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THE PERMEABILITY TO WATER OF THE CUTICLES OF SOME ADULT WATER BUGS

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INTRODUCTION

Studies of the water balance in adult water bugs have been reported in previous papers (Staddon, 1963, 1964). The adults lose water in the rectal fluid and gain water as a result of drinking and by osmotic uptake through the cuticle. The rate of osmotic uptake was estimated indirectly, from a knowledge of the rates of rectal fluid production and drinking, to lie between 2 and 7% of the body weight per day at 18° C. Similar values for the osmotic water uptake have been found in several aquatic insect larvae. For example, Shaw (1955) gives a value of 4% of the body weight per day for the osmotic uptake in *Sialis lutaria* larvae; Sutcliffe (1961) a value of 7% for the uptake in several caddis larvae. Thus it would appear that adult water bugs may be at least as permeable to water as these aquatic larvae; possibly more permeable, since the possession of gas films and elytra must restrict the area of cuticle through which water absorption occurs. On the other hand, the figures given by Holdgate (1956) and Beament (1961) for evaporation rates from a wide variety of aquatic insects, including adult water bugs, caddis and *Sialis* larvae, indicate that the adult forms are less permeable to water than the larvae. Thus a need for further quantitative information on the cuticular permeability and osmotic water influx in adult water bugs is indicated. The work to be described in this paper goes some way towards supplying this information.

MATERIALS AND METHODS

Adult water bugs were collected from Kenfig Pool, Glamorgan. In the laboratory they were kept without food, each in a small volume of de-ionized water, until required. Small fragments of nylon cloth were provided for anchorage. At all times the specimens were handled carefully to avoid possible damage to the cuticle. Permeability measurements were made on the following species: *Ilyocoris cimicoides* (L.), *Corixa dentipes* (Thoms.) and *Notonecta glauca* L. In the work on *Ilyocoris* only adult females were used, these being appreciably larger than the males, but no such selection was made in the case of the other species in which the difference between the sexes is not so marked.

The permeability to water of the cuticles of the adults was measured using deuterium oxide (heavy water) as tracer. This technique was previously used by Shaw (1955) in a study of the permeability of *Sialis lutaria* larval cuticle.

To measure the concentration of deuterium oxide in the haemolymph advantage was taken of the fact that the freezing-point of deuterium oxide (3·8° C.) is much higher than that of water. Distillates were prepared from the haemolymph and

freezing-point measurements were made by the method of Ramsay & Brown (1955). The author is indebted to J. Shaw of the Zoology Department, University of Newcastle upon Tyne, for suggesting this application of the freezing-point method.

Haemolymph was obtained from *Ilyocoris* adults by amputating one of the forelegs below the base of the stout femur. A droplet of haemolymph was forced from the wound by gently squeezing the abdomen. Haemolymph was obtained from *Corixa* and *Notonecta* adults by detaching one of the wings.

Samples of haemolymph were distilled in thin-walled, hard-glass capillary tubes each measuring about 1·5 mm. wide and 50 mm. long. The unsealed tube was drawn to a taper at one end, and approximately 10 mm. from the other end was bent at an angle of about 45°. As the haemolymph exuded from the wound a small quantity,

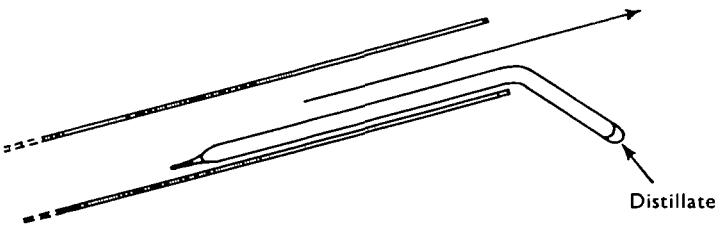

Fig. 1. Distillation of haemolymph.

about 0·5–1 μl., was drawn by capillarity into the tapered end of the tube. The tube was then sealed at both ends in a small flame. The longer arm of the sealed tube which contained the sample at its extremity was immersed in steam by slipping it into the side arm of a distillation flask which contained boiling water (Fig. 1) and left there until the whole of the water from the haemolymph had condensed at the end of the short exposed arm of the capillary. The tube was then removed, dried and snapped to expose the distillate, which was immediately covered with liquid paraffin from a capillary pipette. The same pipette was used to transfer the distillate to a silicone-lined watch-glass containing liquid paraffin. The freezing-point of the distillate was measured shortly afterwards and whenever possible determinations were made on the distillates of four or five samples of haemolymph collected from the same specimen.

The relationship between freezing-point and D_2O concentration was determined empirically over the range 0–20% (20 g./100 ml.) D_2O. Distillates were prepared from standards and the freezing-point was determined with reference to the freezing-point of distillates of de-ionized water prepared in the same way. The freezing-points of the distillates are shown plotted against the D_2O concentration in Fig. 2. The straight line drawn through the points is the line of regression of concentration on freezing-point. The regression coefficient was 26·85 and made equal to m in the estimating equation $y = mx$, where y is the D_2O concentration and x the freezing-point of the distillate. The standard deviation from regression was $\pm 0{\cdot}21$ and the number of measurements 19.

Distillates prepared from haemolymph yielded a significantly lower freezing-point than distillates prepared from de-ionized water. The results of a typical series of measurements on *Ilyocoris* haemolymph, together with the results of a similar series of measurements on de-ionized water, are shown for comparison in Table 1. On

average, the freezing-point of a distillate of *Ilyocoris* haemolymph was 0·04° C. lower than that of a distillate prepared from de-ionized water. Presumably volatile substances are released from the haemolymph during the distillation process and dissolve in the distillate to bring about a lowering of the freezing-point. Errors due to this cause were minimized by estimating the concentration of D_2O in a distillate of haemolymph with reference to the freezing-points of distillates prepared from normal haemolymph. Thus the concentration of D_2O in the haemolymph was calculated by making the difference between the freezing-points of normal and D_2O-containing haemolymph equal to y in the estimating equation.

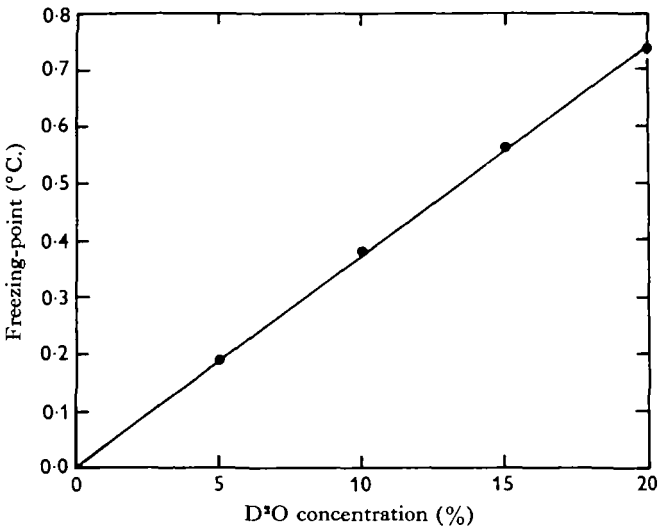

Fig. 2. Relationship between freezing-point and concentration of heavy water in distillates prepared from standard D_2O solutions.

Table 1. *A comparison of the freezing-point of distillates of de-ionized water and* Ilyocoris *haemolymph*

Sample	Mean (°C.)	S.D. (± °C.)	No. of measurements
De-ionized water	+0·02	0·0037	10
Haemolymph	−0·02	0·0063	11

PERMEABILITY MEASUREMENTS

To measure the penetration of heavy water adults were placed in about 150 ml. of a solution containing 20 g. (Norsk Hydro 99·7%) D_2O/100 ml. The solution was well stirred as shown in Fig. 3. and maintained at a constant temperature with a water bath. After intervals of time which extended up to 8 hr. from the start the adults were removed and determinations were made of the D_2O concentrations of the haemolymph. The penetration of D_2O into *Ilyocoris*, *Corixa* and *Notonecta* adults was measured at 18° C.; additional measurements were made on *Ilyocoris* adults at 8 and 28° C. The results of these experiments appear in Figs. 4 and 5 where the concentration of deuterium oxide in the haemolymph is shown plotted against the time that the adults had been in the 20% D_2O solution.

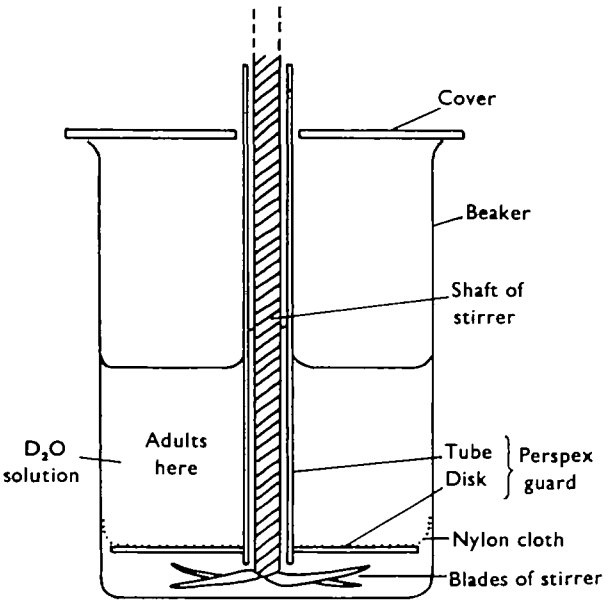

Fig. 3. Apparatus for the stirring of the D_2O solution such that the adults would be protected from the stirrer yet able to visit the surface. The adults were provided with nylon cloth for anchorage. The stirrer was regulated to give a velocity at the surface of about 5 cm./sec.

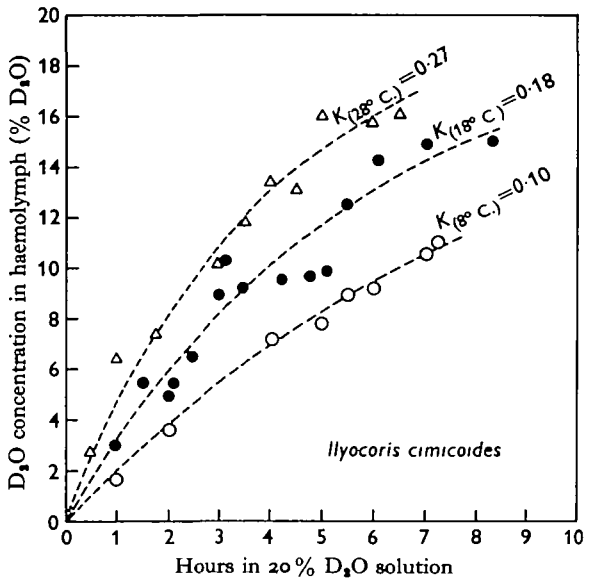

Fig. 4

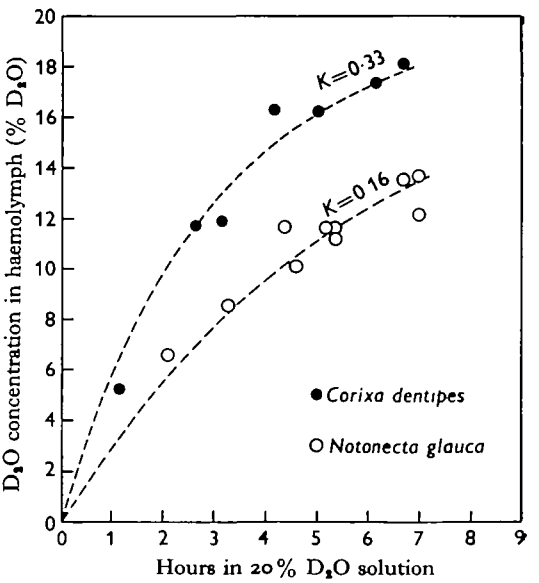

Fig. 5

Fig. 4. Time course for the penetration of heavy water into *Ilyocoris cimicoides* adults from a 20% D_2O solution ○, at 8° C., ●, at 18° C., △, at 28° C.

Fig. 5. Time course for the penetration of heavy water into *Corixa dentipes* and *Notonecta glauca* adults from a 20% D_2O solution at 18° C.

If Fick's law applies to the penetration of deuterium oxide through the cuticle then the concentration of D_2O in the haemolymph (C_i) at any time (t) should be given by the equation $C_i = C_0(1 - e^{-Kt})$, where C_0 is the external concentration of D_2O and K the rate constant of the influx. Curves for this equation are shown plotted in Figs. 3 and 4 for comparison with the experimental results. These curves were fitted statistically by calculating the coefficient of the line of regression of $\ln(C_0 - C_i)/C_0$ on time and substituting for the rate constant K. Although the points do not lie consistently near the curves (the deviations could be attributed partly to differences between individual specimens) there is sufficient correspondence between them to suggest that this relation holds.

Table 2. *The rate constants of the influx and efflux of deuterium oxide*

(Mean ± standard deviation (no. of measurements).)

Species	Temp. (°C.)	Rate constant of influx (hr.$^{-1}$)	Q_{10} (in.)	Rate constant of efflux (hr.$^{-1}$)	Q_{10} (eff.)
Ilyocoris cimicoides	8	0·10 ± 0·007(8)	1·8	—	—
	18	0·18 ± 0·034(15)		0·17 ± 0·034(6)	2·2
	28	0·27 ± 0·047(10)	1·5	0·37 ± 0·028(5)	
Notonecta glauca	18	0·16 ± 0·019(10)	—	—	—
Corixa dentipes	18	0·33 ± 0·046(7)	—	—	—

Table 3. *The rate of water uptake by drinking in the* 20% D_2O *solution*

(Mean ± standard deviation (no. of measurements).)

Species	Temp. (°C.)	Water uptake (μl./hr.)
Ilyocoris cimicoides	8	Less than 0·01(8)
	18	0·1 ± 0·46(5)
	28	1·0 ± 0·55(10)
Notonecta glauca	18	1·0 ± 0·38(10)
Corixa dentipes	18	0·14 ± 0·13(6)

Rate constants of the influx calculated according to this relation are presented in Table 2. From the rate constants of the influx through *Ilyocoris* adults Q_{10} values can be calculated. The Q_{10} of the influx had an average value of 1·8 over the temperature interval 8–18° C. and a value of 1·5 over the interval 18–28° C. Thus a slight decrease in Q_{10} with increasing temperature is indicated.

The possibility that the adults had been drinking during the time that they had been in the 20% D_2O solution was also investigated. Amaranth in known concentration (0·01 M/l.) was incorporated in the 20% D_2O solution (the inclusion of amaranth has no detectable effect on the rate of influx of D_2O) and from the ratio of dye uptake to concentration the uptake of water was calculated. The amaranth content of the gut was estimated by the technique of Treherne (1957). When present the dye was usually confined to the lumen of the anterior midgut (it was not absorbed from the gut), which suggests that the anterior midgut is the principal site of water absorption. The results of these determinations are presented in Table 3. These results show that negligible drinking had been occurring except by the *Ilyocoris* adults at 28° C. and by the *Notonecta* adults. Nonetheless, the rate of drinking by these adults (av. 1 μl./hr.),

although significant in terms of the water economy, would be insufficient to account for more than a small proportion of the total D_2O influx. The total influx was therefore equated with the influx through the cuticle.

A few measurements were made of the efflux of D_2O from *Ilyocoris* adults at 18 and 28° C. for comparison with the influx rates at the same temperatures. Several adults were kept in a 20% D_2O solution for several days by which time it was assumed that the internal concentration of D_2O had reached that of the outside solution. The specimens were then transferred to about 150 ml. of de-ionized water which was well stirred while maintained at a constant temperature with a water bath. The adults were taken from the water at intervals of from 1 to 6 hr. and measurements were made of the concentration of D_2O in the haemolymph. The rate constant of the efflux was calculated according to the relation $C_t = Ce^{-Kt}$, where C is the concentration of D_2O in the haemolymph at the start. The best value of K was obtained by calculating the coefficient of the regression line of $\ln C_t/C$ on time and substituting for K. The results of these measurements are included in Table 2.

At 18° C. the mean values obtained for the influx (0·18/hr.) and efflux (0·17/hr.) were very similar, indicating that the permeability of the cuticle to water may be the same in the two different directions. At 28° C., however, the means were significantly different, the mean for the efflux (0·37/hr.) being higher than that of the influx (0·27/hr.); but whether this difference implies an asymmetry in the permeability of the cuticle or a difference in the experiments it is difficult to say. The asymmetry in the conditions on either side of the cuticle makes the experiments different in the two cases, although the difference may be slight. The Q_{10} for the efflux was 2·2.

The permeability constant (P) for the penetration of heavy water through the cuticle was calculated from the relation $P = KV/A$, where K is the rate constant of the influx, V the volume and A the surface area.

The volume was equated with the total body water on the assumption that the adults could be treated as a one-compartment system. Any error in this assumption is likely to be small, since the work of Shaw (1955) has shown that the D_2O exchange between the haemolymph and tissues is rapid compared with the exchange across the body surface. The total body water was calculated in the usual way from a knowledge of the wet and dry weights of the adults.

The surface area was measured on the assumption that the dorsum of head and prothorax, the scutellum and the limbs (excluding the coxae) would be the only sites through which water could penetrate. The surface area of these structures was measured while they were flattened between two slides, having first cleared the specimen in 10% KOH. Minute irregularities in the surface were not taken into account. The cuticular covering of the ventral surface and coxae was ignored since it is precluded from contact with liquid water by a bubble of gas. The elytra were ignored since they appear to lack an efficient circulation of haemolymph except through a small region close to the point of attachment. It was assumed that the cuticle would be uniformly permeable to water over its whole surface and some support for this assumption is provided by the work of Beament (1961).

The results of these determinations are presented in Table 4. The units chosen were A in cm.^{2}, V in ml. and K in hr.$^{-1}$; thus P is in cm./hr. According to these values *Corixa dentipes* has the most permeable cuticle, with a permeability at 18° C. of

$P = 2{\cdot}3 \times 10^{-2}$ cm./hr., the least permeable being that of *Notonecta glauca* with $P = 1{\cdot}4 \times 10^{-2}$ cm./hr.

The osmotic water uptake (W) was calculated from the D_2O permeability constant using the relation $W = PA(M_1 - M_2)$, where $(M_1 - M_2)$ is the difference between the mole fractions of water and haemolymph. It was assumed that the D_2O permeability constant would be equally applicable to water and that the permeability of the cuticle to water diffusing across it would be the same in the two different directions. The mole fraction of water in the haemolymph was calculated from a knowledge of the haemolymph osmotic pressure. The results which are presented in Table 5 indicate that the osmotic water uptake would be greatest in *Corixa dentipes*, with an uptake at 18° C. of about 3% of the body weight per day, and least in *Notonecta glauca*, with an uptake of 1·8%.

Table 4. *The estimated* D_2O *permeability constant of the cuticle at* 18° C.

Species	Total body weight (mg.)	Total body water (mg.)	Effective surface area ($cm.^2$)	Rate constant of influx ($hr.^{-1}$)	Permeability constant (cm./hr.)
Ilyocoris cimicoides	129	94·4	1·0	0·18	0·017
Notonecta glauca	136	94·4	1·05	0·16	0·014
Corixa dentipes	90	68·8	0·93	0·33	0·023

Table 5. *The estimated osmotic water uptake at* 18° C.

Species	Haemolymph osmotic pressure (mM/l. NaCl)	Osmotic water uptake		
		mg./$cm.^2$/24 hr.	mg./adult/24 hr.	% total body weight
Ilyocoris cimicoides	203	2·98	2·98	2·3
Notonecta glauca	185	2·3	2·4	1·8
Corixa dentipes	149	2·98	2·8	3

DISCUSSION

It is interesting to compare the results obtained in the present paper for the permeability of the cuticle of adult water bugs with those obtained by Shaw (1955) in a similar study of the larval cuticle of *Sialis lutaria*. The permeability of the cuticle of the adults varied from $1{\cdot}4 \times 10^{-2}$ to $2{\cdot}3 \times 10^{-2}$ cm./hr. at 18° C. From the results given by Shaw the permeability of *Sialis* larval cuticle at the same temperature can be calculated to be $1{\cdot}4 \times 10^{-2}$ cm./hr. Thus the adults appear to be at least as permeable to water as the larva. On the other hand, the Q_{10} for the penetration of D_2O through *Sialis* larval cuticle (3·8) is high when compared with Q_{10}'s of 1·5 and 1·8 for *Ilyocoris* cuticle. This difference could reflect a possible difference in the lipid covering of the cuticle. Beament (1961) detected a difference in the temperature/evaporation curves of larval *Sialis* and adult *Notonecta* cuticles and from comparative evidence postulated that the cuticle of the *Sialis* larva was grease-covered (the grease may be lacking over the gills) whereas that of *Notonecta* adults was covered by a harder wax.

The comparison based on the heavy water experiments must be viewed with some caution, however. Evidence that the gills of *Sialis* larvae lack an efficient covering of lipid and are more permeable to water than the rest of the body surface has been

obtained by Beament. Shaw made his calculations on the assumption that the permeability of the cuticle would be the same over the whole surface.

Shaw used the D_2O permeability constant to calculate the osmotic water uptake in *Sialis* larvae. The value thus derived was confirmed by measurements of the weight increase in ligatured larvae. Similar attempts to obtain confirmation of the values for the osmotic uptake given in this paper have not been successful. The rigid nature of the cuticle together with the difficulty of applying ligatures makes it difficult to measure the osmotic uptake in living adults by means of a simple volumetric method. However, a comparison can be made with earlier indirect estimates based on a comparison of the rates of rectal production and drinking (Staddon, 1963, 1964). For *Corixa dentipes* adults the uptake was predicted to lie between the limits 2 and 7% of the body weight per day; the value of 3% obtained from the heavy water experiments falls within these limits. On the other hand, a value of about 7% of the body weight per day was needed to account for differences in rectal fluid production and drinking in *Notonecta glauca* adults, which is high compared with the value of 1·8% predicted from the permeability measurements. Further work would be necessary to resolve the problem presented by this discrepancy.

SUMMARY

1. The permeability to water of the cuticles of some adult water bugs has been measured using deuterium oxide (heavy water) as tracer.

2. The D_2O permeability constant of *Ilyocoris cimicoides* cuticle was $1{\cdot}7 \times 10^{-2}$ cm./hr. at 18° C. There was no apparent difference between the rates of D_2O influx and efflux at 18° C., but at 28° C. a possible difference between them was indicated. The Q_{10} of the influx was 1·8 over the temperature interval 8–18° C., 1·5 over the interval 18–28° C. The estimated osmotic water uptake was 2·3% of the body weight per day at 18° C.

3. In *Corixa dentipes* adults the D_2O permeability constant of the cuticle was $2{\cdot}3 \times 10^{-2}$ cm./hr. at 18° C. and the derived osmotic water uptake 3% of the body weight per day. In *Notonecta glauca* adults the corresponding values were $1{\cdot}4 \times 10^{-2}$ cm./hr. and 1·8% of the body weight per day.

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