THE PERMEABILITY TO WATER OF THE CUTICLE OF THE LARVA OF *OPIFEX FUSCUS* (HUTTON) (DIPTERA, CULICIDAE)

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INTRODUCTION

Mosquito larvae have been extensively used as models for the investigation of osmotic and ionic regulation in insects. They are phylogenetically well understood, and have colonized a wide variety of different environments, so that comparative studies have been possible on related species from widely differing habitats. Larval mosquitoes are easily obtained and reared in large numbers, are of convenient size for experimental treatment, and the structures considered important in osmoregulation are relatively easily isolated. As a result of the detailed studies carried out by a number of workers a tolerably complete knowledge of the mechanisms involved in salt and water balance has emerged, at least for the freshwater larva of *Aedes aegypti* (Stobbart, 1971a–c). In this insect most of the transepithelial exchange of salts and water between medium and haemolymph occurs through the anal papillae (Wigglesworth, 1933a, b; Treherne, 1954). The papillae are also the sites of active uptake of sodium, potassium and chloride ions into the haemolymph against a steep concentration gradient (Koch, 1938; Ramsay, 1953; Treherne, 1954; Stobbart, 1959, 1960, 1971b).

Less information is available regarding the mechanisms underlying the well-developed powers of hypo-osmotic regulation of mosquito larvae inhabiting salt water. The cuticle of *Aedes detritus* was regarded as being almost impermeable to water by Beadle (1939), and any osmotic loss of water which took place by this route was thought to be replaced by drinking the medium and the production of a concentrated rectal fluid (Ramsay, 1950).

Shaw & Stobbart (1963) have suggested that the cuticle of marine insects would prove to have a permeability to water at least an order of magnitude less than that of related freshwater forms. As yet, however, no data have emerged to support this hypothesis. Previous experiments (Nicolson, 1972) established that the general pattern of salinity tolerance and osmotic regulation in larvae of the New Zealand marine mosquito, *Opifex fuscus*, was very similar to that of *Aedes detritus*. The present paper reports the results of experiments to test the validity of the hypothesis of Shaw & Stobbart (1963) by measuring the permeability of the cuticle of this larva to water.

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Table 1. Composition of the artificial sea water used in the experiments

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>g/l</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sodium chloride (NaCl)</td>
<td>23.960</td>
</tr>
<tr>
<td>Sodium sulphate (Na₂SO₄)</td>
<td>7.954</td>
</tr>
<tr>
<td>Sodium bicarbonate (NaHCO₃)</td>
<td>0.210</td>
</tr>
<tr>
<td>Potassium chloride (KCl)</td>
<td>0.746</td>
</tr>
<tr>
<td>Magnesium chloride (MgCl₂.₆H₂O)</td>
<td>10.979</td>
</tr>
<tr>
<td>Calcium chloride (CaCl₂.₆H₂O)</td>
<td>2.191</td>
</tr>
</tbody>
</table>

MATERIALS AND METHODS

Larvae of *Opifex fuscus* were collected and maintained in the laboratory as described previously (Nicolson, 1972). Unless otherwise stated, all experiments were carried out at a temperature of 20 °C. Only fourth-instar larvae were used in the experiments. They were acclimatized for several days in artificial sea water (Table 1), and starved for 2 days, to empty the gut, before use in experimental work.

Ligaturing, when necessary, was performed with fine human hair or Nylon thread. A knot was tied between head and thorax to prevent drinking and/or between the seventh and eighth abdominal segments to prevent excretion. Although this restricted the respiratory siphon, larvae with such an anal ligature remained alive and active for many hours. Addition of osmotically insignificant (less than 0.001 M) amounts of amaranth (Azorubin S, B.D.H.) to the experimental solution provided a useful check on the effectiveness of the ligatures. Any ligatured larvae still able to drink could be detected by the appearance of the red stain in the mid-gut, while damaged larvae showed colour in the haemolymph. Such animals were rejected.

The water content of the larvae was determined by measuring the weights of individual larvae before and after drying to constant weight at 104 °C. The rate of osmotic loss of water through the cuticle of larvae in sea water was determined by measuring the change in weight of larvae immersed in sea water after ligation at neck and anus. The larvae were weighed in air after careful blotting to remove adherent moisture. The approximately constant weight of control animals, treated in the same way but without ligatures, suggests that this is a consistent method, and also that the larvae lose little weight by evaporation during their exposure to air. All weighings were carried out on a Sartorius balance accurate to 0.001 mg. Results were expressed as mean ± standard deviation.

The thickness of the wax layer of the cuticle and the total surface area of the larva were measured by the method of Shaw (1955). Larvae were killed by immersion in ammonia solution, and after gentle dissection a rectangular piece of cuticle was cut from the abdominal wall. The inside of the test piece was scraped free of adhering material, and carefully washed with petroleum ether. The area of the portion was determined using a graticule in a microscope eye-piece, and the sample was then extracted with petroleum ether in a small test tube at 45 °C. The petroleum ether with its dissolved cuticular wax was then poured onto the surface of a Langmuir trough made of Perspex, of dimensions 11 × 2 × 1.5 cm, and allowed to evaporate. The wax layer left behind was compressed, by means of a screw thread attached to a Perspex slide, against a waxed paper float suspended from a sensitive balance by fine glass rods. An abrupt change in the reading of the balance gave the point of compres-
Permeability to water of cuticle of larva of O. fuscus

sion of the wax monolayer, and its area in the trough could then be measured. A similar procedure was adopted for whole moulted skins of the larvae, although in this case the amount of petroleum ether used for extraction was carefully measured, the extraction tube was sealed, and a small part of the total volume was used for the area determination. A correction was then made for the whole area.

The flux of water across the cuticle was determined using tritiated water. To determine the inward flux of water, a solution of artificial sea water was made up to contain tritiated water at an activity of approximately 10 $\mu$curies/ml. Ligatured and unligatured animals were then placed in the solution, and at suitable times after immersion a group of five ligatured animals and a group of five unligatured animals were removed, washed rapidly and carefully in two lots of inert sea water, and blotted to remove surface moisture. The animals in each group were then placed in turn on a Perspex slide under a microscope, pierced with a fine tungsten needle, and 1 $\mu$l of the haemolymph escaping from each animal was taken up in a calibrated capillary tube. This sample was transferred to a scintillation vial containing 5 ml of scintillation fluid (Triton X 100: 1 part; toluene containing 3 g PPO/l and 0.3 g POPOP/l: 2 parts). Samples of the medium were also taken.

For the determination of efflux rates of water through the cuticle, experimental animals were immersed in the radioactive solution for 24 hr, in which time it had been previously established that they had come into equilibrium with the medium. A number of animals was then removed from the medium, washed rapidly and placed in a second container through which sea water was allowed to flow slowly. Samples of haemolymph were taken as before. Activity of the samples was determined in a Packard TriCarb Scintillation Counter (model 3375), programmed for tritium counting, with automatic background subtraction, and with automatic channels-ratio printout to enable correction to be made for differential quenching.

The inward flux of water was also measured using deuterium oxide, by the method used by Shaw (1955) and Staddon (1966). 50 ml of artificial sea water was made up containing 10 g deuterium oxide. Groups of larvae were placed in this solution and removed after known intervals. Haemolymph samples were removed as before, and the deuterium oxide and water in the mixture were determined by freezing-point elevation using a modification of the technique of Ramsay (1949). The freezing-point apparatus was calibrated using standards over the range 0–20 % deuterium oxide to ensure that the relationship between freezing-point and the deuterium oxide concentration was linear. Distillates were prepared from these standards and their freezing-points were determined with reference to that of de-ionized water. Distillates of the blood of the control animals showed a significantly lower freezing-point than that of de-ionized water, presumably owing to volatile substances passing over into the distillates (Staddon, 1966). The haemolymph samples were used as a baseline for the freezing-point elevations, and it was assumed that there was a similar amount of solute contamination in all tubes.
RESULTS

(1) The osmotic permeability of the cuticle

(a) The net osmotic flux of water across the body wall

Fig. 1 shows the loss of weight with time of larvae immersed in sea water after ligation at neck and anus. The larvae were found to lose 3.95 ± 1.51 % of their original weight in 4 h, corresponding to 0.98 ± 0.38 % body weight/hour.

The mean individual weight of ten larvae was found to be 7.61 ± 0.50 mg. After drying to a constant weight at 104 °C the mean weight was found to be 1.12 ± 0.21 mg, corresponding to a water content of 85.2 ± 2.1 % wet weight. The low standard deviation of these results lends some justification to the assumption that the larvae are fairly uniform, and to the use in subsequent calculations of the mean weight of body water, 6.49 mg, derived from these figures. A loss of weight of 1 % per hour corresponds to a net outward flux of water through the cuticle, of ligatured animals, of 0.076 μL.h⁻¹.

(b) The surface area of the cuticle

Table 2 shows the results of determination of the number of monolayers of wax on pieces of freshly dissected cuticle from the abdomens of Opifex larvae. The numbers of monolayers were estimated by dividing the area of the wax monolayer film by the area of the piece of cuticle from which it was extracted. The average of the measurements was 81 monolayers with a standard error of 5 monolayers. Since the average thickness of a monolayer of insect wax is 3.7 nm (Shaw, 1955), the thickness of the wax layer of the larva can be estimated to be 0.30 μm.

Similar treatment of whole moulted cuticles to determine the total surface area gave the results shown in Table 3. Only a fraction of the dissolved wax could be measured in the Langmuir trough at one time so that it was necessary to apply a
Permeability to water of cuticle of larva of O. fuscus

Table 2. The estimated number of monolayers of wax on pieces of cuticle from the abdomen of Opifex fuscus

<table>
<thead>
<tr>
<th>Length and width of piece of cuticle (mm)</th>
<th>Area (mm²)</th>
<th>Length of monolayer in trough 13 mm wide (mm)</th>
<th>Area of monolayer of wax on cuticle (mm²)</th>
<th>Number of monolayers of wax on cuticle</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.2 x 1.3</td>
<td>1.6</td>
<td>5.5</td>
<td>72</td>
<td>45</td>
</tr>
<tr>
<td>1.6 x 1.7</td>
<td>2.7</td>
<td>13.0</td>
<td>169</td>
<td>63</td>
</tr>
<tr>
<td>0.9 x 1.0</td>
<td>1.8</td>
<td>11.0</td>
<td>141</td>
<td>78</td>
</tr>
<tr>
<td>1.8 x 1.0</td>
<td>1.8</td>
<td>13.0</td>
<td>169</td>
<td>94</td>
</tr>
<tr>
<td>1.0 x 1.3</td>
<td>1.3</td>
<td>4.0</td>
<td>52</td>
<td>40</td>
</tr>
<tr>
<td>1.4 x 0.9</td>
<td>1.3</td>
<td>10.0</td>
<td>130</td>
<td>100</td>
</tr>
<tr>
<td>0.6 x 1.7</td>
<td>1.0</td>
<td>8.5</td>
<td>111</td>
<td>111</td>
</tr>
<tr>
<td>0.8 x 1.7</td>
<td>1.4</td>
<td>8.0</td>
<td>104</td>
<td>74</td>
</tr>
<tr>
<td>1.6 x 2.3</td>
<td>3.7</td>
<td>24.0</td>
<td>312</td>
<td>84</td>
</tr>
<tr>
<td>1.7 x 1.2</td>
<td>2.0</td>
<td>13.5</td>
<td>176</td>
<td>88</td>
</tr>
<tr>
<td>0.9 x 1.8</td>
<td>1.6</td>
<td>13.5</td>
<td>176</td>
<td>110</td>
</tr>
<tr>
<td>1.2 x 2.2</td>
<td>2.6</td>
<td>13.0</td>
<td>169</td>
<td>65</td>
</tr>
<tr>
<td>1.0 x 1.3</td>
<td>1.3</td>
<td>7.5</td>
<td>98</td>
<td>75</td>
</tr>
<tr>
<td>1.8 x 0.9</td>
<td>1.6</td>
<td>10.0</td>
<td>130</td>
<td>81</td>
</tr>
<tr>
<td>1.6 x 1.2</td>
<td>1.9</td>
<td>15.0</td>
<td>195</td>
<td>103</td>
</tr>
<tr>
<td>0.9 x 1.5</td>
<td>1.4</td>
<td>9.5</td>
<td>124</td>
<td>88</td>
</tr>
</tbody>
</table>

Mean number of monolayers is 81. Standard error is 5.

Table 3. Determination of the surface area of the larva of Opifex fuscus

<table>
<thead>
<tr>
<th>Length of monolayer in trough 13 mm wide (mm)</th>
<th>Area of monolayer (mm²)</th>
<th>Fraction of extract</th>
<th>Area of whole extract (mm²)</th>
<th>Surface area (mm²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>33.0</td>
<td>429.0</td>
<td>0.166</td>
<td>2574</td>
<td>31.7</td>
</tr>
<tr>
<td>21.0</td>
<td>273.0</td>
<td>0.125</td>
<td>2184</td>
<td>27.0</td>
</tr>
<tr>
<td>22.0</td>
<td>286.0</td>
<td>0.125</td>
<td>2288</td>
<td>28.2</td>
</tr>
<tr>
<td>23.0</td>
<td>299.0</td>
<td>0.125</td>
<td>2392</td>
<td>29.5</td>
</tr>
<tr>
<td>24.5</td>
<td>318.5</td>
<td>0.125</td>
<td>2548</td>
<td>31.4</td>
</tr>
<tr>
<td>22.0</td>
<td>286.0</td>
<td>0.125</td>
<td>2288</td>
<td>28.2</td>
</tr>
</tbody>
</table>

Mean surface area is 29.3 mm².

correction for the whole sample. Such a calculation gives a total surface area for a fourth-instar Opifex larva of 29.3 mm².

It is obvious that this method of determining the surface area is subject to many sources of error, perhaps the most serious of which is the assumption that the wax layer is uniformly distributed over the surface of the insect. However, the small size of the animal precludes an attempt to estimate the extent of variation of the thickness of the wax layer in this insect. A small change in the thickness of the wax in various parts of the body would obviously lead to a considerable error in estimation of total surface area. If, however, the simplifying assumption is made that the animal is a perfect cylinder, then an approximate figure for the surface area can be calculated from the elementary dimensions of the larva. The average length of a fourth-instar larva of Opifex is 9 mm, and the diameter of most of the body is 1 mm. If the animal is treated as a right cylinder having these dimensions, then a figure of 30 mm² is
obtained for the surface area. Taking the figure of 29.3 mm² as the surface area of the larva gives a value for the net outward flux of water through the cuticle of $0.076/29.3 = 0.0026 \mu l. mm^{-2}. h^{-1}$ assuming that the cuticle is uniformly permeable to water.

(c) **The osmotic permeability coefficient**

The relation between the osmotic flux of water and the osmotic permeability coefficient is given by the expression

$$J_{os} = P_{os} A \frac{V_w}{A} \Delta C,$$

where $J_{os}$ is the net osmotic flux of water (ml.h⁻¹), $P_{os}$ is the osmotic permeability coefficient (cm.h⁻¹), $A$ is the surface area (cm²) across which the flux is occurring, $V_w$ is the partial molar volume of water, and $\Delta C$ is the osmolar concentration difference of the solute across the cuticle. In sea water the haemolymph of *Opifex* is about 400 mOsm (Nicolson, 1972), and the medium itself has an osmotic concentration of 1000 mOsm. Substitution of the values found above for the net flux and the surface area gives a value for the osmotic permeability coefficient $P_{os}$ of $2.4 \times 10^{-2}$ cm.h⁻¹.

(2) **The diffusional fluxes of water across the cuticle**

(a) **The influx of tritium**

Bearing in mind the assumptions pointed out by Shaw (1955), the entry of tritiated water into a small animal should follow an exponential curve having the form

$$C_i^* = C_o^* (1 - \exp (-t/T)),$$

where $C_i^*$ and $C_o^*$ are the internal and external activities of tritiated water respectively, $t$ is the time, and $T$ is the time constant of exchange of tritiated water. A plot of $\log_{10} (1 - C_i^*/C_o^*)$ against $t$ should give a straight line with a gradient of $-1/2.3 T$. Such
Permeability to water of cuticle of larva of O. fuscus

Fig. 3. The rate of penetration of deuterium oxide into ligatured and unligatured larvae of *Opifex*, shown as the rate of change of the function, \((1 - C_i/C_o)\), with time, where \(C_i\) is the internal concentration of deuterium oxide at time \(t\), and \(C_o\) is the external concentration of deuterium oxide.

![Graph showing rate of penetration of deuterium oxide into larvae](image)

Fig. 4. The percentage decrease in activity with time of larvae fully loaded with tritiated water, and immersed in flowing unlabelled sea water. Each point is the mean of at least five determinations.

![Graph showing percentage decrease in activity with time](image)

A plot is shown in Fig. 2. Calculation of the coefficient of regression gives values for \(T\) of \(4.0 \pm 0.3\) h for unligatured animals and \(4.54 \pm 0.3\) h for ligatured ones. The insignificant difference between the two groups suggests that uptake of water by drinking is small compared with the diffusional influx of water through the cuticle.

The diffusional permeability coefficient \(P_d\) is related to the time constant by the expression

\[
P_d = V/TA,
\]

where \(V\) is the volume of body water and \(A\) the surface area of the cuticle. If \(V\) is measured in ml and the surface area in cm², then the units of \(P_d\), the diffusional permeability coefficient for water, are cm·h⁻¹. Substitution of the determined values in the above expression gives a value for \(P_d\), in ligatured animals, of \(4.8 \times 10^{-3}\) cm·h⁻¹.

The inward flux of water was checked by the use of deuterium oxide. Results given
by this method are shown in Fig. 3. The value of the time constant of exchange found by this method was 4.16 h, and no significant difference was found between ligatured and unligatured larvae over the short period of the experiment. These figures are in sufficiently good agreement with those obtained by the use of tritium to suggest that the time constant of exchange of the heavy isotopes of water is close to that of normal water.

(b) The efflux of tritium

Fig. 4 shows the percentage decrease in activity with time of 1 µl samples of haemolymph from larvae loaded with tritium and immersed in unlabelled sea water. Calculation of the regression coefficient as before gives a value for the time constant of exchange of 4.08 ± 0.08 h.

DISCUSSION

Insects living in a medium hyperosmotic to the haemolymph are exposed to dehydration by outward diffusion of water down an osmotic gradient across the body wall. Such an osmotic loss is generally held to be small in marine insects, and its replacement is thought to be achieved by uptake of the medium by drinking and by excretion of a rectal fluid slightly more concentrated than the medium. The marine larva of *Aedes detritus* is known to drink considerable quantities of the medium, and in the course of the present work it was found that the gut of the larva of *Opifex fuscus* quickly became filled with dye when the larva was placed in sea water to which amaranth had been added, indicating that this larva too takes up the medium by drinking.

The cuticle of the larva of *Aedes detritus* was regarded by Beadle (1939) and by Ramsay (1950) as being virtually impermeable to water; thus the osmotic loss of water by this route would be negligible. Stobbart (1971c) found, however, that larvae of this insect showed considerable shrinkage when immersed in sea water after ligation of mouth and anus, suggesting an appreciable permeability to water. In the present work it was likewise found that the cuticle of the body wall of *Opifex* was measurably permeable, the ligatured larva losing weight at about 1 % body weight/hour in sea water. The osmotic permeability coefficient $P_{os}$ calculated from these data was $2.4 \times 10^{-2}$ cm h$^{-1}$. Other factors remaining unchanged, in fresh water (50 mOsm) the larva would be expected to gain weight at the rate of approximately 0.5 % body weight/h, or 12 %/day. This is a figure of the same order as that found by direct weighing of freshwater insects. Sutcliffe (1961) gives a value of about 7 % of the body weight per day for the osmotic uptake of water through the cuticles of several caddis larvae, Shaw (1955) gives a value of 4 % per day for the larva of *Sialis lutaria*, and Staddon (1969) a figure of 10 % per day for osmotic uptake through the cuticle of adults of *Ilyocoris cimicoides*. In the same paper, Staddon (1969) suggests that the larva of *Aedes aegypti* must gain water at the rate of about 20 % of the body weight per day to account for the observed rate of rectal fluid production (Ramsay, 1950).

Table 4 gives values of the diffusional permeability coefficient of water for the body surface of some freshwater insects and of *Opifex*. The brine shrimp *Artemia salina*, another hypo-osmotic regulator, is also included. Unfortunately no data are available for freshwater mosquito larvae.
Table 4. The permeability of some aquatic insects and Artemia to water

<table>
<thead>
<tr>
<th>Species</th>
<th>Diffusional permeability coefficient (cm·h⁻¹)</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ilyocoris cimicoides</td>
<td>1.7 x 10⁻¹</td>
<td>Staddon (1966)</td>
</tr>
<tr>
<td>Notonecta glauca</td>
<td>1.4 x 10⁻¹</td>
<td>Staddon (1966)</td>
</tr>
<tr>
<td>Corixa dentipes</td>
<td>2.3 x 10⁻¹</td>
<td>Staddon (1966)</td>
</tr>
<tr>
<td>Sialia lutaria</td>
<td>1.8 x 10⁻¹</td>
<td>Shaw (1955)</td>
</tr>
<tr>
<td>Artemia salina</td>
<td>2.5 x 10⁻¹</td>
<td>Smith (1969)</td>
</tr>
<tr>
<td>Opifex fuscus</td>
<td>4.8 x 10⁻²</td>
<td>Present work</td>
</tr>
</tbody>
</table>

Shaw & Stobbart (1963) predicted that the permeability of the cuticles of marine insects would prove to be at least an order of magnitude less than that of freshwater forms. This hypothesis is supported by the present finding that the value for $P_d$ in *Opifex* is markedly lower than in those freshwater insects for which this parameter has been measured. The figure for *Artemia* is for the most permeable part of the body surface, the gill epithelium.

Smith (1969) has compared the osmotic and diffusional water permeabilities of the gill epithelium of *Artemia*. $P_{os}$, calculated from the drinking rate, is 0.35 cm·h⁻¹ and $P_d$, calculated from the influx of tritiated water, is 2.5 x 10⁻² cm·h⁻¹, some 14 times smaller than $P_{os}$. Similar discrepancies have often been observed in recent work on other epithelial tissues.

In common with such findings, the diffusional permeability coefficient, $P_d$, for the cuticle of *Opifex* larvae was considerably less than the osmotic permeability coefficient, $P_{os}$. Although the value of $P_d$ in the present work may be criticized on the grounds that it was not measured under the necessary condition of zero volume flow (Dainty & House, 1966), it seems none the less to be much less than $P_{os}$. The difference between the values of $P_d$ and $P_{os}$ in other tissues has been attributed either to the fact that the penetration of water takes place through water-filled pores or channels (Koefoed-Johnsen & Ussing, 1953), or, as seems more likely in the present case, that the value of $P_d$ has been underestimated owing to inadequate mixing and the presence of unstirred layers within or adjacent to the cuticle (Dainty & House, 1966).

Although the permeability of the cuticle of *Opifex* is low when compared with that of freshwater insects, the directly measured osmotic loss of water through the cuticle must be a significant factor in the water economy of the insect. If the animal is to remain in balance, then the transcuticular water loss must be met by a net gain of water elsewhere. In *Aedes detritus* this has normally been held to be achieved by the uptake of the medium by drinking, followed by the production of a hypertonic rectal fluid. However Ramsay’s (1950) figures for the rectal fluid of *Aedes detritus* in sea water showed only three cases out of seven in which the fluid was hypertonic to the medium. It is possible that in the other four cases the rectal fluid had not reached equilibrium, and it may be argued that it is sufficient to show that the hind gut can elaborate a fluid more concentrated than the medium.

A net osmotic gain of water may also be achieved by uptake of the medium by drinking, and the excretion of salts at an extra-renal site at a rate exceeding that of the osmotic outflow of water. In *Drosophila melanogaster* larvae, Croghan & Lockwood
602 SUSAN W. NICOLSON AND J. P. LEADER

(1960) suggested that adaptation to a hyperosmotic medium involved the active excretion of sodium chloride through ventral plates on the abdomen. The anal papillae of freshwater mosquito larvae are known to be important sites of ion uptake (Stobbart, 1971a, b), but until recently their role in marine forms has been regarded as an insignificant or minor one. Like Aedes detritus and A. campestris, Opifex has anal papillae greatly reduced in size as compared with freshwater species.

Phillips & Meredith (1969) and Meredith & Phillips (1973) have shown, however, that the anal papillae of Aedes campestris may be concerned in the active extrusion of chloride ions into a hyperosmotic medium. If it is the case that the anal papillae can in fact transport ions from the haemolymph into the medium, then the load on the excretory system will be correspondingly reduced. Further work is in progress on the role of anal papillae in osmotic and ionic regulation of the larva of Opifex fuscus.

SUMMARY

1. Fourth-instar larvae of Opifex fuscus were found to have a mean body weight of 7.61 mg, a water content of 85.2%, and a mean cuticular surface area of 29.3 mm².

2. Larvae ligatured at neck and anus were found to lose 1% of the body weight per hour when immersed in sea water.

3. The osmotic permeability coefficient of the cuticle was calculated from these data to be $2.4 \times 10^{-3}$ cm h⁻¹.

4. Measurement of the diffusional permeability coefficient ($P_d$) by means of tritiated water and Deuterium oxide gave a value for this parameter of $4.8 \times 10^{-3}$ cm h⁻¹.

5. The diffusional permeability of the cuticle of Opifex fuscus is thus less than that of typical freshwater insects, as predicted by Shaw & Stobbart.

The initial study which led to this work was carried out by one of us (S. W. N.) as part requirement for the degree of B.Sc. (Hon.) of the University of Auckland.

Thanks are due to Professor R. E. F. Matthews, of the Department of Cell Biology, University of Auckland, for permitting the use of a Packard Scintillation Spectrometer, and to Professor A. L. Odell, of the Department of Chemistry, University of Auckland, for the gift of a sample of tritiated water. Mr T. P. Warren and Mr J. G. Moorhouse provided valuable technical assistance.

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REFERENCES


