THE WATERPROOFING MECHANISM OF ARTHROPODS

II. THE PERMEABILITY OF THE CUTICLE OF SOME AQUATIC INSECTS

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INTRODUCTION

Recent investigations into the permeability of the integument of terrestrial insects (Beament, 1958a, 1959) have given new information about their waterproofing mechanisms. The cuticles of such insects have a range of permeability: from less waterproof plant-eating larvae to very impermeable pupae. In order to explain the properties of the more permeable cuticles, it might be sufficient to assume merely that there is a layer of wax of appropriate thickness. However, the extreme resistance to water-loss of insects such as *Rhodnius* cannot be accounted for in this way; it is postulated that there is a special molecular configuration of the wax, which has exceptional impermeability to water. When the effect of temperature upon permeability is also considered, it appears that even the more permeable terrestrial forms may show transition phenomena, which are characteristic of organized monolayers of lipid. Such studies of the effect of temperature also indicate, through the nature of transition and the reversibility of the process, whether the lipid on the cuticle is a grease or a wax.

Although such detailed physico-chemical information can only be revealed by technically complex methods, an earlier investigation by Holdgate (1956) showed conclusively that many aquatic insects are altogether more permeable to water than their terrestrial counterparts, though some aquatic species might approach the degree of waterproofing of the more permeable terrestrial forms. The object of the present study is to investigate further the cuticular permeability of some representative aquatic insects, and to suggest underlying mechanisms.

MATERIAL AND METHODS

Since waterproofing structures are known to be very delicate and are easily damaged by handling (Beament, 1959), the insects were collected, and later transferred from one medium to another, in small nylon nets. Most experiments were carried out soon after capture in the field. The animals were killed by introducing cyanide, azide or hydrogen sulphide into the water containing them, but it was often necessary to raise the concentration of carbon dioxide in the water to the point of anaesthesia, before adding the poison, in order to prevent self-inflicted damage. As soon as death had occurred, animals were washed repeatedly in tap water, while resting on nylon gauze, and transferred to the measuring equipment after superficial water had been
allowed to drain away. The equipment for determining water-loss, surface and air temperatures under controlled conditions has already been described (Beament, 1958a, b, 1959).

**EVAPORATION FROM INTACT ANIMALS**

At the commencement of an experiment, an animal has free moisture on its surface, so that desiccation must be continued for a time sufficient to establish equilibrium conditions in the surrounding air and a uniform gradient across the cuticle, before measurement is meaningful. With the less permeable animals, the criteria for such conditions are readily obtained: an initial high rate of evaporation, representing the removal of superficial water, falls within a very few minutes to a steady value, and the difference in temperature between the air and the cuticle surface correspondingly decreases. Only for animals as small as *Gyrinus* adults, which are also relatively waterproof, is it then necessary to take any special precautions to conserve the water reserves of the specimen during a determination of the permeability/temperature characteristic. When the cuticle is very permeable, on the other hand, high wind speeds are needed in order to ensure that conditions obtain in which evaporation is limited by the cuticle, rather than by diffusion in the air (see Beament, 1961a). In dry air, such circumstances then lead to the very rapid depletion of the animal's internal water reserves. In order to obtain the rates of evaporation from some of the more permeable insects which appear in Table 1, values determined in high-velocity dry air have been compared with others obtained in moving air of higher humidities; the more permeable insects moreover appear to have rather greater variation between individuals, compared with the consistent values obtained with more waterproof species. The figures quoted in Table 1 are means from both kinds of measurement. It must be borne in mind that some authors have claimed that curious effects may result from promoting high rates of evaporation in strong currents of air; Holdgate (1956) suggests that a nymph of *Anax* loses much less water when exposed to a jet of dry compressed air, than when in an unstirred desiccator. Edney (1951) believes that attempts to create high rates of desiccation can actually decrease the permeability of the cuticle of woodlice. No evidence lending decisive support to these contentions was obtained in the present series of experiments.

In Table 1 the rates of evaporation obtained at 20°C are expressed both per animal and per unit area of surface. The latter are included primarily in order that they may be compared with corresponding measurements by Holdgate (1956) who quoted his results in this form. However, the difficulty of arriving at a meaningful figure for the evaporating surface of an insect is well known. The areas used for present calculations have been obtained through geometric considerations, and from camera lucida drawings of dissected specimens. But as these figures (and those of Holdgate, in his own estimation) are liable to an error of perhaps 50%, only major divergences between the two investigations need receive serious consideration. Relatively close agreement has indeed been obtained for the majority of the more waterproof insects, such as the dytiscids and the anisopteran dragonflies. The rates obtained with the more permeable insects in the present study are apparently rather higher than those given by Holdgate; but, in particular, the value for *Sialis* is much closer to Holdgate's figure than to the substantially higher figure given by Shaw (1955). The divergence of the present
The waterproofing mechanism of arthropods. II

Table I

<table>
<thead>
<tr>
<th>Species</th>
<th>Rate of evap. at 20° C. in mg./unit s.d./hr.</th>
<th>Rate of evap./animal/unit s.d./hr. at 20° C.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Per animal</td>
<td>Per cm.***</td>
</tr>
<tr>
<td>Tipula larva</td>
<td>0-95-1-1</td>
<td>0-9-1-2</td>
</tr>
<tr>
<td>Sialis larva</td>
<td>0-25-0-3</td>
<td>0-2-0-3</td>
</tr>
<tr>
<td>Coenagrion nymph</td>
<td>0-3-0-4</td>
<td>0-075-0-1</td>
</tr>
<tr>
<td>Notonecta adult</td>
<td>0-27</td>
<td>0-1-0-15</td>
</tr>
<tr>
<td>Corixa adult</td>
<td>0-25-0-5</td>
<td>0-070-0-1</td>
</tr>
<tr>
<td>Aeshna nymph</td>
<td>0-14</td>
<td>0-013</td>
</tr>
<tr>
<td>(Hydrobus)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Agabus adult</td>
<td>0-05-0-06</td>
<td>0-03-0-04</td>
</tr>
<tr>
<td>Dytiscus adult</td>
<td>0-12-0-2</td>
<td>0-01-0-15</td>
</tr>
</tbody>
</table>

* Data calculated from Holdgate (1956).
† From Beament (1959).

results from those of Holdgate, when more permeable insects are considered, may be explained on a number of counts. Owing to the greater efficiency of the circulation of air in the present apparatus, the conditions of the experiment may have approached more closely to the idealized state in which evaporation is limited by the membrane alone (Beament, 1961a). Holdgate's figures were calculated on the assumption that the air was at 0 % R.H. throughout each desiccation period, whereas the values here presented have been compensated for the rise of humidity of the air in the desiccation chamber during each period of desiccation. But further, the present values are quoted at cuticle temperature; those of Holdgate are quoted at air temperature. Because the temperature of the cuticle is lowered by evaporation, the present values might more correctly be compared with values which Holdgate would have obtained at air temperatures a few degrees above 20° C.

Finally, Holdgate (1956) observed that the pupa of Tenebrio at 20° C. was at least ten times as impermeable to water as the least permeable of the aquatic insects he examined. According to more recent measurements (Beament, 1959), this extremely efficiently waterproofed animal, with two wax layers, may be as much as fifty times as impermeable as terrestrial larvae, such as those of Nematus or Pieris. The more impermeable of the aquatic insects which have now been investigated are of precisely the same order of permeability as the less waterproof terrestrial forms, and there is no a priori reason for suggesting that they differ materially in waterproofing mechanism on this evidence alone.

The permeability of different parts of the cuticle

When insects are as permeable to water as some of those under present consideration, there is a more serious objection to the expression of results in terms of a rate of evaporation per unit area of surface. If an animal is excessively impermeable to water, then the simplest hypothesis assumes that its surface is uniformly covered by lipid, and has uniform impermeability; for if any unwaterproofed areas were to exist, then the permeability of the remainder would have to be impossibly low. Indeed the
krypton adsorption method for measuring the surface area of insects (Lockey, 1960) gives such high values of area as to lead to a similar difficulty. When, however, an animal has a high rate of evaporation, in relation to its order of size, the cuticle giving rise to this could equally well comprise some areas of high permeability, the remainder having an efficient covering of lipid, or it could have uniformly intermediate permeability throughout the surface. Very little indeed is known about the distribution of cuticular lipids in aquatic insects. From the effect of ligating the anal gills of mosquito larvae (Wigglesworth, 1934) it is possible to deduce that the gill cuticle must be much more permeable to water than that over the rest of the animal; Shaw (1955) found that there was not significantly less lipid on the gill surface of Sialis as compared with the general abdominal integument, and in order to explain the greater evaporation rate from this insect than from typical terrestrial forms, he suggested that the waxes of Sialis must have 'different chemical and physical properties'. He points out that such a layer of wax on the gill would not greatly resist the diffusion of oxygen, while it would still control the movement of both water and salts. In the absence of direct measurement of the comparative permeability of such wax to oxygen and to water, the larger size of the oxygen molecule must be set against the validity of such an assumption. Shaw does, however, say that the surface of the abdomen and gill cuticle does not give a polyphenol reaction with ammoniacal silver nitrate, whereas the remainder of the integumental surface does so. Since the nature of the substrate is believed to be of very great importance in relation to the formation of organized monolayers of lipid (Beament, 1945, 1958a, 1960), further investigation into the permeability of different parts of some of the more permeable insects which appear in Table 1 is desirable.

Table 2

<table>
<thead>
<tr>
<th>Species</th>
<th>Rate of evap. (mg./animal/unit s.d./hr.)</th>
<th>Percentage area left uncovered by 'Newskin'</th>
<th>Percentage of original evaporation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Intact</td>
<td>Gills covered in 'Newskin'</td>
<td></td>
</tr>
<tr>
<td>Ephemera nymph</td>
<td>1.2-1.3</td>
<td>0.8-0.9</td>
<td>65</td>
</tr>
<tr>
<td>Coenagrion nymph</td>
<td>0.3-0.4</td>
<td>0.2-0.27</td>
<td>70</td>
</tr>
<tr>
<td>Sialis larva</td>
<td>0.25-0.3</td>
<td>0.06-0.07</td>
<td>50</td>
</tr>
</tbody>
</table>

Following the measurement of the over-all rate of water-loss from a specimen of one of the more permeable species, the gill area was painted over with a solution of 'Newskin', a proprietary solution of pyroxylin in alcoholic solvents. This material was shown, in separate experiments, to be relatively (but certainly not outstandingly) impermeable to water; it has the valuable property, however, of extremely high adhesion to cuticle surfaces and its local application does not seem materially to affect the property of the surrounding cuticle. The very greatest care was exercised during this process so that mechanical damage to the cuticle was avoided. In Table 2 the rates of evaporation from intact animals are compared with those when the gills have been so covered. Now some reduction in the rate of water-loss would result from such a treatment, even if the animal had a uniformly permeable surface; and unless the film of 'Newskin' were entirely impermeable to water, the degree of reduction would not even be proportional to the area which was covered, compared with the area left
uncovered. The mere fact, as Table 2 shows, that some reduction obtained from this treatment is of little consequence; what is important is that in the cases both of the damselfly and also of *Sialis* the degree of reduction was significantly greater than would have occurred had the animal been uniformly permeable all over, and had the gill area been made completely impermeable. No explanation is possible other than the conclusion that the gills of these animals are substantially more permeable to water than the remainder of the cuticle. The experiments on mayflies are inconclusive on this point. Because neither cuticle, nor ‘Newskin’ films, are likely to obey Fick’s Law at all closely, we are not justified in using the data in Table 2 to calculate the actual permeability of the gill cuticle. That information has been obtained in the following section.

**THE EFFECT OF PERMEABILITY ON TEMPERATURE**

The assessment of the actual permeabilities of different areas of the cuticle on one insect is based on the measurement of surface temperature; Beamant (1958a) shows that the temperature of the cuticle surface is lower than that of the surrounding air by an amount proportional to the rate of evaporation proceeding from it. In exploratory experiments in which a thermocouple probe was successively applied to different points on the surface of insects suspended in a stream of dry air (the reference junction being in the air, upstream of the specimen) it was found that the cooling of some gill surfaces exceeded that of the head or thoracic cuticle by some three or four centigrade degrees. In other cases, little difference occurred between the temperatures of different areas of a specimen. The results of a series of experiments conducted in this way, in an air stream controlled to a temperature of 15° C. at a point immediately before the air reached the specimen, are reported in Table 3.

<table>
<thead>
<tr>
<th>Species</th>
<th>Temperature depression on thorax (centigrade degrees)</th>
<th>Other area tested</th>
<th>Temperature depression of other area (centigrade degrees)</th>
<th>Calculated evap (mg./cm.²/unit s.D./hr)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Ephemera</em> nymph</td>
<td>0.9–1.05</td>
<td>Gill</td>
<td>1.0-1.1</td>
<td>Gill</td>
</tr>
<tr>
<td><em>Coenagrion</em> nymph</td>
<td>0.3</td>
<td>Gill</td>
<td>0.55</td>
<td>Gill</td>
</tr>
<tr>
<td><em>Sialis</em> larva</td>
<td>0.15</td>
<td>Gill and abdomen</td>
<td>0.65</td>
<td>0.3</td>
</tr>
<tr>
<td><em>Dytiscus</em> larva</td>
<td>1.0</td>
<td>Abdomen</td>
<td>2.0</td>
<td>0.75</td>
</tr>
<tr>
<td><em>Notonecta</em> adult</td>
<td>&lt; 0.1</td>
<td>Plastron region</td>
<td>&lt; 0.1</td>
<td>0.3</td>
</tr>
</tbody>
</table>

It is seen from Table 3 that a number of insects which have exposed gills—mayfly and zygopteran larvae, and *Sialis*—are evaporating water very readily from those gills. The damselfly and *Sialis* are, further, losing water much more rapidly from their gills than from the rest of the cuticle, for the remaining cuticle is at a much higher temperature. *Ephemera* does not show this distinction; like the larva of *Dytiscus* it appears to be equally permeable over its entire surface. The adult water-beetles, anisopteran nymphs and *Notonecta* appeared to be equally impermeable over their entire surface, as judged by the readings of the thermocouples. While such a result is not unexpected, it is worth noting that the thermocouples do not indicate any significant difference
in permeability between the main, hydrophilic areas and the extremely hydrofuge plastron region of *Notonecta* despite the great difference in surface property.

As an approximation—and certainly as accurately as surface areas can be estimated—the rate of evaporation may be assumed to be directly proportional to the depression of the surface temperature. The total recorded evaporation rate from a specimen is made up of the sums of the products of each local evaporation rate multiplied by each local area. There is no reason why an insect should not have continuously varied permeability over its surface; but simplifying the situation through the assumption that a specimen has two levels of permeability—over the gill area and over the remaining cuticle—we can derive those permeabilities. If we divide the total rate of water-loss of a specimen in the ratio of

\[
\frac{\text{area of gill} \times \text{temperature depression of gill}}{\text{area of remainder} \times \text{temperature depression of remainder}}
\]

we obtain figures for the respective permeabilities of the two regions. Values obtained in this manner have been included in Table 3 for the two species where higher rates of evaporation were demonstrated from the gills. The results obtained in the following section have been treated in a similar manner.

**THE EFFECT OF TEMPERATURE UPON PERMEABILITY**

According to Holdgate (1956) the greater 'porosity' of the general cuticle of the aquatic insect would give rise to a permeability/temperature relationship in which permeability was very dependent on temperature. But now that we have demonstrated the differences which can occur in the permeability of two areas of one individual, the relationship between permeability and temperature must be interpreted in this light. From the measurements given in Tables 1 and 3, the aquatic insects used in this paper can be divided into three categories: (a) insects of uniformly low permeability, (b) insects of uniformly high permeability, (c) insects with more permeable gills. Insects of type (a) were expected to compare closely in property with typical terrestrial insects, and the method of measuring their permeability/temperature characteristic (Beament, 1958a, 1959) was applied to them without modification. The results of these experiments are represented in Fig. 1. With the addition of precautions to ensure that permeability rather than diffusion was the limiting factor (p. 278), animals in category (b) were similarly investigated. Despite the short time-intervals during which readings can be obtained at any one temperature in the apparatus, the water reserves of some of these very permeable insects are so rapidly depleted that some of the curves in Fig. 2 are constructed from data obtained from more than one specimen.

The recording of two independent surface temperatures simultaneously, during a permeability/temperature determination, presents a difficult technical problem. The beam of the balance on which the specimen is exposed to desiccation has a bifilar suspension; the thermocouple array (consisting of a probe junction against the cuticle and a reference junction in the air) is connected to the galvanometer through the two elements of that suspension. Thus an additional thermocouple array, to measure a second cuticle temperature, cannot be added, for there is no way of increasing the number of suspension elements. In consequence, three methods of procedure were
adopted. A preliminary experiment was conducted on a number of specimens, with the probe junction either against the thorax, or against the gill-area; the ranges of temperature and of desiccation rate which were likely to be of particular interest were thus determined. By rapid manipulation, and by judicious selection of temperatures, it was then possible to carry out a determination of the permeability/temperature characteristics, with the probe against the thorax, followed by a similar determination over the whole temperature range with the probe against the gills, on one specimen, without depleting its water reserves too greatly. Correspondence of the rates of desiccation during the second run, with those obtained in the first run, acted as a check on depletion. This experiment relies on the assumption that the cuticle is not irreversibly affected by its first experience of higher temperatures; the validity of this assumption was confirmed with the material used. Secondly, a single series of permeability measurements was made, over a selected range of temperature, but the position of the probe junction was alternated between thorax and gill for each successive reading. After a number of experiments of the preceding types, the form of the curve for total evaporation against air temperature was well established. In the third series of experiments, the balance beam was therefore clamped, and a second thermocouple array was introduced into the desiccation chamber so that both thoracic and gill temperatures could be recorded simultaneously against air temperatures, without the corresponding values of evaporation rate. It was fortunate that the material, whose properties are represented in Fig. 3, showed a relatively high degree of uniformity within a species.

Two types of waterproofing system are represented in the graphs of Fig. 1. *Dytiscus* and *Gyrinus* adults produce a curve of Z shape, similar in general form to that of the cockroach, *Periplaneta* (Beament, 1958a). The adults of *Agabus*, *Ilybius* and *Notonecta* produce the L-shaped curve, which is more typical of the majority of terrestrial insects so far examined (Beament, 1959). From the theories proposed in the two papers which have just been cited, we should conclude that *Dytiscus* and *Gyrinus* have a grease as their waterproofing material, whereas the others are covered by a harder wax. Greases are believed to have lower mean molecular weights than waxes, so that it is not surprising to find that the transition temperatures of *Dytiscus* and *Gyrinus* are appreciably lower than those of the others. But attention is drawn to the extremely low transition temperatures of all of these insects: that of *Dytiscus*, for example, is ten degrees lower than the corresponding value for *Periplaneta*.

There is little of note that we can deduce from the permeability/temperature characteristics of the very permeable insects. On the one hand they are so permeable to water over the entire temperature range which has been examined, and on the other the circumstances in which this high permeability has been measured differ so greatly from those in the natural environment that one cannot attempt to attribute biological significance to the effect of temperature which has been recorded.

The dissection of the over-all permeability/temperature curves for the three larvae with external gills—*Ephemera*, *Coenagrion* and *Sialis*—into the component curves for the gill area and for the remaining cuticle reveals some interesting biological information. The mayfly can be summarily dismissed; if the gills were allowed to collapse on to the surface of the animal, no difference in temperature depression could be demonstrated anywhere on the surface of the animal, but if the gills were lifted, they
shrivelled rapidly and their surface temperature was then higher than that of the rest of the animal. While *Ephemera* would seem to have a cuticle rather less permeable to water than would be expected from a completely lipid-free surface, it is clear that the gill cuticle is not specially permeable to water, and, by inference, not specially permeable to oxygen either. This would agree with the observation (Thorpe, 1933) that these insects can respire adequately through the main cuticle in well-aerated water, but that the gills may become important only when the animal is in less favourable circumstances of oxygen supply or demand. That evaporation from the exposed

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**Fig. 1.** The relationship between permeability of the cuticle (expressed as evaporation rate per unit of saturation deficiency of dry air) and cuticle surface temperature, for a number of less permeable aquatic insects. *Note.* The ordinate consists of three sections, each with a different linear scale.
and shrunken gill falls rapidly should occasion no great surprise, for the collapse of the structure may limit the supply of water from the haemolymph purely mechanically; there is no need to postulate a change in permeability with desiccation, such as Edney (1951) suggests may occur in isopods. The damselfly (Fig. 3a), on the other hand, has two kinds of cuticle with very different temperature coefficients of permeability. Neither the curve for the gill nor that for the thorax shows any indication of transition phenomena. In view of the comparatively high permeability of both cuticles, none would be expected. At lower temperatures the permeability to water of the gill is the greater; its permeability is also relatively unaffected by temperature. As permeability to water is likely to reflect permeability to oxygen also, this may indicate that the gill has the greater respiratory role at lower temperatures. The permeability of the remaining cuticle rises uniformly with temperature, so that at 24°C, the gill is actually less permeable, and by 30°C, the main cuticle may transmit water twice as rapidly as the gill. Both the temperatures quoted are probably above the survival range of this species, but the experiment shows how different the temperature relationships of two parts of the cuticle of one individual may be—and, perhaps, how different their relative roles in, for example, respiration may be at different temperatures.

The experiments with \textit{Sialis} (Fig. 3b) reveal an entirely different state of affairs. The curve for total evaporation against temperature is of complex form, and it would be unwise to infer that either of the irregular portions of it were evidence for a transition phenomenon; they could fall within the limits of experimental error of the method. When the depression of the temperature of the gill is considered, the permeability of this region is seen to be very dependent on temperature. But the
permeability of the thoracic cuticle, judged by the change in its temperature depression with rising temperature, is little affected by temperature except for one pronounced discontinuity of Z type, occurring at around 35°C. Interestingly enough, that discontinuity does correspond with one of the small irregular regions of the curve for total water-loss. The validity of the procedure of dissecting the curve for total

Fig. 3a. The relationship between air temperature and the temperature depression (below air temperature) of the cuticle surface, due to evaporation. The curves have been obtained from the thorax and from the gill of Coenagrion (Zygoptera, Odonata) larvae.

Fig. 3b. The relationship between air temperature, surface temperature depression and permeability, for the thorax and for the gill of Sialis larvae. For further explanation, see text.
water-loss into its component parts in this manner is particularly supported by the form of the permeability/temperature relationship of the thoracic cuticle, calculated by dividing the values for total water-loss in the ratio of corresponding thoracic to gill temperature depressions as outlined above (p. 282). Though all three functions from which this relationship is derived (total water-loss/temperature, temperature depression of gill/temperature, and temperature depression of thorax/temperature) are of complex form, the calculated curve shows sections which are linear and parallel with the temperature axis, both below and above the transition region; in other words, two simple functions joined by a transition region, with precisely the same form as one obtains from cockroach grease (Beament, 1958a) and from the cuticles of *Dytiscus* and *Gyrinus* adults in the present investigation. Furthermore, when specimens of *Sialis* were examined after the experiment, the head and thorax were shiny, as though grease-covered, whereas the abdomen was matt in appearance. There seems little doubt that the gill of *Sialis* (and possibly the whole abdomen) is without an efficient covering of lipid, but the head and thorax have organized grease over them; one could deduce therefore that in this insect the gill is the only region of important respiratory consequence.

**DISCUSSION**

Even within this small sample of aquatic insect species a wide variety of permeability of the cuticle and of distribution and type of cuticular lipid is present. The animals sharing one specific environment—a pond—may be, like most terrestrial insects, covered by harder wax, or by softer grease, and may be comparatively impervious to water; they may have an effective barrier to water over only a part of their surface, or they may, apparently, be quite devoid of an impermeable lipid barrier. But where organized cuticular lipid is present, all these animals share the property of having a low transition temperature, as compared with many terrestrial forms. It has been argued from the time of the early studies in this field (e.g. Wigglesworth, 1945) that the higher the transition temperature the more impermeable the insect—a correlation confirmed by later investigations (e.g. Lees, 1947; Beament, 1959) and with which present results agree; why impermeability should be so related to the mean molecular weight of the lipid is at present without adequate physico-chemical explanation (see Beament, 1961a). To a submerged insect, as compared with an insect exposed to dry air, impermeability may have an entirely different physiological significance, but it is clear that the ecological consequences of very low transition temperatures may be considerable (see Beament, 1961b). The adult *Dytiscus* has a transition temperature at around 24°C. It dies in water two to three degrees hotter than this. Dissection suggests that the internal organs are then waterlogged and that death might be attributed to excessive entry of water when the animal becomes less impervious at this higher temperature (a matter which is now under active investigation). Now by comparison, adults of the related beetles *Agabus* and *Ilybius* survive in the laboratory in water at temperatures up to 29°C.; they have very much higher transition temperatures. Whether this observation is reflected in the distribution of these animals in Britain is unknown, but O'Farrell (private communication) says that in Australia *Agabus* is certainly found in water in areas very much hotter than those where *Dytiscus* occurs. Without careful assessment of the characteristics of the actual
species (and even varieties) concerned, ideas of this kind must be limited to generalization, but the implication is clear.

From the study of the larvae bearing gills it is apparent that great care must be taken in interpreting the form of a curve representing the total evaporation rate against temperature; indeed, from such a curve alone it may be impossible to deduce anything of consequence. Whatever the property of the rest of the cuticle on such a larva, the gill is very permeable to water—a natural correlate of its primary function of transmitting oxygen efficiently. These nymphs (or perhaps pre-adult stages might be more correct) endure a degree of terrestrial exposure in the process of metamorphosis and adult emergence. In none of the species examined did there appear to be any differences in waterproofing between the final larval stage and the earlier instars; larvae actually in the process of emerging were not examined. There would appear to be a considerable difference between the resistance to desiccation, for example, of the two kinds of dragonfly nymphs; but the atmosphere into which they emerge, immediately over their aquatic environment, must in any case have a very high humidity.

Caution is necessary in interpreting any of the information in this paper in terms of the osmotic protection which waterproofing may confer on the aquatic insect. There can be no doubt that a lipid barrier which restricts evaporation must also reduce the inward flow of water in the submerged insect—but because of the phenomenon of the asymmetric permeability of insect cuticle (Beament, 1961a) we cannot use the values for permeability to outwardly moving water for quantitative estimates of influx rates. In this respect, the most surprising finding in this study is the great difference between the adult beetle and its larva. Both occupy essentially the same habitat and have very similar diets. The larva of *Dytiscus*, for example, has a pair of terminal spiracles, surrounded by hydrofuge hairs, and no obvious adaptations for cutaneous respiration. Yet on present evidence its cuticle is thoroughly permeable to water, and presumably cutaneous respiration could occur. And it must have to offset a considerable osmotic influx of water. The adult is efficiently waterproofed and we have already seen that it cannot sustain the osmotic invasion of its body when that waterproofing loses its efficiency.

Finally, we must consider the actual form of some of the permeability/temperature curves which have been obtained, for these suggest reasons for what may otherwise appear as a sporadic representation of lipids. The actual permeabilities of *Dytiscus* and *Gyrinus* adults, below transition, could be the result of a relatively thick layer of molecularly unorganized lipids. The existence of a sharp transition phenomenon eliminates this possibility, whereupon the permeability is consonant with one monomolecular layer alone. Now the increment in permeability at transition (about a fivefold increase) corresponds closely with the characteristics of a monolayer of cockroach grease spread on water, instead of with the behaviour of the much thicker layer of grease on intact *Periplaneta* (Beament, 1958). The permeabilities of *Corixa*, *Notonecta*, *Agabus* and *Ilybius*, all of which show sharply defined transition phenomena, are also of the right order if their surfaces are covered by one monolayer of lipid, though in some of them the form of the permeability/temperature function indicates that the lipid must be a wax rather than a grease. The particular property shared by all the animals mentioned in this paragraph is that they have at least some areas of surface which are highly hydrofuge, even after prolonged immersion in water, and functioning
variously as plastons, air-reservoirs, or flotation devices. It may well prove that osmotic protection is not the key to the distribution of cuticular lipids in the aquatic insects, and that highly organized monolayers are associated with their hydrofuge properties. This suggestion will be explored in a later paper in this series.

SUMMARY

1. The permeability to water of the cuticles of a number of aquatic insects has been measured under conditions of evaporation. The range of rates of evaporation amongst these animals is very great; some adult aquatic beetles are quite as waterproof as more permeable terrestrial insects (caterpillars for example), whereas their larvae would seem to be without specific mechanism for restricting the passage of water.

2. Two methods are described, whereby local differences in permeability may be detected, and from one of these methods it is possible to calculate the permeability/temperature characteristics of different areas of cuticle of an intact insect.

3. The more impermeable insects have uniform permeability over their entire surfaces; these include adult beetles, and both mature and immature stages of Hemiptera. Larvae of mayflies and of some beetles, which are very permeable to water, likewise show no local differences in permeability.

4. The gills of all the species examined are very permeable to water; those of zygopteran dragonflies are more permeable than the rest of the cuticle at lower temperatures, those of Sialis are much more permeable at all temperatures.

5. The effect of temperature upon permeability has been measured. The more waterproof insects reveal transition phenomena characteristic of organized monolayers of lipid, such as are found in terrestrial insects, but by comparison with them the temperatures at which transition occurs are very low. In particular, the temperature at which adults of Dytiscus and Gyrinus become suddenly more permeable (ca. 25°C.) could obtain in natural circumstances, and the evidence suggests that these animals die through osmotic invasion of water when placed at higher temperatures. Other beetles and aquatic Hemiptera, with higher transition temperatures, survive temperatures above 25°C. These ecological implications are discussed.

6. A transition phenomenon occurs in organized lipid over the main cuticle of Sialis, whereas the more permeable gill shows no evidence of this. There is no evidence for organized lipid on the main cuticle of zygopteran larvae, but the temperature/permeability relations of the general cuticle and of the gill are very different.

7. The absolute permeability, and the shape of the permeability/temperature function, of the cuticle of those insects which have very hydrofuge areas of surface suggest that their waterproofing system consists of only one monolayer of organized lipid, which may be either grease or wax.

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