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A SITE OF WATER AND IONIC EXCHANGE WITH THE MEDIUM IN *PODURA AQUATICA* L. (COLLEMBOLA, ISOTOMIDAE)

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INTRODUCTION

Specialized areas of the body surface are implicated in water and ionic uptake in many insects (Nutman, 1941; Drummond, 1953; Wigglesworth, 1933; Treherne, 1954; Stobbart, 1959, 1960) and the cuticle over these areas is usually permeable more than that over the remaining body surface (Beament, 1961). Drummond (1953) suggested that the eversile vesicles of Thysanura were organs of water uptake. He also included the vesicles of Dipleura and Collembola in this category. Davies (1928) described the use of the ventral tube vesicles of the collembolan *Sminthurus viridis* L. in conveying droplets of water to the mouth. However, not all collembolans possess ventral tube vesicles which can be everted far enough to reach the mouth, and any role of the vesicles in water uptake in these collembolans must be other than direct transference of water droplets to the mouth. De Geer (1743), following experiments in which he partially desiccated specimens of *Podura aquatica* and then allowed them to recover on a water surface, suggested that the ventral tube was used directly in the uptake of water. This was the first record of a function being assigned to the ventral tube in Collembola. Nutman (1941) described experiments using vital stains as indicators of the movement of water which demonstrated that possible points of entry were the ventral tube vesicles and the tips of the limbs in *Onychiurus armatus* (Tullb.). These findings were verified by myself, using other soil Collembola and placing them on a solution of methylene blue. In addition to the entry through the ventral tube vesicles and the tips of the limbs, I also found that the stain entered the anterior part of the gut through the mouth. The entry of the stain into the ventral tube and the tips of the limbs does not, of course, imply that the cuticle overlying these areas is more permeable to water, or to the larger molecules of the dyes, than the cuticle over other parts of the body; it may merely be a reflexion of the contact of these areas with the medium, as Nutman also suggested (see also Noble-Nesbitt, 1963*c*). Indeed it may well be that the differential wettability of the cuticle determines those areas through which exchange with an aqueous medium is possible.

Ruppel (1953) found no evidence for water uptake by the ventral tube vesicles in *Orchesella villosa* and *Tomocerus vulgaris*, although imbibition through the mouth occurred freely. But these experiments are open to the criticism that the animals had not been induced to evert the ventral tube vesicle. Ruppel's apparatus has been used

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by the author with specimens of *Podura aquatica*, and complete eversion obtained. Eversion also occurs after contact of the limbs with a water surface (see Noble-Nesbitt, 1963*c*). These and other preliminary experiments indicated that the ventral tube could be a site for the exchange of water and ions with the external medium.

MATERIALS AND METHODS

Podura aquatica L. was collected from the surfaces of ponds and ditches in the Cambridgeshire area and kept in the laboratory in stock cultures, as outlined elsewhere (Noble-Nesbitt, 1963*a*, *b*).

For experiments involving the use of radioactive isotopes large specimens were transferred from the stock culture onto a balanced salt solution without food in it. One to three days later they were transferred onto a medium, chemically the same,

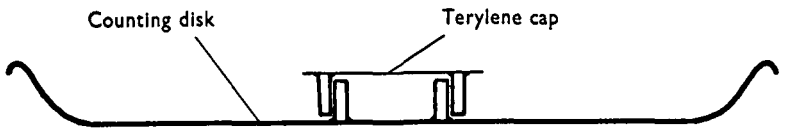

Fig. 1. The specimen holder used to count the radioactivity of living *Podura aquatica*.

but containing the radioactive isotope ^{24}Na in place of some of the sodium, or ^{42}K in place of some of the potassium. The composition of the balanced salt solution was based upon that used by Treherne (1954) and was as follows:

	mM/l.		mM/l.
NaCl	3·00	KCl	1·00
$MgCl_2$	0·20	NaH_2PO_4	0·10
$CaCl_2$	0·50	NaOH	0·059

The NaH_2PO_4-NaOH buffer was used to give a pH of 6·3.

Counting of radioactivity was carried out using a 'Labgear' thin-window G.M. counter and Dekatron scaler unit. In order to make successive counts of the radioactivity of whole, live insects, a special chamber to hold the insect was mounted on a counting disk (see Fig. 1). A 2 mm. length of 3 mm. external diameter brass tubing having an internal diameter of 2 mm. was cemented in the centre of the disk by means of Araldite. A 2 mm. length of brass tubing having an internal diameter of 3·5 mm. was sealed at one end with Terylene 0·004 in. thick. This formed a cap which could be fitted over the narrower brass tubing, enclosing a small chamber into which the insect could be placed. Control experiments showed that the Terylene cap caused no appreciable lowering of the count rate. This chamber ensured that the living insects were confined to an almost identical geometrical position for every count. The same chamber was used for all determinations in each series of experiments, and for many of the series of experiments.

The theoretical value of 15·0 hr. for the half-life of ^{24}Na, used in applying corrections for decay, was checked with a dried down, small volume of the external medium. In all determinations a single micropipette was used, which had been calibrated with a ^{32}P solution of known activity. In this way the count rate of a known volume of the external medium was obtained in each experiment.

The insects during the course of an experiment were individually confined in stoppered tubes containing 5 ml quantities of balanced salt solution labelled with the radioactive isotope. Ten insects, of which at least two were controls, were used in each series of experiments. They were removed from the surface of the solution at various time intervals and transferred briefly (approximately for 10 sec.) to the surface of distilled water, to wash off traces of the radioactive isotope on the body surface. They were then transferred to the counting chamber and their count rates were determined. It was assumed that self-absorption was negligible, and constant for every insect. Counting was carried out for the standard time of 2 min. in order to obtain sufficient determinations for every insect in the experimental series. After counting, the insects were returned to the surface of the medium and allowance was made for the time spent off the medium. At the end of each experimental series, the individual insects were weighed and desiccated in the apparatus described elsewhere (Noble-Nesbitt, 1963*c*), using the nylon-noose method of attachment to the glass hook for weighing purposes.

Equations have been worked out (Harris & Burn, 1949; Davson, 1951; Treherne, 1954; Stobbart, 1959) from which the transfer constants for ionic penetration may be calculated. They apply equally well to the isotopes of sodium or potassium. The symbols used here follow those used by Treherne (1954). They are as follows: k_{in} = transfer constant in the direction out to in; k_{out} = transfer constant in the direction in to out; $[Na_{in}]_\infty$ = internal concentration of labelled sodium at infinite time; $[Na_{out}]$ = external concentration of labelled sodium; $[Na_{in}]_t$ = concentration of labelled sodium within the cell at time, t. The transfer constants are related to the concentration of sodium in the cell and the external medium by the formula:

$$k_{in} = k_{out} \frac{[Na_{in}]_\infty}{[Na_{out}]}. \tag{1}$$

Following conventional methods, the cell is placed in a solution of labelled sodium and $[Na_{in}]_t$ is determined at intervals of time. Using the equation

$$-k_{out}\, t = \ln\left(1 - \frac{[Na_{in}]_t}{[Na_{in}]_\infty}\right), \tag{2}$$

$\log_{10}\left(1 - \frac{[Na_{in}]_t}{[Na_{in}]_\infty}\right)$ is plotted against time, and the slope of the line gives the value of k_{out}. The error in determining the points increases as the exchange nears completion (Solomon, 1952). The slope of the line cannot, therefore, be obtained by calculating a regression line and the slope has been found as the mean of the slopes of the lines joining the individual points to the origin (cf. Treherne, 1954).

The equations given above require that $[Na_{in}]_\infty$ be known independently of the experiment. In the absence of methods sensitive enough to measure the concentration of sodium within a single insect, the value for $[Na_{in}]_\infty$ had to be estimated from the asymptotic value of the total body count, which indicated a figure of approximately 75 m-equiv./l. (Table 1).

The time for half exchange ($T_{\frac{1}{2}}$) of the sodium in the system may be found from the relationship

$$k_{out} = 0{\cdot}693/T_{\frac{1}{2}}. \tag{3}$$

The constants k_{in} and k_{out} are independent of cell volume and surface area, having the dimensions of 1/time.

The above equations have been worked out for single-cell systems. For a multicellular insect, the permeability constants describe only the overall process of permeation. It was assumed that, as in the mosquito larva (Ramsay, 1953; Treherne, 1954), the sodium content of the tissues was low, so that the exchange between the tissues and the haemolymph might not significantly affect the exchange between the haemolymph and the external medium; if this were otherwise, then a straight line would not be expected from a plot of $\log_{10}(1-[Na_{in}]_t/[Na_{in}]_\infty)$ against time. A satisfactory straight line was obtained, however (see Fig. 4), and the body was therefore considered as a single compartment.

The volume of the body compartment into which the labelled sodium passed was taken as the volume of water lost when the insect was desiccated. It was therefore possible to estimate the count rate of a volume of the external medium equal to the total body fluid volume from the count rate of the known volume of the external medium (see above) and to calculate the ratio of the internal count rate to the external count rate, so obtaining a value of $[Na_{in}]_t$ for each determination, since the value of $[Na_{out}]$ was known to be 3·00 m-equiv./l. $[Na_{in}]_t$ was plotted against time and the asymptote ($=[Na_{in}]_\infty$) obtained. These values were substituted in equations (1), (2), (3) to obtain values for k_{in}, k_{out}, and $T_{\frac{1}{2}}$.

All experiments were conducted at room temperature (18–22° C.).

EXPERIMENTS AND RESULTS

Uptake of water

Some attempt has been made to measure the effect of supplying water to the ventral tubes of desiccated specimens of *Podura aquatica*, using a technique similar to that of Ruppel (1953). After short treatment with carbon dioxide to anaesthetize it the specimen was attached to a glass rod with 'Newskin' adhesive applied to its dorsal surface. The glass rod was held in one arm of a micromanipulator and a fine-bore glass tube, through which the water could be fed to the ventral tube, was held in the other arm. Though contact between the ventral tube vesicles and the water surface was readily established, the maintenance of this contact in a living insect over the long periods required to obtain survival times was more difficult; for example, rapid desiccation was clearly produced by heat from the lamp illuminating the apparatus. Under such conditions, the limbs of a specimen became motionless after 30 min. unless water was supplied to the ventral tube; with a supply of water movement continued up to 90 min., but death occurred usually within 120 min. even so. In less drastic desiccating conditions the limbs usually are affected first, and these experiments suggest that water uptake occurs through the ventral tube vesicles.

In a further series of experiments the state of the ventral tube was noted when insects were placed on a water surface after they had been partially desiccated by heating for a period of time at 35° C in a dry tube. After treatment for 8 min. water was continuously imbibed through the mouth; at first the ventral tube was retracted and it appeared shrivelled. After 3 min., the vesicles opened slightly, but it seemed that there was insufficient haemolymph pressure to produce complete eversion. Eight minutes after drinking had started the vesicles were everted sufficiently to bring them into contact with the water surface, and so they remained for a further half-hour. As

recovery from desiccation continued the mouth was raised from the water, and drinking occurred with ever decreasing frequency. By the end of half-an-hour mobility had largely returned, but springing was not observed until about an hour after this.

By comparison, desiccation at 35° C. for 5 min. did not prevent immediate eversion of the ventral tube when the specimen was transferred to water; full recovery and mobility could be achieved even if the mouth was blocked with wax. However, after dessication for 10 min. all movement was stopped and only partial recovery was possible.

These results are interpreted as follows: desiccation stops springing movements, ventral tube eversion and limb movements progressively in that order. When free water is subsequently available, water uptake occurs through the mouth. If the haemolymph pressure is sufficient, i.e. if desiccation has not gone to far, the ventral tube is everted and uptake occurs across its vesicles too. If desiccation has been carried too far, then eversion of the vesicles is only possible after sufficient water has been imbibed through the mouth. Both the mouth and the ventral tube are used in water uptake until most of the body water has been regained, but the later stages of recovery are accomplished mainly by uptake by the ventral tube. If the mouth is blocked but eversion of the ventral tube is possible, then water uptake over the ventral tube vesicles alone is sufficient for full recovery.

The uptake of labelled sodium

Large specimens, previously kept on a balanced salt solution, were placed on a balanced salt solution containing 3·00 m-equiv. Na/l. in which the sodium was labelled with its radioactive isotope ^{24}Na. The uptake of sodium into the whole body was followed over a period of 50–100 hr. A typical time course of ^{24}Na uptake, expressed as counts per animal per minute, is shown in Fig. 2. The same data expressed as the ratio $[Na_{in}]_t/[Na_{out}]$ and as $[Na_{in}]_t$ is shown in Fig. 3. It will be seen that at the end of the experiment the ratio had risen to a value equivalent to a level of approximately 100 m-equiv. Na/l. of body fluids, which corresponds well with the value found for the haemolymph in mosquito larvae (Ramsay, 1953; Treherne, 1954). These results show that the sodium exchanges with that in the external medium.

Using equation (2) applied to the above data, a semi-logarithmic plot results in a fairly satisfactory straight line (see Fig. 4). The line shown in Fig. 4 is the mean of the slopes calculated from the lines joining the individual readings to the point of origin. This result suggests that the whole body may be considered as a single compartment containing sodium. This supports the assumption made in the theoretical considerations.

The time for half-exchange ($T_{\frac{1}{2}}$) is approximately 16 hr. and the values of the constants k_{in} and k_{out} are 1·01 hr.$^{-1}$ and 0·044 hr. $^{-1}$, respectively (see Table 1, controls).

The site of uptake of sodium

To determine the site of uptake of sodium, the entry of ^{24}Na into the whole body was followed in (i) normal insects, (ii) insects with mouths blocked, (iii) insects with ventral tube vesicles blocked, and the rates of entry were compared.

Blocking was achieved by applying hot paraffin wax to the ventral tube or mouth, or by destroying them with a cautery.

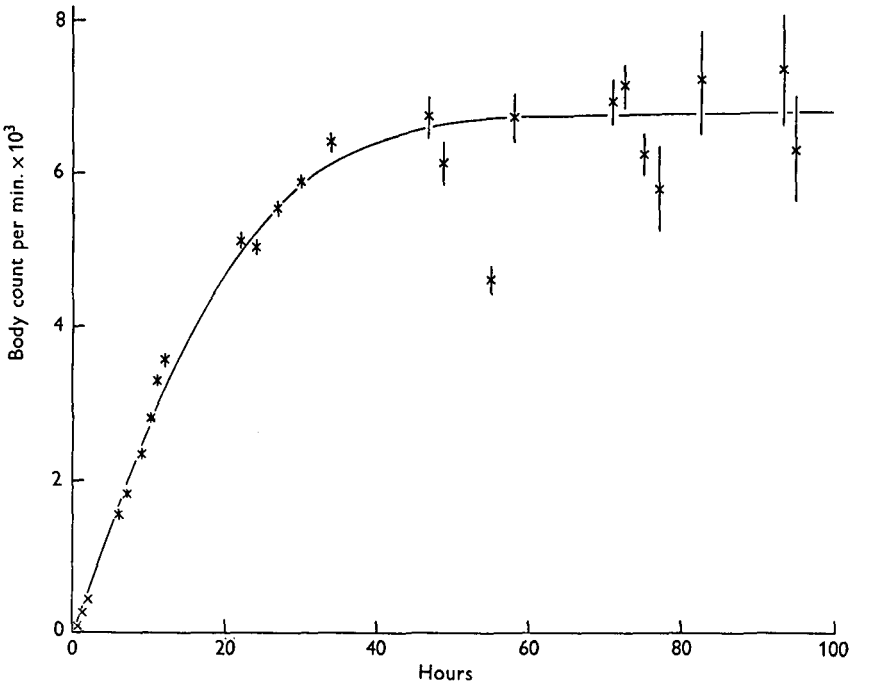

Fig. 2. The uptake of ^{24}Na into the body of a single insect over a period of 100 hours. The crosses show single determinations of the radioactivity of the insect, and the vertical lines show the standard deviations due to counting errors.

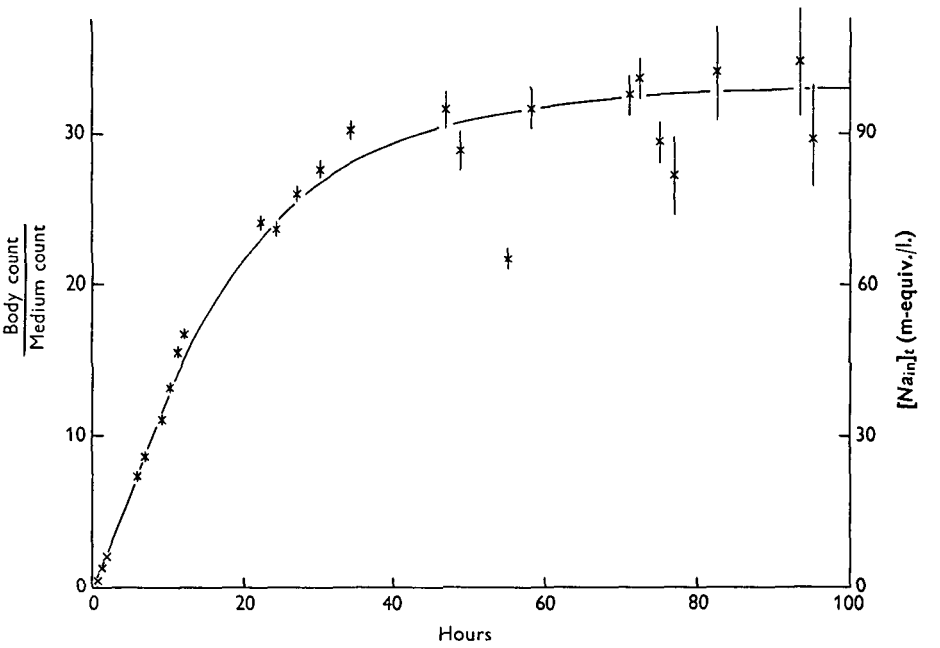

Fig. 3. The data shown in Fig. 2 are here expressed as the ratio of the body count:medium count (= $[Na_{in}]_t : [Na_{out}]$) and $[Na_{in}]_t$ against time. Symbols as for Fig. 2.

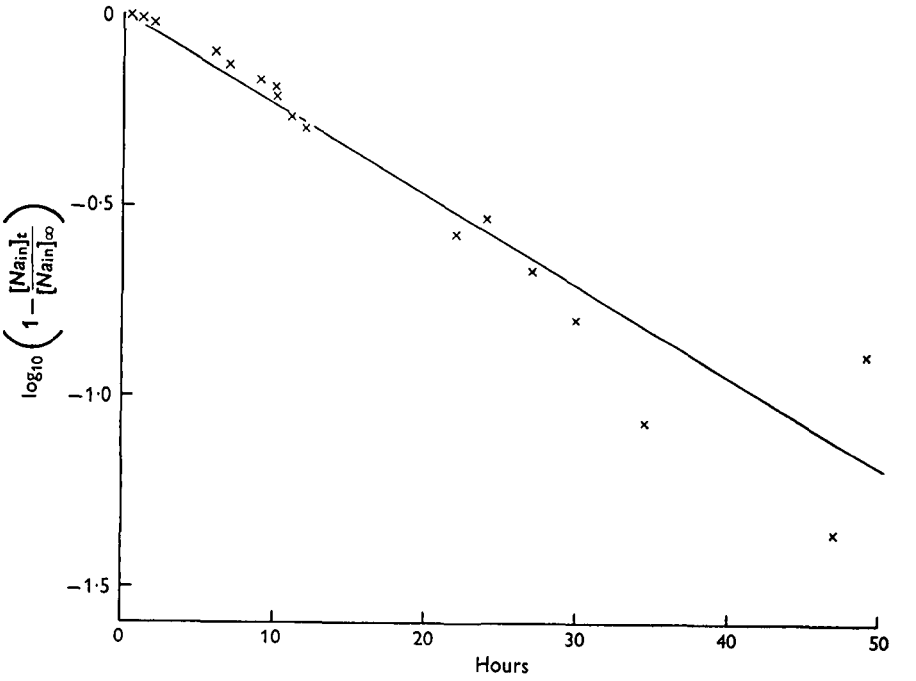

Fig. 4. The data shown in Figs. 2 and 3 are here plotted according to equation (2). The slope of the line drawn through the points is the mean of the slopes of the lines joining the individual points to the origin.

Table 1. *The site of uptake of labelled sodium*

Specimen	State of insect	$[Na_{in}]_\infty$ (m-equiv./l.)	k_{in} (hr.$^{-1}$)	k_{out} (hr.$^{-1}$)	$T_{\frac{1}{2}}$ (hr.)
1	Control	99 (*a*)	1·43	0·043	16·4
2	Control	48 (*a*)	0·77	0·048	14·3
3	Control	75 (*a*)	1·11	0·044	15·6
4	Control	45 (*a*)	0·53	0·035	19·8
5	Control	39 (*a*)	0·62	0·048	14·5
6	Control	99 (*a*)	1·57	0·047	14·6
Average	Control	69	1·01	0·044	15·9
7	Mouth blocked	108 (*a*)	1·48	0·041	16·8
8	Mouth blocked	54 (*a*)	0·69	0·038	18·0
9	Mouth blocked	75 (*c*)	1·03	0·041	16·8
10	Mouth blocked	60 (*a*)	1·50	0·075	9·9
11	Mouth blocked	75 (*c*)	2·34	0·094	7·4
Average	Mouth blocked	75	1·41	0·058	13·8
12	Ventral tube blocked	90 (*c*)	0·19	0·006	111·5
13	Ventral tube blocked	90 (*c*)	0·16	0·005	130·9
14	Ventral tube blocked	90 (*c*)	0·08	0·003	273·6
15	Ventral tube blocked	90 (*c*)	0·03	0·001	752·5
16	Ventral tube blocked	90 (*c*)	0·08	0·003	250·8
Average	Ventral tube blocked	90 (*c*)	0·11	0·004	303·9
12	Ventral tube blocked	45 (*c*)	0·19	0·013	54·7
13	Ventral tube blocked	45 (*c*)	0·16	0·011	64·0
14	Ventral tube blocked	45 (*c*)	0·08	0·008	91·2
15	Ventral tube blocked	45 (*c*)	0·03	0·002	376·3
16	Ventral tube blocked	45 (*c*)	0·08	0·006	107·5
Average	Ventral tube blocked	45 (*c*)	0·11	0·008	138·7

a = $[Na_{in}]_\infty$ estimated from asymptote. *c* = $[Na_{in}]_\infty$ derived from asymptotes of control insects.

The results of these experiments are summarized in Table 1, and Fig. 5 illustrates data from one insect in each class. Blocking of the ventral tube vesicles greatly cuts down the rate of uptake of labelled sodium, whereas blocking of the mouth does not. In arriving at the values of $T_{\frac{1}{2}}$, k_{in} and k_{out} given in Table 1, the value for $[Na_{in}]_\infty$ was taken as the asymptote where this was obtainable; as an approximate value of 75 m-equiv./l. (obtained from the control insects) for the mouth-blocked insects for

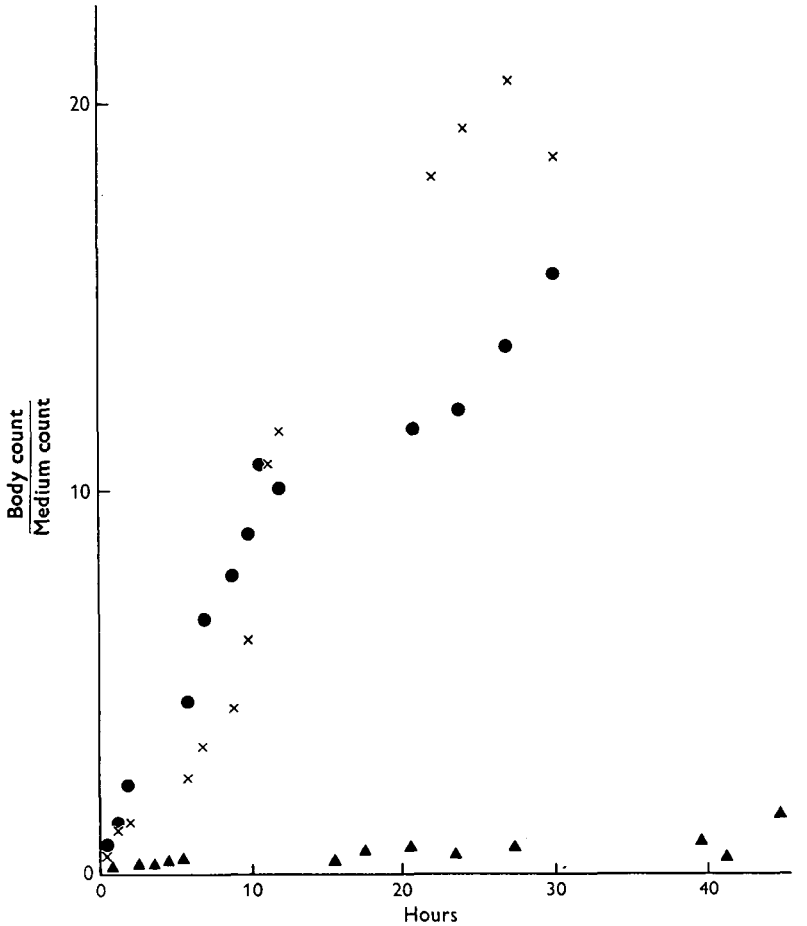

Fig. 5. The effects of blocking the mouth and the ventral tube vesicles on the uptake of ^{24}Na. Crosses show readings from a control insect, solid circles show readings from an insect with its mouth blocked, and solid triangles show readings from an insect with its ventral tube vesicles blocked.

which an asymptote was not obtainable; and as minimum and maximum values of 45 and 90 m-equiv./l. (obtained from the control insects) for the insects with their ventral tubes blocked, to give minimum and maximum values for $T_{\frac{1}{2}}$ and k_{out}. Even the minimum values so arrived at are greatly in excess of the control values. It is clear, therefore, that most of the sodium enters the body via the ventral tube vesicles, very little entering through the gut or across the general body surface.

Individuals which moulted subsequent to the blocking treatment could not be distinguished from control insects, indicating that the blocking treatment had had few side effects.

The uptake of labelled potassium

In experiments conducted to determine the rate of uptake of labelled potassium into the whole body no appreciable increase in radioactivity could be detected in the body, even after 24 hr. This would be expected if (i) the total body potassium were very low (less than 0·001 m-equiv/g. tissue wet weight); (ii) the tissue concentration were high, the haemolymph concentration low (less than 1·0 m-equiv./l.) and the exchange between haemolymph and tissues were extremely slow; (iii) the exchange between medium and haemolymph were extremely slow. In the absence of sufficiently sensitive methods to carry out potassium determinations on single individuals it was not possible to determine which of these alternatives was correct. However, a comparison with results reported for other arthropods indicates which alternative is the most probable. Shaw (1955, 1958, 1959) has shown that the potassium content of the tissues is high in crustaceans (approximately 0·12 m-equiv./g.). Tissue potassium is likewise high in insects (cf. Buck, 1953). Even with a large blood volume, tissue concentrations of this order should give a measurable count. It is probable, therefore, that a low body potassium level is not the full answer. Low concentration of potassium in the haemolymph is a feature of some insects (Buck, 1953; Treherne, 1961; Sutcliffe, 1962), but the values quoted are nearer to 10 m-equiv./l. than to 1·0 m-equiv./l. Furthermore, Treherne (1961) has shown that the fluxes of potassium between the haemolymph and the nerve cord are rapid in the cockroach and Shaw (1958) has shown that potassium freely exchanges between the blood and muscle tissues in the crab. Therefore a slow exchange of potassium between haemolymph of low potassium concentration and tissues of high potassium concentration also may not provide the full answer. This leaves the third alternative, that exchange between the medium and the haemolymph is very slow. If this is true, then it indicates that passive diffusion is a very slow process and must play only a small part in the processes producing high rates of exchange. This suggests that the high rates of exchange of sodium may be due to an active mechanism.

DISCUSSION

Most of the movement of water and ions between *Podura aquatica* and the external medium is apparently confined to the ventral tube vesicles. The role of the ventral tube vesicles in this function dates from the observations of De Geer (1743), but this has often been dismissed in favour of other functions (e.g. as a holdfast, an observation also dating from De Geer). As in many controversies, both observations are true to a certain extent, and the ventral tube clearly performs more than one function.

The results described above indicate that sodium ions are absorbed from dilute solutions by the ventral tube vesicles. The maintenance of a steep gradient in concentrations across the vesicles suggests that an active mechanism operates, though a process of active transport cannot be strictly inferred without some knowledge of the electrical potential across the membrane (Ussing, 1949). The results obtained using labelled potassium indicate that passive diffusion of ions plays only a small part in the exchange of sodium, which perhaps would be expected if an active process were involved (Wilbrandt, 1954). Furthermore, the fine structure of the underlying epithelium (see Noble-Nesbitt, 1963*a*) indicates that it may be involved in active

transport (cf. Smith & Littau, 1960). The greatly increased surface of the epidermal cells in this region may be expected to provide a large surface for the active uptake of ions, which may exchange across the whole surface. Some specific mechanism which distinguishes between sodium and potassium ions must be present. There is some evidence that pinocytosis may occur at the bases of the microvilli of the cell surface (Noble-Nesbitt, 1963*a*). This process by itself would not be expected to differentiate between ions, and therefore seems unlikely to provide the active mechanism for sodium exchange.

The area of the ventral tube vesicles is small compared with the total body surface area. The anal papillae of mosquito larvae also have an area small in comparison with the total body surface area. Exchange of sodium over both of these structures has a similar $T_{\frac{1}{2}}$, k_{in}, and k_{out} ($T_{\frac{1}{2}}$ = 16 hr. for *Podura aquatica*; Treherne (1954) and Stobbart (1959) quoted values of 62 and 10 hr. respectively for starved and fed larvae; k_{out} = 0·044 hr.$^{-1}$ for *Podura aquatica* as against 0·011 hr.$^{-1}$ and 0·069 hr.$^{-1}$ for starved and fed larvae). This suggests that the areas of the exchanging structures are commensurate with the degree of exchange required. Wigglesworth (1933) has shown that the anal papillae of mosquito larvae undergo hypertrophy in very dilute external media.

The ventral tube vesicles of *Podura aquatica* therefore are visualized as performing in this insect the functions ascribed to the anal papillae in mosquito larvae. Further, the sodium concentration in the body corresponds well with the concentration found in mosquito larvae.

The exchange of sodium over the ventral tube vesicles implies a high cuticular permeability to sodium, and it is to be expected that the vesicles will also be highly permeable to water. This adds further weight to the results from the uptake of water, which imply that water uptake also takes place over the ventral tube vesicles.

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SUMMARY

1. In the collembolan *Podura aquatica* L., uptake of water occurs both via the mouth and across the ventral tube vesicles.

2. Drinking is important in water-stress situations and when the haemolymph pressure is too low for eversion of the vesicles. Everted vesicles alone are sufficient for recovery from partial desiccation.

3. Experiments in which insects with their mouths or ventral tubes blocked were exposed to a medium containing ^{24}Na indicated that most of the exchange of sodium between insect and medium occurred over the ventral tube vesicles, and that this exchange was rapid.

4. In experiments using ^{42}K, no measurable exchange occurred between the insect and the medium.

REFERENCES

BEAMENT, J. W. L. (1961). The waterproofing mechanism of arthropods. II. The permeability of the cuticle of some aquatic insects. *J. Exp. Biol.* **38**, 277–90.
BUCK, J. (1953). In Roeder, *Insect Physiology*. New York: Wiley.
DAVIES, W. M. (1928). The effect of variation in relative humidity on certain species of Collembola. *J. Exp. Biol.* **6**, 79–86.
DAVSON, H. (1951). *A Textbook of General Physiology*. London.
DE GEER, C. (1743). *Acta Soc. Reg. Scient. Upsal.* **1**, 279 (as reported by Miall, 1934).
DRUMMOND, F. H. (1953). The eversile vesicles of *Campodea* (Thysanura). *Proc. Roy. Ent. Soc. Lond.* **28**, 145–8.
HARRIS, E. J. & BURN, G. P. (1949). The transfer of sodium and potassium ions between muscle and the surrounding medium. *Trans. Faraday Soc.* **45**, 508–28.
MIALL, L. C. (1934). *The Natural History of Aquatic Insects*. London: Macmillan.
NOBLE-NESBITT, J. (1963*a*). The fully-formed intermoult cuticle and associated structures of *Podura aquatica* (Collembola). *Quart. J. Micr. Sci.* **104**, 253–70.
NOBLE-NESBITT, J. (1936*b*). The cuticle and associated structures of *Podura aquatica* at the moult. *Quart. J. Micr. Sci.* **104**, 369–91.
NOBLE-NESBITT, J. (1963*c*). Transpiration in *Podura aquatica* L. (Collembola, Isotomidae) and the wetting properties of its cuticle *J. Exp Biol.* **40**, 681–700.
NUTMAN, S. R. (1941). The function of the ventral tube in *Onychiurus armatus* (Collembola). *Nature, Lond.*, **148**, 168–9.
RAMSAY. J. A. (1953). Exchange of sodium and potassium in mosquito larvae. *J. Exp. Biol.* **30**, 79–89.
RUPPEL, H. (1953). Untersuchungen über die Bedeutung des Ventraltubus und die Atmung der Collembolan. *Zool. JB. (Allg. Zool.)* **64**, 429–69.
SHAW, J. (1955). Ionic regulation in the muscle fibres of *Carcinus maenas*. I. The electrolyte composition of single fibres. *J. Exp. Biol.* **32**, 383–96.
SHAW, J. (1958). Further studies on ionic regulation in the muscle fibres of *Carcinus maenas*. *J. Exp. Biol.* **35**, 902–19.
SHAW, J. (1959). Salt and water balance in the muscle fibres of the East African freshwater crab, *Potamon niloticus* (M. Edw.). *J. Exp. Biol.* **36**, 145–56.
SMITH, D. S. & LITTAU, V. C. (1960). Cellular specialisation in the excretory epithelia of an insect, *Macrosteles fascifrons* Stal. (Homoptera). *J. Biophys. Biochem. Cytol.* **8**, 103–33.
SOLOMON, A. K. (1952). The permeability of the human erythrocyte to sodium and potassium. *J. Gen. Physiol.* **36**, 57–110.
STOBBART, R. H. (1959). Studies on the exchange and regulation of sodium in the larva of *Aedes aegypti* (L.). I. The steady-state exchange. *J. Exp. Biol.* **36**, 641–53.
STOBBART, R. H. (1960). Studies on the exchange and regulation of sodium in the larva of *Aedes aegypti* (L.). II. The net transport and the fluxes associated with it. *J. Exp. Biol.* **37**, 594–608.
SUTCLIFFE, D. W. (1962). The composition of haemolymph in aquatic insects. *J. Exp. Biol.* **39**, 325–43.
TREHERNE, J. E. (1954). The exchange of labelled sodium in the larva of *Aedes aegypti* L. *J. Exp. Biol.* **31**, 386–401.
TREHERNE, J. E. (1961). Sodium and potassium fluxes in the abdominal nerve cord of the cockroach *Periplaneta americana* L. *J. Exp. Biol.* **38**, 315–22.
USSING, H. (1949). Transport of ions across cellular membranes. *Physiol. Rev.* **29**, 127–55.
WIGGLESWORTH, V. B. (1933). The function of the anal gills of the mosquito larva. *J. Exp. Biol.* **10**, 16–26.
WILBRANDT, W., (1954). Secretion and transport of non-electrolytes. *Symp. Soc. Exp. Biol.* **8**, 136–61