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THE EFFECT OF SOME ANIONS AND CATIONS UPON THE FLUXES AND NET UPTAKE OF SODIUM IN THE LARVA OF *AËDES AEGYPTI* (L)

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INTRODUCTION

It is now well established that the anal papillae of culicine larvae secrete salts from the dilute external medium into the body (Koch, 1938; Ramsay, 1953; Stobbart, 1960). In the case of the larvae of *Aëdes aegypti* the exchange and net transport of sodium through the papillae have been studied in some detail (Treherne, 1954; Stobbart, 1959, 1960) and it appears that the sodium pump in the papillae operates in conjunction with something resembling an exchange-diffusion mechanism (Stobbart, 1959, 1960). The anal papillae of this species are also the site for the exchange of phosphate between body and medium (Hassett & Jenkins, 1951).

The work to be described in the present paper is an attempt to characterize more closely the mechanism for sodium transport in the larva of *Aëdes aegypti*.

MATERIALS AND METHODS

Fourth-instar larvae starved for 60–72 hr. were used throughout. The larvae were of the same colony (London School of Hygiene and Tropical Medicine) as those used earlier (Stobbart, 1959) and they were reared in the same way.

Measurements of sodium flux using the radioactive isotope ^{22}Na

(1) Measurements of influx. To make a measurement of influx 40 or 50 larvae were placed in a solution containing sodium labelled with ^{22}Na in a hard glass or polypropylene beaker covered with aluminium foil. After a suitable time the larvae were removed from the solution, washed with deionized water, and weighed in groups of ten to the nearest 0·5 mg. with a torsion balance. The groups of ten larvae were then ashed (450 °C. for 5 hr.) on platinum foil or silica, and the ash was converted to chlorides and transferred with 10μl. of 0·9 M dextrose (as a spreader) to planchettes, for measurement (when dry) of its radioactivity. The specific counting rate of the sodium in the external solution was found in the presence of 10μl. of 0·9 M dextrose, and from this and a knowledge of the radioactivity of the larvae the mean influx of sodium and its standard deviation for the four or five groups of ten larvae were calculated and expressed as mμM Na/mg. wet wt./hr. The time intervals were kept as short as possible to minimize the amount of net sodium uptake, which was always very small, and errors due to 'back movement' of tracer; they varied between 10 min. for the most concentrated, and 5 hr. for the most dilute solutions.

(2) Measurements of outflux. To make a measurement of outflux 40 larvae suitably labelled with ^{22}Na (Stobbart, 1959) were placed in 80 ml. of unlabelled solution. After a suitable time the larvae were removed and weighed and were then discarded. The radioactivity in the solution was now measured. To do this the solution was evaporated down with 620μl. of 0·9 M dextrose on to a planchette. The larger amount of dextrose was needed to give adequate spreading of the sample in the case of the more concentrated solutions. Control tests showed that the additional amounts of salts in the more concentrated solutions did not cause a significant lowering in the counting rate when the samples were counted for a total of 1000 pulses. The outflux was calculated from a knowledge of the radioactivity of the external solution and of the specific counting rate (measured in the presence of 620μl. of 0·9 M dextrose) of the sodium with which the larvae had been labelled; it was also expressed as mμM Na/mg. wet wt./hr. In some cases the groups of forty larvae were split into four groups of ten larvae in 20 ml. of solution to allow the standard deviations of the samples to be found.

The specific activities of the media varied between 192 mc. ^{22}Na/g. Na (used for measuring influxes at very dilute external concentrations) and about 1 mc. ^{22}Na/g. Na (used for labelling larvae with ^{22}Na).

Measurements of radioactivity

These were made with an end-window (mica) Geiger counter and suitable scaling equipment. In the case of the influx measurements 1000–4000 pulses were counted from each planchette, and 1000 pulses in the case of the outflux measurements. The standard deviations of the points in Figs. 2–5 and 7–9 are due to (i) errors inherent in the measurement of radioactivity, and (ii) the variability of the larvae. As the observed standard deviations are much larger than those expected on the basis of statistical fluctuations in counting rate, it must be supposed that most of the variability is due to the larvae. By reason of the long half-life of ^{22}Na (2·6 years) no correction was neccessary for decay during the course of the experiments.

Measurement of haemolymph volume

A minimal estimate of the haemolymph volume was obtained by weighing groups of five larvae on the torsion balance and then puncturing them on filter-paper, and reweighing them. To ensure that as much haemolymph as possible was absorbed by the filter-paper the thorax, the abdomen, the head capsule, and the papillae were all punctured and the larvae were pressed firmly into the filter paper.

Accuracy of the weighings

The larvae were dried on filter-paper according to a standardized procedure which took about 2 min. The accuracy was about $\pm$ 1·7% (standard deviation). There was no rapid loss of weight when dried larvae were left on the balance, so it was assumed that all the surface water had been removed.

Measurements of sodium and potassium

These were made with the 'Eel' flame photometer. Sodium and potassium in the haemolymph were measured as described earlier (Stobbart, 1959, 1960) except that

for each sample about 7 μl. of haemolymph were collected from ten larvae and diluted in 2 ml. of deionized water. The higher concentration of haemolymph was to facilitate the measurement of potassium. Measurements of total sodium and potassium in the larvae were made by ashing groups of five larvae and, after converting the ash to chlorides, analysing it with the flame photometer.

Measurements of chloride

Wigglesworth's (1937) micro-adaptation of the Volhard titration was used, the sodium thiocyanate being discharged from an 'Alga' micrometer syringe. The silver chloride was not filtered off, but a small drop of nitrobenzene was used to stabilize the end-point. For measurements of haemolymph chloride pooled haemolymph from five larvae was used for each sample. For measurements of total chloride five larvae were thoroughly macerated in a small drop of deionized water by means of a glass rod. The macerate was then allowed to stand for about an hour, being stirred occasionally, before the titration was performed. In some experiments total chloride was also measured by estimating the radioactivity of macerates of larvae which had been reared in a medium containing ^{36}Cl. The results did not differ significantly from the results of the Volhard titrations, indicating that the extraction of chloride by deionized water was adequate. The titrations were performed on 'fluon' or polythene slabs.

Measurements of the potential difference between haemolymph and medium

A Pye Universal pH and millivoltimeter modified to give a reading of 80 mV at full-scale deflexion was used for these, with a backing-off potential of about -20 mV applied to the positive electrode. The thorax and anterior abdomen of a larva were carefully sealed into a bed of beeswax-resin cement (Krogh & Weis-Fogh, 1951). The cement at some distance from the larva was melted with a hot wire and the bed was then tilted so that the molten but cooling cement made contact with the desired parts of the larva. The posterior abdomen was left free and was surrounded by a drop of experimental medium arranged so that the respiratory siphon of the larva could make contact with the air. The larva was left to settle down for about 30 min. then the tip of a mercury-calomel-saturated KCl agar electrode was inserted into the medium; the thorax of the larva was now torn open and the tip of a similar electrode inserted into the pool of haemolymph and the potential was measured. The cement (which had been extracted with boiling deionized water to remove any ions) is hydrofuge and providing that the larvae had been properly sealed into it there was no tendency for the haemolymph to creep along the sides of the larva to the medium. The medium could be rapidly changed when necessary. The experimental media used were all dilute (for constitution see Stobbart, 1959) and contained 2 mM/l. Na and 3·9 mM/l. Cl. Fairly large electrodes (tip diam. 0·5–0·25 mm.) had to be used to prevent the instrument behaving as if on open circuit, but control experiment showed that the amount of KCl diffusing out of the electrodes during the time taken for the measurements was quite negligible. Once the thorax of a larva had been opened measurements were taken from it for a period of only 2 or 3 min.

All the experiments were conducted at a temperature of 28° C. except for the measurements of potentials which were made at 23–25° C. The lines through the points

in the figures were drawn in by eye except where it is stated otherwise. The usual convention of significance was used in the statistical tests.

In this paper the terms 'influx' and 'outflux' are used to describe the sodium fluxes found by means of the tracer, and the terms 'net loss', 'loss', 'net uptake' and 'uptake' are used to describe changes in sodium content.

RESULTS

Salt balance

Larvae of *Aëdes aegypti* and *Culex* can withstand prolonged treatment with deionized water (Koch, 1938; Wigglesworth, 1938; Ramsay, 1953; Treherne, 1954; Stobbart, 1959, 1960). In view of their well-developed ability to take up salts, it is of interest to know the lowest external concentration at which they can keep themselves in salt balance. To discover this larvae were put into deionized water at densities of 10, 20, 40 and 80 larvae per 20 ml. of water and the increase in sodium and potassium in the medium was measured at suitable times. The results for larval densities of 10 and 80 per 20 ml. are shown in Fig. 1. After about 20 hr. larvae at the low density have come into sodium balance at the low external concentration of 5 μM/l. and remain in this state till at least the 95th hr. A true potassium balance is not achieved and the external potassium concentration rises slowly to about 10 μM/l. at 95 hr. At the higher density sodium balance is achieved some time after 20 hr. at about 20 μM/l., so the larvae have clearly lost proportionately less sodium than at the lower density; in addition the proportionate loss of potassium is somewhat greater, and as before potassium balance is not achieved. The intermediate larval densities gave intermediate results. Similar results for potassium have been obtained by Shaw (unpublished) from *Astacus pallipes*. When placed in a large volume (1 l.) of dilute potassium chloride solution this animal achieves a very erratic potassium balance, but a much greater

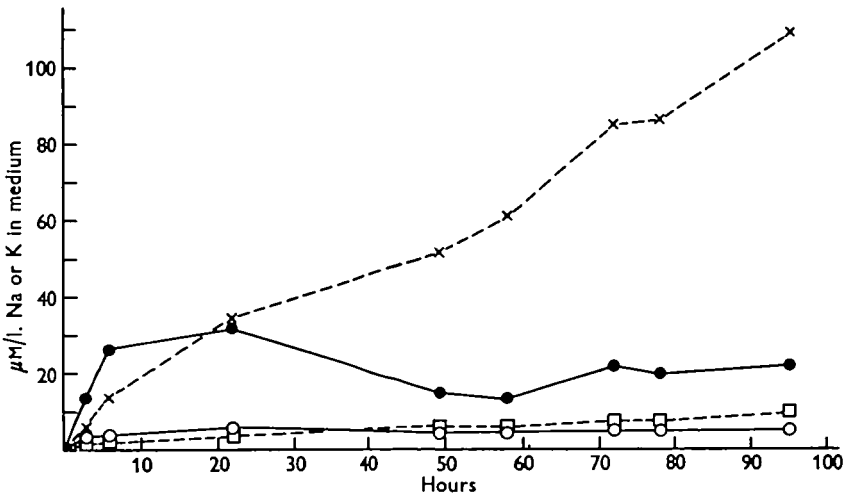

Fig. 1. The net loss of sodium and potassium to deionized water from starved larvae. O—O sodium, 10 larvae/20 ml.; □---□, potassium, 10 larvae/20 ml,; ●—●, sodium, 80 larvae/20 ml.; x---x potassium, 80 larvae/20 ml.

loss of potassium occurs when it is put into a small volume of deionized water. In contrast the crab *Potomon niloticus* Shaw (1959*b*) achieves a definite potassium balance when placed in 1 l. of deionized water.

When larvae which have been brought into sodium balance at the low density are placed in 2 mM/l. NaCl flux values are found similar to those described from starved larvae grossly deficient in sodium (Stobbart, 1960). So it appears that (as in *Astacus*) the balancing procedure activates the sodium transporting mechanism more or less fully. It also causes the haemolymph sodium to drop from 103·6 mM/l. ± 1·15 (standard deviation), N(degrees of freedom) = 19 to 92·9 mM/l. ± 0·711, N = 37, and the chloride to drop from 60·3 mM/l. ± 1·92, N = 4 to 48·1 mM/l. ± 2·56, N = 3. The change in haemolymph potassium is small and erratic. In terms of *overall* ionic content there is a 10% drop in sodium, a 26% drop in chloride and a 6% drop in potassium (approximate values). The effects of balancing are therefore similar to those found in *Astacus pallipes* (Shaw, 1959*a*), *Potomon niloticus* (Shaw, 1959*b*) and *Gammarus pulex pulex* (Shaw & Sutcliffe, 1961), but the external concentration at which sodium balance is achieved by *Aëdes* larvae is only about one-tenth as great.

'Balancing' the larvae at low density in deionized water is a most convenient way of stimulating the sodium transport mechanism, and all the measurements of sodium fluxes and some of the measurements of potentials were made with larvae balanced in this way for 24 hr. Many experiments have now been done with such larvae and in every case they have come into sodium balance at an external concentration of about 5 μM/l.

Flux measurements

The effect of the external concentration of different sodium salts upon the sodium fluxes

Sodium chloride. Measurement of influx and outflux at different external concentrations of sodium chloride are shown in Fig. 2. The relationship between influx and external concentration is similar to that found in *Astacus* (Shaw, 1959*a*) and *Gammarus duebeni* and *G. pulex pulex* (Shaw & Sutcliffe, 1961), and may be described fairly well by the Michaelis equation $M = KC/(Km+C)$ where M = influx, K = maximum influx, Km = external sodium concentration for half maximum influx, C = external sodium concentration (Shaw, 1959*a*). The broken line in Fig. 2 was calculated according to this equation for K = 12 mμM/mg. wet wt./hr. and Km = 0·55 mM/l. These values were found by trial and error to give the best fit. At the higher concentrations, however, the increase in influx is slower than that predicted by the equation. This is indicated by the dotted line in Fig. 2. This situation also occurs in solutions of sodium bicarbonate and sodium sulphate (see below). A similar increase in outflux of sodium also occurs with increasing external sodium concentrations. When larvae are put to balance in deionized water the initial net loss of sodium measured over the first 3 hr. at a larval density of 10/20 ml. is 0·83 mμM/mg./hr. (Fig. 1). When such larvae are put into fresh deionized water the net loss is now much lower (Figs. 2–5), 0·215 mμM/mg./hr. ± 0·0484. Clearly the larvae can reduce the net loss very considerably after treatment with deionized water. These results agree well with earlier work (Stobbart, 1959). Here it was shown that the steady-state outflux of sodium (which one might expect to be similar to the initial net loss into deionized water) was about 1 mM/l. of haemolymph/hr. (or 1 mμM/mg./hr., see below)

and it may be estimated from Fig. 1 (Stobbart, 1959) that the net loss into *flowing* distilled water from larvae which have lost about 10% of their haemolymph sodium is about 0·55 mM/l. of haemolymph/hr. A similar ability to reduce the rate of net loss of sodium is also seen in *Gammarus duebeni* and *G. pulex pulex* (Shaw & Sutcliffe 1961). In all three cases the fall in haemolymph sodium concentration is insufficient to cause the reduction in net loss. In Fig. 2 the difference between the values for influx and outflux at any external concentration clearly represents the net uptake of sodium at that concentration, and similarly the space between the abscissa and the line of outflux represents the overall exchange component in the fluxes. At an external concentration of 2 mM/l. NaCl the influx is about 9·3 mμM/mg./hr. the outflux about 1·7 and the net uptake about 7·6.

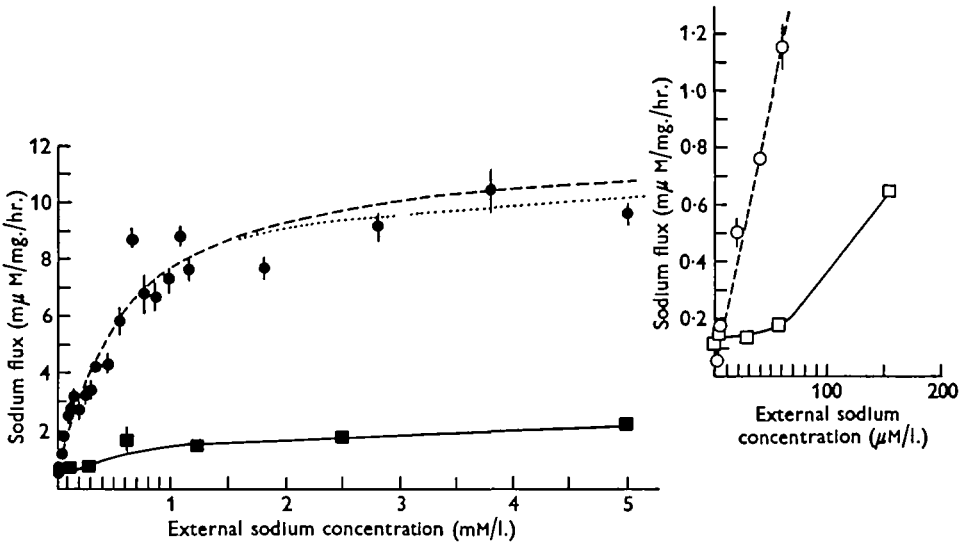

Fig. 2. The relationships between external concentration of sodium chloride and sodium influx and outflux in starved balanced larvae. The fluxes at very dilute external concentrations are shown in the inset. O---O and ●---●, sodium influx; □—□ and ■—■, sodium outflux. The broken line drawn through the points for influx was calculated according to the Michaelis equation (see text). The dotted line is drawn by eye to fit the points. The balance point is shown by the intersection of the lines for influx and outflux. In this fig. and in Figs. 3–5 and 7–9 the vertical extents of the lines emerging from the symbols indicate the standard deviations. In general the standard deviations are not shown if they are smaller than the vertical extents of the symbols. In the case of the measurements for outflux shown in Figs. 2–5 the standard deviation was found for only one point in each series.

In the earlier work (Stobbart 1960) the influx in larvae in a medium containing 2 mM/l. NaCl was found to be 10–18, the outflux 3–9, and the net uptake 7–9 mM/l. of haemolymph/hr. Now about 90% of the sodium of the larva must be in the haemolymph (because the haemolymph is at least 62·5 ± 1·44% of the body weight, and because the total body sodium is 60–70 mM/kg. (Ramsay, 1953, and confirmed by myself) so the larvae can be considered to consist approximately of only the haemolymph compartment. Therefore movements of sodium calculated as mμM/mg./hr. are roughly comparable to those calculated as mM/l. of haemolymph/hr. Bearing in mind the roughness of some of the earlier estimations, the different degree of sodium deficiency of the larvae, and the fact that in the earlier work the medium in addition

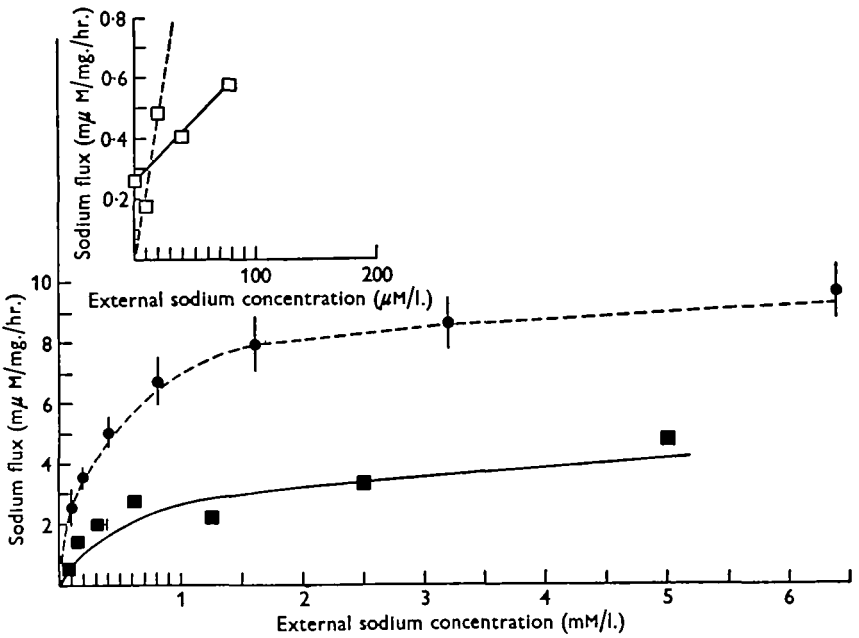

Fig. 3. The relationships between external concentration of sodium bicarbonate and sodium influx and outflux in starved balanced larvae. The fluxes at very dilute external concentrations are shown in the inset. ●—●, Sodium influx; □—□ and ■—■, sodium outflux. In the inset the open squares, representing measurements of outflux, relate to the continuous line. The broken line joins the origin to the first measurement of influx; but this measurement being made at an external concentration of 0·1 mM/l. Na, lies outside the scope of the inset.

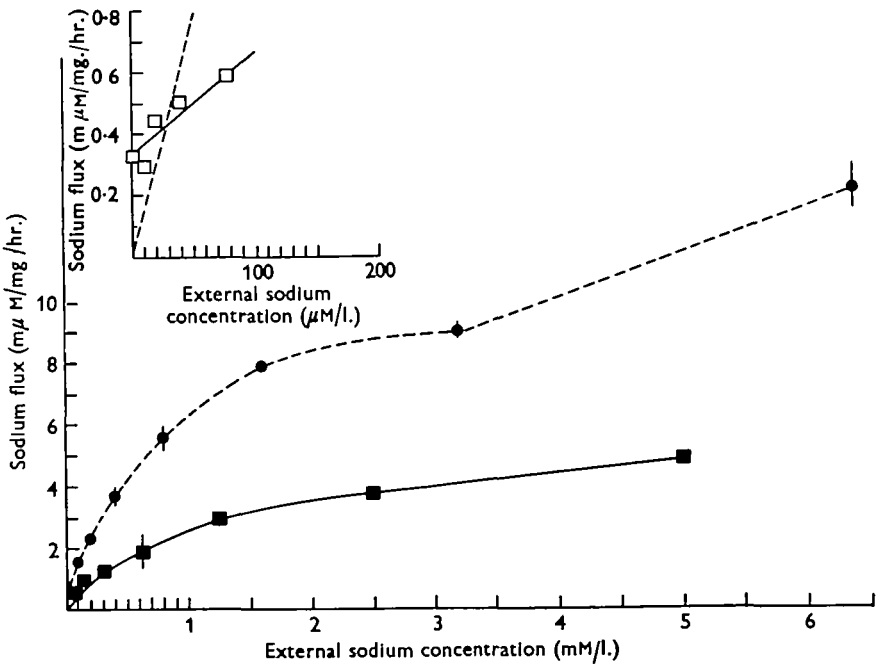

Fig. 4. The relationships between external concentration of sodium nitrate and sodium influx and outflux in starved balanced larvae. Symbols are as in Fig. 3.

to potassium, calcium and magnesium, contained more chloride (3·9 as opposed to 2·0 mM/l., see later)—the agreement between the two sets of data seems reasonable.

Sodium bicarbonate, nitrate, and sulphate. Sodium fluxes at different external concentrations of sodium bicarbonate, nitrate and sulphate are shown in Figs. 3, 4 and 5.

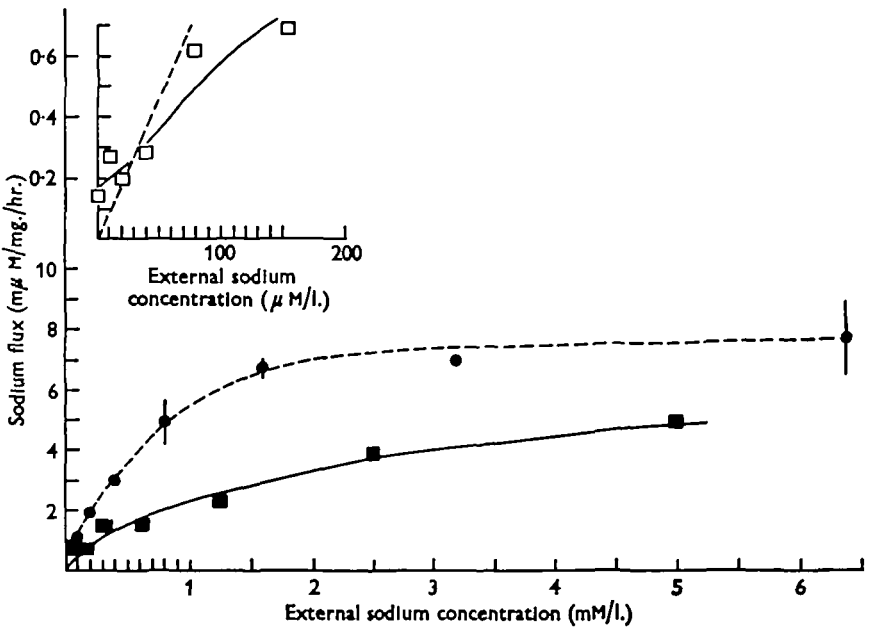

Fig. 5. The relationships between external concentration of sodium sulphate and sodium influx and outflux in starved balanced larvae. Symbols as in Fig. 3.

In general the results are similar to those obtained with sodium chloride, but the following points should be noted.

(i) In every case the outflux is significantly greater than in sodium chloride (about twice as great at an external sodium concentration of 5 mM/l.), while the influx is roughly the same, except in the case of sodium sulphate in which it seems to be significantly lower.

(ii) As far as can be judged from the intersection of the lines for influx and outflux the larvae balance at a rather higher external sodium concentration than in sodium chloride. These balance points cannot be measured directly because of net chloride loss from the larvae.

(iii) At high concentrations nitrate causes a rapid influx of sodium. The effect is not found when nitrate is applied to the larvae as its potassium, calcium or magnesium salts.

In the experiments with sodium bicarbonate and sodium nitrate a rather higher outflux into deionized water was found than in those with sodium chloride. There is no obvious reason for this difference as the larvae which were put into deionized water did not come into contact with the salt solutions, and it could possibly be due to a change in the larvae, as several generations elapsed during the course of the experiments. However, no alterations were found in the balance point for sodium during the pretreatment with deionized water.

The effect of the external anion upon the net uptake of sodium

Figure 6 shows the net uptake of sodium from the solutions of the different salts as a function of the external sodium concentration. The values of net uptake were obtained by subtracting the results for outflux from those for influx in Fig. 2 (lower line for influx used) and Figs. 3–5. In the case of the outflux measurements the standard deviation was found for only one point in each treatment in order to economize in time and effort. It is therefore impossible to do more than guess at the statistical errors of the values for net uptake. I think it probable that net uptake from NaCl is significantly greater than from $NaHCO_3$, Na_2SO_4 and $NaNO_3$, and that the net uptake from Na_2SO_4 is probably significantly smaller than from $NaHCO_3$ and $NaNO_3$. The increased net uptake from the higher concentrations of $NaNO_3$ must be regarded with caution as the single group of larvae which gave a high value for influx (Fig. 4) might also have given a high value for outflux.

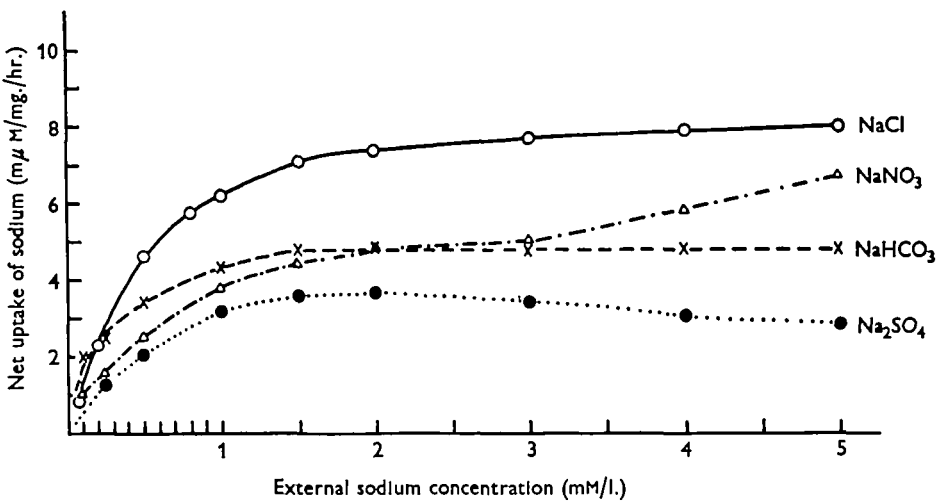

Fig. 6. The relationship between net uptake of sodium and external sodium concentration in starved balanced larvae; O—O, from NaCl; △–·–△, from $NaNO_3$; ×---×, from $NaHCO_3$; ●····●, from Na_2SO_4.

The effect of different cations upon the influx of sodium from 0·1 mM/l. *sodium chloride*

The effect of K^+, NH_4^+, Ca^{++} and Mg^{++} applied to the larva at different concentrations as sulphates or nitrates in 0·1 mM/l. sodium chloride is shown in Figs. 7 and 8. The general picture is of no inhibition of the flux ($CaSO_4$ and $MgSO_4$) or a slight to a considerable inhibition, the most active inhibitors being K_2SO_4, KNO_3 and $Ca(NO_3)_2$. This is in marked contrast to the case where the cations are applied as chlorides (Fig. 9). Here the influx is roughly doubled except for the case of NH_4Cl where it is increased to about 140% of the original.

Measurement of potential difference between haemolymph and medium

These measurements (which have been described briefly elsewhere (Shaw & Stobbart, 1963) were preliminary in nature and designed as a supplement to earlier

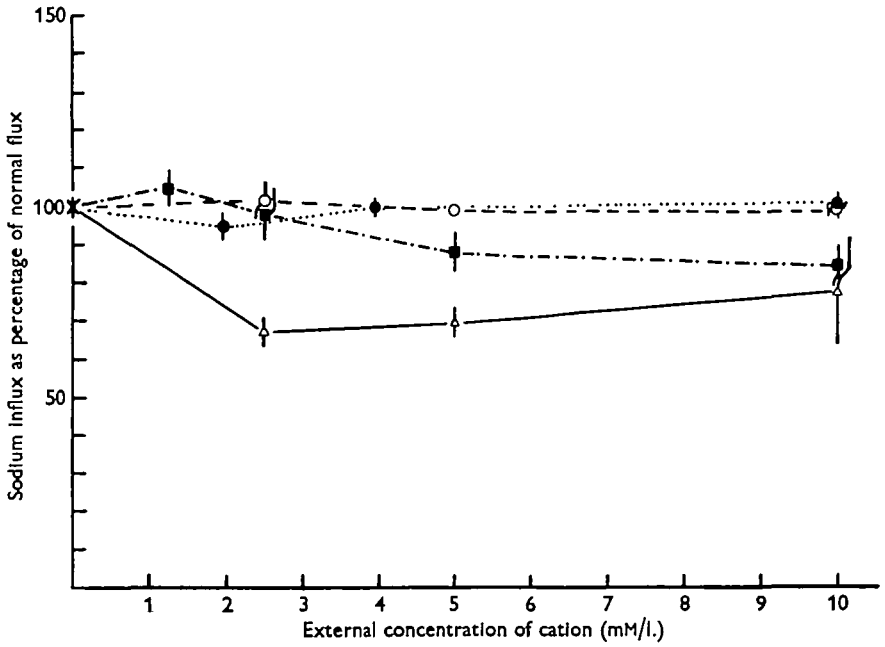

Fig. 7. The effect, in starved balanced larvae, of various sulphates upon sodium influx from 0·1 mM./l. NaCl; ■-·-■, $(NH_4)_2SO_4$; O---O, $MgSO_4$; △—△, K_2SO_4; ●····●, $CaSO_4$.

work (Stobbart, 1959, 1960). In this work it was demonstrated that sodium-deficient larvae secrete sodium from the medium into the haemolymph, but as no potential measurements were made it was not possible to decide definitely that active transport of sodium was occurring.

It was necessary first to verify that the balanced larvae were capable of a net uptake of sodium when immobilized in the beeswax-resin cement. To do this thirty balanced larvae were set in the cement with the papillae covered by a drop of the medium (see Stobbart, 1959, for the constitution of the medium, which contained 2 mM/l. sodium and 3·9 mM/l. chloride). One hour later they were freed from the cement and their haemolymph was collected to give three samples. These samples were analysed and compared with three similar samples from balanced larvae which had not had access to the medium. In the case of the experimental larvae the haemolymph sodium concentration was 101·8 mM/l. ± 1·827, $N = 2$. In the control larvae the concentration was 95·5 mM/l. ± 1·18, $N = 2$. The difference is significant ($P < 0{\cdot}05$).

The results of the measurements of potential difference (P.D.) and the conditions under which they were made are given below.

(*a*) Normal larvae in a steady state with respect to the medium: P.D. = +9·2 mV (sign refers to haemolymph), ± 2·8, $N = 19$.

(*b*) Larvae previously balanced in deionized water and taking up sodium and presumably chloride (Wigglesworth 1938):

(i) P.D. measured a few minutes after setting up in the beeswax resin cement:

$$\text{P.D.} = -3{\cdot}1 \text{ mV} \pm 7{\cdot}0, \quad N = 9.$$

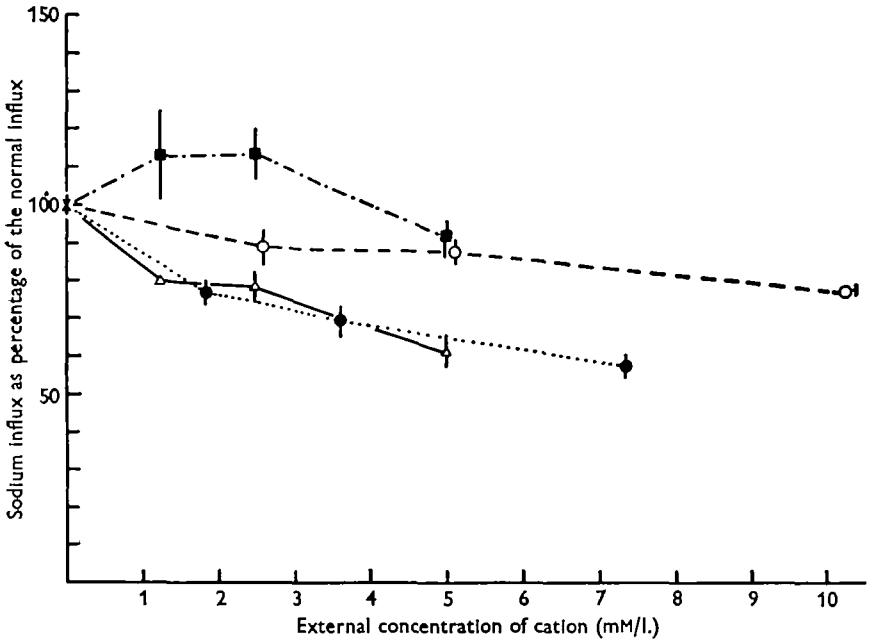

Fig. 8. The effect, in starved balanced larvae, of various nitrates upon sodium influx from 0·1 mM/l. NaCl; ■– –■, NH_4NO_3; O– – –O, $Mg(NO_3)_2$; △—△, KNO_3; ●· · · ·●, $Ca(NO_3)_2$.

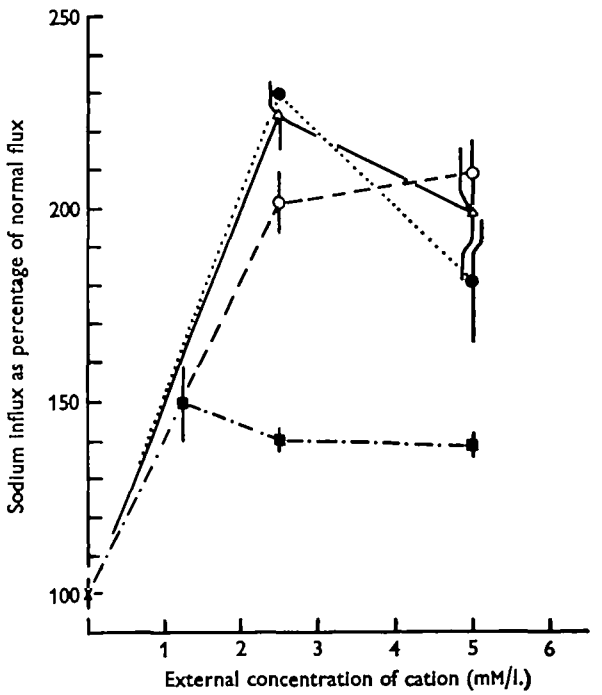

Fig. 9. The effect, in starved balanced larvae, of various chlorides upon sodium influx from 0·1 mM/l. NaCl; ■–·–■, NH_4Cl; O– – –O, $MgCl_2$; △—△, KCL; ●· · · ·●, $CaCl_2$.

(ii) P.D. measured 15–50 min. after setting up:

$$\text{P.D.} = -28{\cdot}8 \text{ mV} \pm 5{\cdot}0, \quad N = 3.$$

The differences between the P.D.'s for (*a*) and (*b*)(ii), and between those for (*b*)(i) and (*b*)(ii) are significant.

The equilibrium concentration of a monovalent ion in the haemolymph for any observed P.D. is given by

$$C_H = C_M \exp(F/RT \times \text{P.D.})^{-1}$$

for cations and

$$C_H = C_M \exp(F/RT \times \text{P.D.})$$

for anions, where C_H = the haemolymph concentration, C_M = the concentration in the medium and R, T and F have their usual meanings.

As the sodium concentration in the medium is 2 mM/l. the equilibrium concentration in the haemolymph for a P.D. of -30 mV. (at 23° C.) is 6·5 mM/l.; the actual haemolymph concentrations were between 94 and 106 mM/l. In the case of chloride the concentration in the medium is 3·9 mM/l. so, for a P.D. of $+9{\cdot}2$ mV the equilibrium concentration in the haemolymph is 5·6 mM/l.; the actual haemolymph concentrations were about 60 mM/l. in normal larvae, and about 50 mM/l. in larvae balanced in deionized water. It is therefore clear that active transport of sodium (and chloride) must occur. When KCN is added to the medium at a concentration of 5 mM/l., the potentials rapidly go negative by 30–60 mV. It seems probable that this may represent a diffusion potential following upon the inhibition of the mechanisms for active transport.

DISCUSSION

Although the relationship in *Aëdes* larvae between sodium influx and external sodium concentration is not accurately described by the Michaelis equation, the results do strongly suggest that sodium ions are moved inwards by a saturable rate-limited system with a high affinity for sodium ions (cf. Shaw, 1959*a*). A similar relationship exists between sodium outflux and external sodium concentration, although the outflux is always much smaller than influx at any given external concentration (except at concentrations approaching zero). As only very small amounts of net uptake occurred during the period taken for the flux measurements, the concentration difference in sodium larva and medium must be greatest at the dilute external concentrations, and so the increase in outflux with increasing external concentrations cannot be explained in terms of passive movement of sodium. The evidence in fact suggests that superimposed upon a passive outflux of sodium (whose greatest value is probably represented by the net loss into deionized water) there is an outflux caused by a mechanism similar to the one which causes influx; this is clearly compatible with the mechanism suggested earlier (Stobbart, 1959, 1960) for exchange and net uptake of sodium. Similar sets of influx data for brackish-water and freshwater Crustacea have been discussed by Shaw (1961) who has shown that acquisition of a high affinity uptake mechanism for sodium overcomes the need for an excessive reduction in permeability of the body surface to maintain balance at low external concentrations.

As the larva of *Aëdes* balances at an external concentration of sodium only about one tenth of that at which *Astacus pallipes* (Shaw, 1959*a*) and *Gammarus pulex pulex* (Shaw & Sutcliffe, 1961) balance, it is interesting that its uptake mechanism has a

lower affinity for sodium than those of the other species. The mechanism in *Aëdes* is half-saturated at an external concentration of 0·55 mM/l., corresponding values for *Astacus* and *Gammarus* are 0·25 and 0·15 mM/l. These differences in Km values however may in part be due to the different temperatures at which the flux measurements were made (12–13° C for *Astacus*, room temperature for *Gammarus*, 28° C. for *Aëdes*). From the standpoint of ability to tolerate dilute media the important thing is to have influx and outflux *equal* at a low external concentration. This might be achieved in a number of ways either singly or in combination: (*a*) by means of an uptake mechanism of high affinity for sodium; (*b*) by reduction in the sodium permeability of the body surface; (*c*) by an increase in the influx without an increase in the affinity of the uptake mechanism for sodium—i.e. an increase in influx brought about by recruitment of more 'sodium carriers'.

The ability of *Aëdes* larvae to reduce the net loss of sodium after being balanced in deionized water is very striking and doubtless plays an important part in maintaining the low balance point. The steady-state outflux into 2 mM/l. NaCl medium is about 1·0 mμM/mg./hr. (Stobbart, 1959) and about 90% of this occurs through the anal papillae. This steady-state outflux is very similar to the net loss observed initially in deionized water. Now if the outflux through structures other than the papillae were abolished as a result of treatment with deionized water but the outflux through the papillae were left unaltered, we should expect to find a net loss of about 0·9 mμM/mg./hr. upon transference to a fresh lot of deionized water. In fact the observed net loss is about 0·2 mμM/mg./hr. It therefore seems very probable that the sodium permeability of the papillae has also been reduced. Information is available which suggests that a similar situation exists in the brackish-water *Gammarus duebeni* (see Lockwood, 1961). In *G. pulex* it may be calculated from the data of Lockwood (1961) that in fresh water about 0·41 μM Na is lost through the urine per day (maximal estimate assuming that the osmotic pressure of the urine is due entirely to sodium chloride); the net sodium loss to deionized water, however, is 4·3 μM/day, which is reduced after further sodium loss to 2·2 μM/day (Shaw & Sutcliffe, 1961). Here again it seems very probable that the sodium permeability of the body surface has been reduced. A similar ability of a tissue to reduce its permeability to an ion moving down the electrochemical gradient is found in the rectum of the locust *Schistocerca gregaria* (Phillips, 1964).

In general in *Aëdes* the external anion, when present in concentrations equal to those of sodium, has little effect upon the sodium influx (Figs. 2–4) and in this *Aëdes* resembles *Astacus* (Shaw, 1960*a*). The sulphate ion, however, causes a reduction in both the net uptake and influx of sodium, and similarly in *Astacus* the net uptake of sodium from sodium sulphate is less than from sodium chloride (Shaw, 1960*a*). Whether in *Aëdes* the net sodium uptake from sodium sulphate occurs without net sulphate uptake (as in *Astacus*) remains to be determined.

In *Aëdes* the anions sulphate, bicarbonate and nitrate, in concentrations equal to those of sodium, cause a reduction, relative to chloride, in the net sodium uptake (Fig. 6). This, and the fact that Ca^{++}, K^{+}, Mg^{++}, and NH_4^{+} as chlorides stimulate sodium influx from 0·1 mM/l. sodium chloride, and as nitrates or sulphates are without effect or reduce the influx (Figs. 7, 8 and 9), suggest that movements of chloride play an important part in ion uptake. Net uptake of chloride has been demonstrated in

Culex, *Chronomus* and *Aëdes* (Koch, 1938; Wigglesworth, 1938) and I have found that in *Aëdes* it can occur independently of sodium from solutions of potassium chloride. The measurements of potential show that the chloride concentration in the haemolymph must be maintained actively. The fact that the potential goes negative after net uptake of salt has been going on for about ½ hr. suggests that under some circumstances active chloride transport may exceed active cation transport. It appears therefore that *Aëdes* resembles anurans (Jørgensen, Levi & Zerahn, 1954; Zadunaisky, Candia & Chiarandini, 1963) *Astacus* (Shaw, 1960*c*) and squid giant axon (Keynes, 1963) in possessing independent mechanisms for the uptake of sodium and chloride. A more detailed consideration of chloride movements in *Aëdes* is reserved for a later paper.

With respect to the alteration of sodium influxes by other cations there are a number of differences between *Aëdes* and *Astacus* (Shaw, 1960*b*). The most striking is that the ammonium ion does not in *Aëdes* have a strong inhibitory effect, but in common with the other cations increases the influx when it is applied as chloride (Fig. 9) and may even have a slight stimulatory effect as nitrate (Fig. 7). This lack of inhibition is perhaps to be expected from an animal which develops in a relatively small volume of stagnant medium. Other differences are the greater degree of inhibition in *Aëdes* by the potassium ion and lack of stimulation by the magnesium ion. The strongly stimulatory effect of calcium, magnesium, potassium and ammonium as chlorides found in *Aëdes* is lacking in *Astacus*, and may be a reflexion of a greater activity of the mechanism for chloride uptake in *Aëdes*.

SUMMARY

1. Starved 4th-instar larvae of *Aëdes aegypti*, when put into deionized water at a density of ten larvae/20 ml., are able to achieve sodium balance at the low external concentration of 5 μM Na/l.

2. The balancing process involves a 10% drop in total sodium content, a more or less complete activation of the mechanism for sodium transport, and a reduction in the permeability of the larva to sodium as measured by the net sodium loss into deionized water. It is very probable that most of this reduction occurs in the anal papillae.

3. The relationship between external sodium concentration and sodium influx in larvae previously 'balanced' in deionized water is described approximately by the Michaelis equation. The sodium outflux also increases with increasing external sodium concentrations.

4. The net uptake of sodium by 'balanced larvae' appears to be significantly greater from solutions of NaCl than from solutions of $NaNO_3$, $NaHCO_3$, and Na_2SO_4.

5. The ions K^+ Ca^{++} Mg^{++} and NH_4^+ when present as chlorides stimulate the influx of sodium from 0·1 mM/l. sodium chloride. When present as nitrates or sulphates they either have no effect or cause an inhibition of influx.

6. The results in 4 and 5 suggest that movements of chloride may be important in sodium uptake, and chloride uptake has been found to occur independently of sodium uptake. Measurements of potential difference between haemolymph and medium demonstrate active transport of both sodium and chloride.

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