

## TRANSPIRATION THROUGH THE CUTICLES OF SOME AQUATIC INSECTS

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### INTRODUCTION

The resistance of terrestrial insects to desiccation is well known to be due to a layer of wax in the epicuticle (Wigglesworth, 1945; Beament, 1945; Way, 1950; Wolfe, 1954; Holdgate & Seal, 1955). This wax can be extracted by solvents and will waterproof membranes on which it is deposited. Both intact insects and their extracted waxes on membranes show a similar, steeply rising, near-exponential relationship between permeability and temperature. Curves of the same type have been obtained from ticks (Lees, 1947), spiders (Edney & Davies, 1952), insect eggs (Beament, 1946, 1948), and mite eggs (Beament, 1951), and wax layers have been demonstrated in the majority of these groups.

But this combination of a low rate of water loss and a high temperature dependence of permeability is only known from undamaged terrestrial arthropods living in dry habitats. It does not occur in soil-inhabiting insects, owing to abrasion of the wax layer by the medium (Wigglesworth, 1945), or in abraded terrestrial insects (Holdgate & Seal, 1955), or in certain other terrestrial arthropods (Edney, 1951). Very little is known about cuticular permeability in aquatic insects, but what observations there are suggest a fairly high level of permeability (Harnisch, 1934; Wigglesworth, 1933). Recently, Shaw (1955) has demonstrated that the larva of *Sialis lutaria* is permeable to water both when exposed to dry air and when under the supposedly more natural conditions of a water/heavy water system. In this larva also, the exponential curve relating permeability and temperature rises less steeply than in terrestrial species.

It is important that further information should be obtained about the waterproofing mechanism in aquatic insect cuticles. If the group as a whole should prove to have permeable cuticles, then the correlation between waterproofing mechanism and habitat attains a greater significance. If they do not, it would suggest that a wax layer, once evolved, had been retained as a protection against the entry of water under osmotic forces. Further, since recent work has necessitated a reinterpretation of the 'critical temperature' (Holdgate & Seal, 1955), the study of as wide a range of insect cuticles as possible has become increasingly important.

## WATER LOSS FROM AQUATIC INSECTS IN DRY AIR

*Material and methods*

The method of Wigglesworth (1945) has been used to determine the rates of water loss in dry air from a number of aquatic insects. Phosphorus pentoxide was used as the desiccating agent, and in later experiments the apparatus was modified by the introduction of a mechanism for stirring the air and setting up a circulation in the desiccator. The final arrangement is shown in Fig. 1.

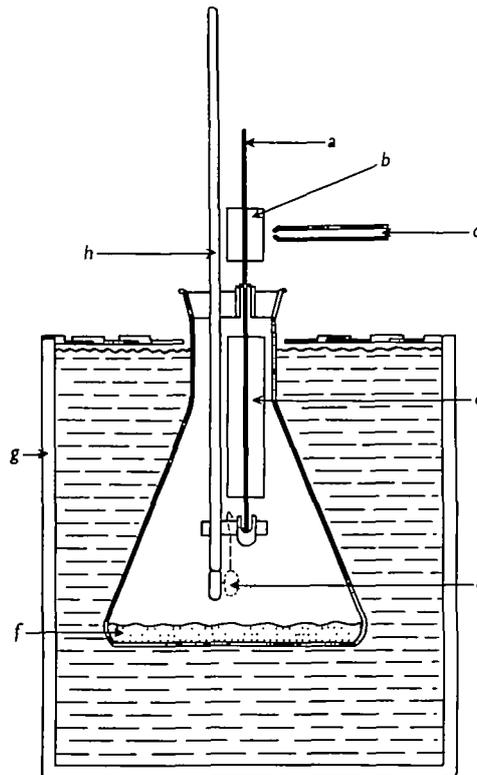


Fig. 1. Apparatus for measuring rates of water loss from insects. *a*, glass fibre; *b*, paper sail; *c*, air jet; *d*, paper sail stirring air in desiccator; *e*, specimen; *f*, phosphorus pentoxide; *g*, water bath; *h*, thermometer.

Rates of water loss over a wide range of temperatures were measured for the following species:

<i>Anax imperator</i> (Odonata; Anisoptera) nymph	<i>Notonecta obliqua</i> (Hemiptera) adult
<i>Coenagrion puella</i> (Odonata; Zygoptera) nymph	<i>Agabus bipustulatus</i> (Coleoptera; Dytiscidae) adult
<i>Limnophilus</i> sp. (Trichoptera) larva	<i>Hydrobius fuscipes</i> (Coleoptera; Hydrophilidae) adult
<i>Corixa punctata</i> (Hemiptera) adult	<i>Sialis lutaria</i> (Neuroptera) larva
<i>Corixa</i> sp. (Hemiptera) adult	<i>Tipula</i> sp. (Diptera) larva

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All specimens were killed by exposure to hydrogen sulphide gas for about 1 hr., this agent being used because it combines high toxicity with an absence of components likely to affect the cuticle. The dead insects were suspended in the desiccator by loops of nylon thread tied loosely around the body; as a control measure to test that this did not damage the cuticle several other groups were suspended in gauze trays.

*Rates of water loss at 20° C.*

The rates of water loss from the species studied, expressed as mg./cm<sup>2</sup>./hr. at 20° C. are given in Table 1. These figures are not precise, the chief causes of inaccuracy being the difficulty of measurement of surface area, and the fact that in some cases measurements were not made at exactly 20° C. so that the values have had to be obtained by extrapolation. But the errors certainly do not amount to more than 50% and are probably much less, so that the values quoted are sufficient for comparative purposes.

Table 1. *Approximate rates of water loss (mg./cm<sup>2</sup>./hr.) at 20° C. for the aquatic insects studied*

Species	Total no. of specimens	No. of experiments	Mean rate of water loss
<i>Tipula</i> sp.	5	2	11.5
<i>Limnophilus</i> sp.	2	1	11 approx.
<i>Sialis lutaria</i>	16	3	6.4
<i>Coenagrion puella</i>	30	4	4.2
<i>Notonecta obliqua</i>	19	6	2.9
<i>Corixa punctata</i>	23	4	2.1
<i>Corixa</i> sp.	6	1	1.8
<i>Anax imperator</i>	5	5	0.9
<i>Agabus bipustulatus</i>	9	2	0.9
<i>Hydrobius fuscipes</i>	16	2	0.3

It is clear from the table that a very wide interspecific variation in cuticle permeability exists in aquatic insects. But all the species are more permeable than normal terrestrial insects. The rate of water loss from *Tenebrio* pupae at 20° C., for example, is 0.02 mg./cm<sup>2</sup>./hr., a figure which is less than one-tenth that of the least permeable aquatic insect and one five-hundredth that of the most permeable.

The rates of water loss from *Tipula*, *Limnophilus* and *Sialis* are nearly of the same order as those observed from a small capsule filled with water and exposed under the same conditions. But, as Shaw (1955) has pointed out, water loss from such a 'free-water surface' is only a small fraction of the theoretical figure, the limiting factor being the slow rate of diffusion of water vapour away from the surface. It is obviously important to ascertain whether gaseous diffusion is similarly limiting for the more permeable insects, and the introduction of the air-stirring mechanism in the desiccator allowed this to be done.

For a free-water surface, stirring the air increases the rate of evaporation considerably. In one experiment, water loss from a polythene capsule brimful with water rose from 120 to 180 mg./cm<sup>2</sup>./hr. when the air was stirred, and reached

600 mg./cm<sup>2</sup>./hr. when the capsule was placed directly under a compressed air jet, even though this air was not dry. On the other hand, an *Anax* nymph placed under the same air jet lost water less rapidly than it did in an unstirred desiccator. A group of *Sialis* nymphs lost water at a rate of 6.6 mg./cm<sup>2</sup>./hr. in still dry air, and at 7.5 mg./cm<sup>2</sup>./hr. when the air was vigorously stirred. With *Notonecta* adults the effect was even less, and the figures obtained using still and stirred air fell on the same curve.

The results seem to indicate that the rate of gaseous diffusion from the cuticle surface to the desiccating agent is not the main limiting factor for water loss from aquatic insects. Instead, the actual permeability of the cuticle appears to be limiting; although the cuticles in these species are more permeable than those of terrestrial insects they do therefore exercise some restriction on the passage of water through them.

*The relationship between water loss and temperature*

The rates of water loss in dry, still air for the above species were determined over a wide range of temperatures. The curves obtained are plotted in Figs. 2, 3 and 4.

These curves are closely similar to that obtained for *Sialis* larvae by Shaw (1955), and they confirm that this animal is in no way atypical among aquatic insects.

In all the species studied water loss increases considerably with temperature, but the curves obtained have a continuous exponential form and show no sharp breaks or 'critical temperatures'. They closely resemble the curve for water-vapour pressure against temperature. This resemblance is not merely fortuitous. The rate of water loss, expressed as mass/unit area/unit time can be shown (Holdgate & Seal, 1955) to be governed by the equation

$$m = \frac{D}{LRT} (p_1 - p_2),$$

Where  $m$  = the rate of water loss,  $D$  the diffusion coefficient,  $L$  = the thickness of the solid barrier,  $R$  the gas constant,  $T$  the temperature,  $p_1$  the vapour pressure of water on the inside of the cuticle, and  $p_2$  the vapour pressure of water on the outside.

It is assumed that there is saturation on the inside of the cuticle, so that  $p_1$  is equal to the saturation vapour pressure of water at temperature  $T$ . Since the insect is in a desiccator,  $p_2$  is zero. Now, since  $p_1$  increases exponentially with increasing temperature, while  $p_2$  remains constant, the expression  $(p_1 - p_2)$ , and in consequence the rate of water loss, will also increase in this way.

In the more permeable aquatic insects—*Tipula*, *Sialis* and *Coenagrion*, for example—the increase in water loss with temperature follows a water vapour-pressure curve closely. When  $\log m$  is plotted against  $1/T$  for these species (for the basis of this treatment see Holdgate & Seal (1955)), the curve obtained has practically the same slope as one relating  $p_1$  to  $1/T$  (Figs. 5, 6). This suggests that in these examples the diffusion coefficient,  $D$ , does not vary very much with temperature.

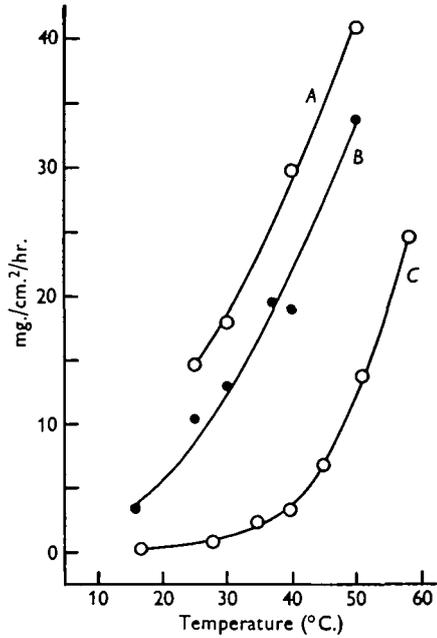


Fig. 2

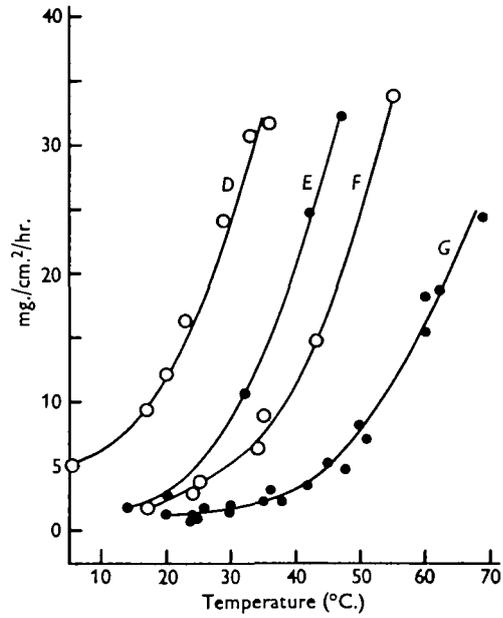


Fig. 3

Fig. 2. The variation in transpiration with temperature in three species of aquatic insect. A, *Limnophilus* sp.; B, *Coenagrion puella*; C, *Hydrobius fuscipes*.

Fig. 3. The variation in transpiration with temperature in: D, *Tipula* sp.; E, *Corixa punctata*; F, *Corixa* sp.; G, *Anax imperator*.

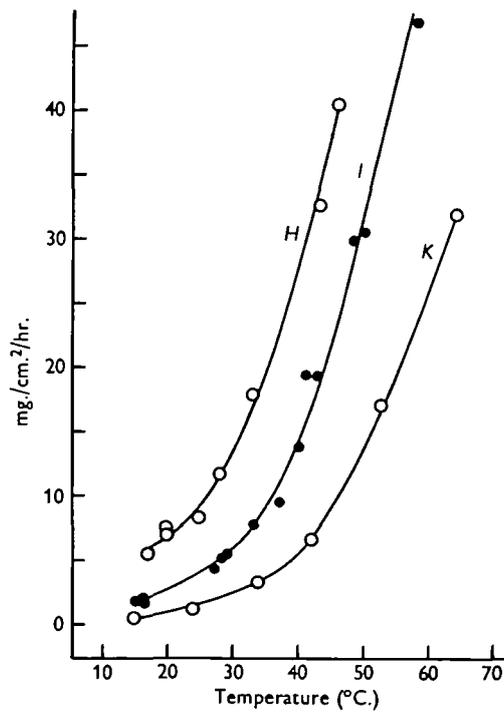


Fig. 4. The variation in transpiration with temperature in: H, *Sialis lutaria*; I, *Notonecta obliqua*; K, *Agabus bipustulatus*.

On the other hand, in the less permeable aquatic species,  $\log m$  increases much more rapidly with increasing temperature, and the curves of  $\log m/T^{-1}$  and  $\log p_1/T^{-1}$  diverge (Figs. 5, 6). In these examples there appears to be an exponential temperature-dependence of diffusion, and the increasing water-vapour pressure difference cannot alone account for the increasing water loss.

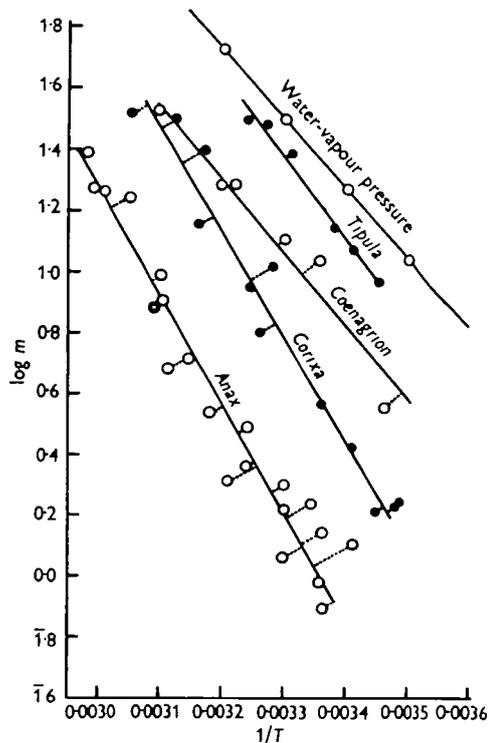


Fig. 5

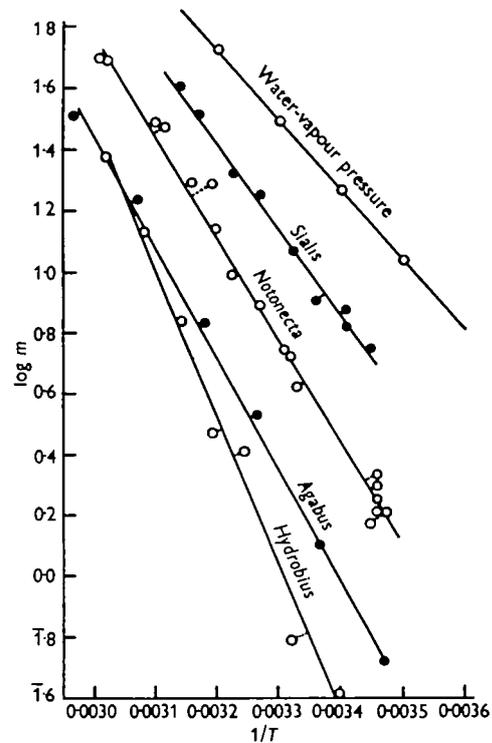


Fig. 6

Fig. 5. Plot of the logarithm of the rate of water loss against the reciprocal of the absolute temperature, for four species of aquatic insect. Note similarity in slope to the curve relating  $\log$  water vapour pressure to  $1/T$ .

Fig. 6. Logarithmic plot of water loss against  $1/T$  for four other aquatic insects.

In all the aquatic species studied, however, the relationship between  $\log m$  and  $1/T$  is linear. The data provide even less evidence for the concept of a 'critical temperature' than do those for water loss from terrestrial species. This linearity of the  $\log m/T^{-1}$  relationship provides added evidence for the validity of the physical theory advanced by Holdgate & Seal (1955).

The insects studied show a correlation between the permeability of the cuticle and the temperature sensitivity of diffusion through it. In the most permeable species  $\log m$  varies with  $1/T$  only, in a way more or less parallel with vapour-pressure changes. In the least permeable species, *Hydrobius*, the curve for  $\log m/T^{-1}$  is much steeper, while in the terrestrial species studied by Holdgate & Seal

(1955), which are the least permeable of all, the temperature-dependence of permeability reaches a maximum. This relationship is brought out in Fig. 7, in which the slope of the log  $m$  curves is plotted against the permeability of the cuticle at 20° C. A similar relationship holds for abraded terrestrial insects (Holdgate & Seal, 1955), and also for gas diffusion through rubber membranes (Barrer, 1941).

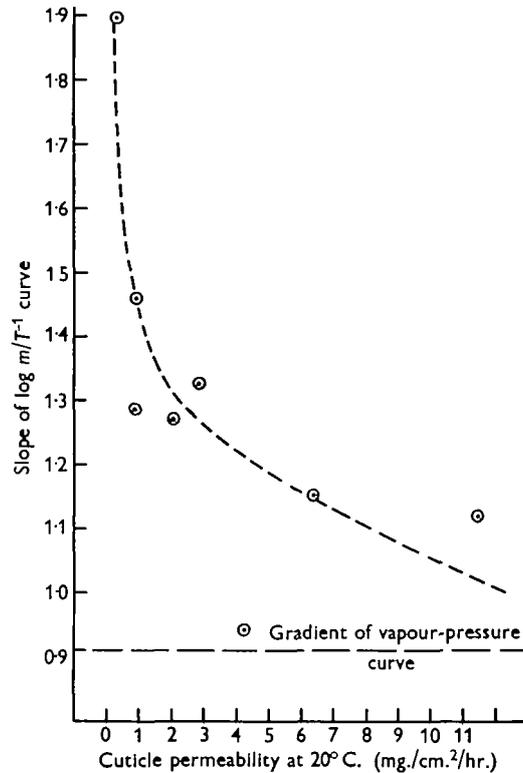


Fig. 7. The relationship between cuticle permeability and the temperature-dependence of diffusion (expressed in terms of the slope of the log  $m/T^{-1}$  curve). Trend line highly conjectural.

*The effect of wax solvents on water loss from aquatic insects*

In none of the data for aquatic insect water loss so far presented in this paper is there any evidence that wax layers are responsible for the resistance of the cuticles to the passage of water. Indeed, if the criteria for the presence of a wax layer were either very low permeability or the presence of a 'critical temperature', it could only be concluded that such a layer was absent. But neither of these criteria are in fact valid (Beament, 1954; Holdgate & Seal, 1955), and Shaw has produced some evidence for the existence of a lipid in the cuticle of *Sialis*, which he suggests may have a waterproofing function.

Table 2 shows the effect of brief immersion in chloroform at room temperature on the subsequent rates of water loss from a number of aquatic species. In all cases

the treatment produced a considerable increase in transpiration, which in several instances rose to more than 5 times its previous level. This increase is of the same order as that produced by wax solvents in the cold on terrestrial insects, and might be held to indicate that the action of the solvent in the two cases was similar. If this is so, then exposed, chloroform-soluble material must play an appreciable part in the resistance of aquatic insect cuticles to the passage of water. If it is not so, then it must be admitted that chloroform can affect the permeability of cuticles in other ways than by solvent action.

Table 2. *The effect of chloroform on the evaporation of water from some aquatic insects. Animals immersed in the solvent for 2-5 min. at room temperature. Rates as mg./cm.<sup>2</sup>/hr.*

Species	No. of specimens	Temperature (°C.)	Initial rate	After treatment
<i>Notonecta obliqua</i>	3	16	2.0	11.3
	3	16	2.2	11.8
	4	15	1.6	10.9
<i>Corixa punctata</i>	2	15	1.7	12.0
	4	14	1.8	9.4
<i>Tipula</i> sp.	2	22	14.0	17.0
<i>Anax imperator</i>	2	20	1.0	17.0
	2	20	—	16.5
	1	20	—	16.4
	1	20	1.1	11.6
<i>Coenagrion puella</i>	10	17	2.9	5.7

#### *The effect of mineral dusts on water loss*

In most terrestrial insects water loss through the cuticle is greatly increased by rubbing the animal with a mineral dust (Wigglesworth, 1945). This abrasion need not be very vigorous nor need the dust involved be very hard, since the impermeable wax layers lie close to the surface and are readily penetrated.

Table 3. *The effect of charcoal dust on water loss from some aquatic insects*

Species	No.	Treatment	Initial rate	Subsequent rate
<i>Coenagrion puella</i>	10	Light dusting	2.87	2.91
<i>Notonecta obliqua</i>	2	Abraded	2.0	3.7
	4	Abraded	2.5	3.0
<i>Corixa punctata</i>	5	Abraded	1.9	4.2

Table 3 shows the effect of abrasion with activated charcoal dust on certain aquatic insects. The dust was ground in a pestle and mortar and spread on filter-paper, and the insects were held lightly between finger and thumb and gently rubbed across the dusted paper for a standard distance. This treatment is sufficient to produce a 40-fold increase in water loss from *Tenebrio* pupae, when these are

abraded in the same way, over about the same area of cuticle. But the table shows that in aquatic insects the increase following dusting is very much less—in some of the experiments so much less as to be of doubtful significance. It might therefore be concluded that the waterproofing layer, such as it is, is not accessible to the dust, although such a hypothesis does not agree with the results of the chloroform treatment. But the actual increase in water loss after abrasion is about the same in aquatic and terrestrial insects. It may be that equal areas of cuticle are abraded to about the same depth, but that the permeability of the aquatic insect is so great already that the proportionate increase is negligible.

*Water uptake by an aquatic insect in distilled water*

The measurement of cuticle permeability for aquatic insects in terms of weight loss in dry air is unrealistic in that it exposes the animals to conditions which they do not meet in nature. It was, however, necessary to use this method for comparison with terrestrial examples. In addition, as a check on the results, another method of estimating cuticle permeability was devised and used on adult specimens of *Notonecta obliqua*, since these were readily obtained and of convenient size.

The animals were killed by  $H_2S$ , and nylon threads tied round them loosely for ease of handling. They were then immersed in a beaker filled with distilled water, being kept below the surface by a glass plate. The beaker was kept at room temperature (*c.* 20° C.), and the animals generally remained in it for 24 hr.

When the animals were removed, the problem was to distinguish between the increased weight due to the actual absorption of water through the cuticle and that due to the adherence of a film over much of their surfaces. This was done by first blotting the animals on dry filter-paper. They were then weighed and transferred to a desiccator, also at room temperature, and thereafter regular weighings were carried out, at half-hourly intervals. During the early period of desiccation the animals lost weight rapidly; this was considered to be due to the removal of superficial moisture. But, after about 30 min. a steady rate of weight loss was attained, comparable with that shown by normal, dead insects, and this was held to be due to transpiration through the cuticle. By extrapolation of this steady rate back to the time of removal from the water, a figure for the actual amount of water taken up by the animal could be obtained.

RESULTS

Table 4 demonstrates that *Notonecta* shows considerable permeability to water under these conditions. In evaluating the results it must be remembered that much of the cuticle surface is covered with a hair pile, which normally retains air, and is only slowly invaded by distilled water. The area of cuticle involved in water uptake may therefore be only about half that concerned in water loss. The physical forces involved in the movement of water are also very different in the two cases. But, while these difficulties make a detailed comparison impossible, it is clear that the permeability of *Notonecta* cuticle is of the same order of magnitude in the two directions, and under the two sets of conditions.

In the desiccation experiments, where the spiracles were not sealed, there was probably an appreciable tracheal component of water loss. But the high rates of transpiration cannot be solely due to the tracheal system, for the cuticle is also shown to be permeable in the water uptake experiments, in which the tracheae remained filled with air.

Table 4 also demonstrates the effect of brief chloroform treatment on the subsequent rates of water uptake. In all cases this treatment produces an appreciable increase in permeability, although this is not so great as is observed under conditions of desiccation.

Table 4. *Water uptake by Notonecta obliqua. Exps. 1-3, untreated specimens. Exps. 4-7, specimens washed in chloroform at room temperature for 2 min.*

Exp.	No. of specimens	Rate of water uptake (mg./specimen/hr.)	Treatment
1	4	0.65	} Untreated specimens
2	4	0.36	
3	5	0.35	
		0.54	
		0.61	
		0.39	
		0.39	
1-3	13	0.48	
4	4	0.78	} Specimens immersed chloroform
5	4	1.30	
6	3	0.90	
7	3	1.77	
4-7	14	1.17	

#### DISCUSSION

There is no doubt that aquatic insects, of whatever species, group, or developmental stage, are permeable compared with terrestrial species. But there is, none the less, a wide range of permeability within the group, and the least permeable examples do not differ very markedly from the more permeable terrestrial species. This in itself suggests that aquatic and terrestrial species may form a continuous series, rather than two discrete groups. Such a view is supported by a study of the water loss/temperature curves. In all cases these have an exponential form, as is to be expected on theoretical grounds (Barrer, 1941; Holdgate & Seal, 1955). Further, the steepness of the exponential is correlated with the level of permeability of the cuticle; a relationship which is well known from non-biological diffusion systems (Barrer, 1941), and the terrestrial insects simply represent one end of the series, in which permeability is minimal, and its temperature-dependence maximal.

Porous membranes in general combine high permeability with a slight temperature-dependence of diffusion (Barrer, 1941). It might therefore be considered that the permeable aquatic insects have porous cuticles, and that the series linking these species with the terrestrial forms is one of decreasing pore size and pore density. If this is so, it would be expected that an artificial increase in the porosity of terrestrial

insect cuticles would produce an increase in permeability and a reduction in temperature-dependence. This is in fact the case. Abrasion of terrestrial insects by mineral dusts can convert the steep exponential water loss/temperature curve that they normally show into a 'vapour-pressure' type curve comparable with that of permeable aquatic species (Holdgate & Seal, 1955). Yet these abraded insects are far from freely permeable, and their rates of water loss are enormously increased by treatment with cold chloroform, which presumably acts by removing the wax layer from some of the undamaged regions of the cuticle. In all these features, therefore, they resemble aquatic species.

As a tentative hypothesis, therefore, it may be suggested that the permeable aquatic insects have cuticles which contain a wax layer, but that this layer is porous. The pores provide an easier pathway for water loss than the wax layer, and as a result the steep and non-linear  $\log m/T^{-1}$  curve which characterizes diffusion through the wax layer is masked. The less permeable species may perhaps have narrower pinholes through which diffusion is more temperature-dependent, while the terrestrial species may have a pinhole system which is still more reduced. But there are other possible systems which could equally well give a graded series of permeability and temperature-dependence of diffusion, and until a great deal more is known about the properties of complex polar insect waxes it would be unwise to lay any stress on this or any tentative explanation.

#### SUMMARY

1. Measurement of the rate of water loss in dry air from ten species of aquatic insects, representing six orders, has shown that the cuticles are in all cases more permeable than are those of typical terrestrial species. There is, however, a wide range of permeability within the group.
2. The rate of water loss increases exponentially with temperature. There is no 'critical temperature'. The steepness of the exponential relating transpiration and temperature is greatest in the least permeable examples.
3. Chloroform treatment produces a marked increase in water loss, but abrasion by mineral dusts has very little effect.
4. Adult specimens of *Notonecta obliqua* immersed in distilled water gain weight at a rate which suggests that the cuticle is comparatively permeable under these conditions also. The rate of water uptake is increased by chloroform treatment.
5. The results support the general theory advanced by Holdgate and Seal (1955), but no sound interpretation of the structural basis of cuticular permeability can yet be advanced.

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