means. The insect is still able to walk over the water surface without using the ventral tube, since the wettable claws hold on to the water surface. About three claws seem to be necessary to bear the weight of the insect. With only two claws penetrating the surface, the hold of the insect is insecure and it usually falls. Provided that this minimum requirement is met, claws can be withdrawn and replaced in the normal walking gait. The ventral tube provides enough support on its own, and provides a useful 'safety factor'.

A perhaps significant feature of these observations is the eversion of the ventral tube vesicles when the claws come into contact with water. This suggests a sensory mechanism in the limbs which, reflexly or otherwise, brings about eversion of the ventral tube (Noble-Nesbitt, 1963c).

DISCUSSION

The implications of the repair to the waterproofing mechanism after treatment with dusts are interesting. Because repair appears to be only partial, it is at best only an emergency measure. Only moulting gives complete repair. Whilst the need for repair may be questioned in soil Collembola, where injury occurs very soon after each moult (Ögel, 1958), it may be much more necessary in an insect that lives on a water surface and which requires a highly hydrofuge cuticle at all times. The retention of moulting throughout the life of the insect, therefore, ensures that a 'safety factor' is present to guard against undue abrasion, and this may help to outweigh the harmful effects due to imperfect ecdysis, recognized by Sweetman (1952).

Richards (1951) quoted values of 6–8 mg./cm.²/mm. Hg/hr. as 'normal' values for plasma membrane permeabilities. These are of the order of ten times those found here for *Podura aquatica* (see Figs. 5, 7) and it is therefore unlikely that the soft cuticle of these insects can be regarded as being as permeable as plasma membranes. It is therefore evident that some degree of resistance to desiccation is provided by the cuticle.

One possible site of high water loss from the insect is the ventral tube vesicles. However, the ventral tube was invariably retracted during the transpiration experiments. Since cuticular flaps close off the vesicles when the ventral tube is retracted excess water loss is expected to be negligible. The high rates of transpiration are not attributable to this cause. They seem to be a real property of the cuticle.

The high transpiration rate requiring no highly orientated wax layer seems to be

incompatible with the greatly hydrofuge nature of the cuticle which implies the presence of a highly orientated wax layer. However, as we have seen, the fine structure of the surface provides a possible explanation. The highly orientated wax layer if confined to the tips of the minute tubercles, as electron micrographs suggest (Noble-Nesbitt, 1963a), could provide a highly hydrofuge surface whilst allowing a high permeability through the cuticle between the tubercles. The absence of a critical temperature could be explained if the wax had a critical temperature higher than 29° C. This view may be in part supported by the observation that death occurs on a water surface at 40–42° C.; the cuticle temperature may be expected to be much lower than this because of the high rate of transpiration (Beament, 1958) and could therefore be well within the range of critical temperatures reported for other insects (Beament, 1959, 1961a). The high rate of transpiration above 29° C. makes it extremely difficult to carry out experiments on the transpiration rate in *P. aquatica* in the low humidity range and a major alteration in the apparatus would be called for, to extend the temperature range, so it has not been possible to extend the transpiration rate/temperature curve to investigate this aspect of the phenomenon further. However, the effect of dusts does support this viewpoint. The mode of action of the dusts could well be the removal of wax from the tubercle tips.

Ögel (1958) concludes that the high desiccation rate is a reflexion of the thinness of the cuticle rather than the absence of an impermeable wax-layer. However, since the great barrier to water loss even in thick cuticles is the wax-layer (Ramsay, 1935; Wigglesworth, 1945, Beament, 1945, 1958, 1959, 1961a), and since measurements of transpiration rate effectively measure the permeability of the most impermeable part of the system, a continuous wax-layer, even over a thin cuticle, would be expected to provide a very low permeability to water and therefore a very low transpiration rate. A discontinuous wax layer, however, means that the permeability of the remaining parts of the cuticle will be measured by the transpiration rate. Under these circumstances the high transpiration rate is a reflexion of the thinness of the cuticle, but only because the impermeable wax-layer is not continuous.

It is thus probable that a highly orientated wax with a critical temperature above 30° C. evolved in these insects as a mechanism to ensure a high degree of unwettability, rather than to prevent desiccation as in pterygote insects. It is possible that the extension of this wax-layer over the whole surface may have been the next step in the exploitation of the terrestrial habitat by insects (Beament, 1962). Coupled with this modification, development of tracheae would be expected to provide for respiratory exchange. Now, the tracheae of insects often are filled with air whilst the insect is still submersed in fluid, and this may be explained by the creation of a hydrofuge surface on the tracheal cuticle by the orientation of a lipid layer (Beament, 1960). This hydrofuge surface is studded with small tubercles and taenidial folds (Locke, 1957, 1958) which increase surface roughness, and, therefore, increase the efficiency of the hydrofuge mechanism. This arrangement closely resembles the arrangement on the external surface of *P. aquatica*. It is possible, therefore, that when the orientated lipid layer over the surface of the cuticle first arose in phylogeny it was involved in the creation of a hydrofuge surface which aided respiration (Beament, 1962). In *P. aquatica*, which has no tracheae, and in *Peripatopsis* (Manton & Ramsay, 1937), this arrangement still prevails over the body, but in the tracheate insects, the lipid

layer over the body also provides a barrier to transpiration. It is therefore probable that the properties of the cuticle of *Podura aquatica* represent an intermediate stage in the evolution of the cuticular properties of insects better adapted to the terrestrial habitat.

SUMMARY

1. In the collembolan, *Podura aquatica*, transpiration through the cuticle is high at all relative humidities. Above 90% relative humidity, cuticular permeability is abnormally high but no adequate explanation of this can be given.

2. Transpiration through the cuticle is high at all temperatures and there is no evidence of a critical temperature over the range 17–29° C.

3. Abrasion increases the transpiration rate, and at least partial recovery occurs within the instar. Moulting fully restores the transpiration rate to the normal level.

4. The hydrofuge nature of the cuticle strongly suggests the presence of an orientated lipid monolayer on its surface. Strong wax solvents render the cuticle wettable, whereas treatment with water under reduced pressure does not.

5. Consideration of the fine structure suggests that the hydrofuge layer is discontinuous and that it occurs on the tips of the minor tubercles. This is compatible with an overall high transpiration rate.

6. The occurrence of hydrophil areas of cuticle is correlated with a smooth cuticular surface.

7. The biological effects of the hydrofuge and hydrophil areas in the life of the insect are discussed.

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