

THE EFFECT OF SOME ANIONS AND CATIONS UPON THE FLUXES AND NET UPTAKE OF CHLORIDE IN THE LARVA OF *AÈDES AEGYPTI* (L.), AND THE NATURE OF THE UPTAKE MECHANISMS FOR SODIUM AND CHLORIDE

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I. INTRODUCTION

Some evidence concerning the influence of various ions upon the fluxes and net uptake of sodium by the aquatic larva of *Aedes aegypti* was described in an earlier paper (Stobart, 1965). One of the main findings was that chloride in the external medium has, in concentrations up to 5 mM/l., a marked stimulatory effect upon sodium influx and net uptake. While the sodium pump (which occurs mainly in the anal papillae) is known in some detail (Ramsay, 1953; Treherne, 1954; Stobart, 1959, 1960, 1965) no information is available about chloride fluxes in this species. However, a sodium-independent net chloride uptake has been demonstrated, and measurements of potential difference between haemolymph and medium show that the haemolymph chloride concentration must be maintained actively against an adverse electrochemical gradient (Stobart, 1965). Furthermore, Koch (1938) showed that net chloride uptake by chloride-depleted larvae of the closely related *Culex*, and of *Chironomus*, occurs only if the anal papillae are intact. The evidence therefore suggests that culicine larvae possess well-developed mechanisms in the anal papillae for the uptake of chloride, and it is my purpose in this paper to describe work aimed at elucidating aspects of the mechanism for chloride uptake in *Aedes aegypti* larvae, and to consider in more detail than was achieved earlier (Stobart, 1965) the question of interaction of sodium and chloride during their passage through the anal papillae. Following earlier practice I shall use the terms 'influx' and 'outflux' to describe ionic fluxes found with radioactive tracers, and the terms 'net loss', 'loss', 'net uptake' and 'uptake' to describe net movements of ions.

II. MATERIALS AND METHODS

Fourth instar larvae reared as described earlier (Stobart, 1959) and starved for 60–72 hr. were used throughout this work, except that fed larvae were also used in some preliminary experiments. Two stocks of *Aedes* were used; the first designated hereafter by L was the same as that used in earlier investigations (Treherne, 1954; Stobart, 1959, 1960, 1965); the adults of this stock were fed naturally on mammals. The second, designated by OSU, was chosen because the adults can be fed artificially, and has been used for most of the work; this stock was originally obtained in Orlando,

Florida, from a colony maintained in the U.S. Public Health Laboratory, and has since been maintained for many years in the department of Zoology and Entomology at Ohio State University. The adult diet since 1956 has consisted of citrated ox blood containing 10 % sucrose. The adult diet used here was a 10 % solution of egg albumen powder (B.D.H.) in 10 % sucrose (Lea, Dimond & Delong, 1956). A small amount of 'Oxoid' bacteriological yeast extract (about 0.05 %) was also added as a source of B vitamins, though this is not essential. So far as the physiology of ionic regulation is concerned, the differences between the two stocks are very small.

Measurements of Na and Cl fluxes using the radioactive isotopes ^{22}Na and ^{36}Cl .

The technique for measurement of fluxes was the same as that used earlier (Stobart, 1965) except that the time-intervals used for the flux measurements in the most dilute solutions were shorter, being about 1 hr. The fluxes were expressed as $\text{m}\mu\text{M}/\text{mg. wet wt.}/\text{hr.}$ Where measurements were made of chloride fluxes alone, or of simultaneous sodium and chloride fluxes, it was necessary to modify the technique as follows.

(1) Measurement of chloride influx alone. The technique was the same as for sodium alone except that it was not possible to ash the larvae, as the ashing procedure drives off all the chloride. Instead, the five groups of ten larvae which constituted a sample were transferred to planchettes (which, as in all the influx measurements, contained $9\mu\text{M}$ dextrose as a spreader) and were macerated on the planchettes by rubbing over them the smooth flattened end of a thick Perspex rod, the rinsings from which were then added to the planchettes. Maceration was continued until no fragments of cuticle or organs could be recognized with the naked eye, and the macerate was dried before its radioactivity was measured. The macerated larval tissue was found to increase by equal amounts the counting rates of both ^{36}Cl and ^{22}Na samples, presumably by altering the sample geometry. A comprehensive series of tests which included (i) macerating unlabelled larvae on to ^{36}Cl and ^{22}Na samples, (ii) ashing the tissues away from samples of macerated larvae labelled with ^{22}Na , showed that the counting rate was increased by a factor of 1.24881 ± 0.02020 (S.D.). This correction factor was applied to the measurements (made with $9\mu\text{M}$ of dextrose on the planchettes) of specific counting rates of labelled chloride before the values for influx were calculated.

(2) Measurement of chloride outflux alone. The technique was the same as for the measurement of sodium outflux except that before the samples of external medium were transferred to planchettes they were made alkaline with a small amount of NH_4OH and dried, in order to guard against the possibility that significant amounts of chloride left the larvae as HCl .

(3) Simultaneous measurement of sodium and chloride influx. Because of the time taken in the preparation of large numbers of samples for the Geiger counter, it was impracticable to use the short-lived ^{24}Na in making simultaneous estimates of sodium and chloride influx. Instead, the following method was used. The groups of ten larvae labelled with both ^{22}Na and ^{36}Cl were transferred with $9\mu\text{M}$ of dextrose to planchettes and were macerated. The radioactivities of the dried macerates were measured and they were then transferred to platinum foil and ashed at 450°C . for 5 hr. This procedure drove off all the labelled chloride. The ashed macerates were now converted to chlorides and transferred back to the planchettes and dextrose was added as before.

Measurement of the ashed macerates gave the radioactivity due to ^{22}Na only, and radioactivity of unashed macerates — (radioactivity of ashed macerates $\times 1.25$) = radioactivity due to ^{36}Cl only. As the specific counting rates of the labelled sodium and chloride were known (measured with $9\ \mu\text{M}$ dextrose on the planchettes) it was possible to find the simultaneous fluxes of sodium and chloride.

(4) Simultaneous measurement of sodium and chloride outflux. The samples of external medium (NaCl solutions) were dried on to planchettes (which as in all the outflux measurements contained $559\ \mu\text{M}$ of dextrose as a spreader) and their radioactivities (due to ^{22}Na and ^{36}Cl) were measured. They were transferred to platinum crucibles and the dextrose was ashed off (450°C . for $\frac{1}{2}$ hr.). Enough $(\text{NH}_4)_2\text{SO}_4$ solution was added to each sample to bring the $(\text{NH}_4)_2\text{SO}_4/\text{NaCl}$ ratio to 9:1 on a molar basis and the samples were dried. Previous tests had shown that heating such a mixture to 450°C . for 5 hr. results in the complete removal of chloride, and in this way the labelled chloride was removed from the samples (the crucibles had to be loosely covered to prevent losses due to spluttering). The samples were now transferred back to the planchettes, dextrose was added as before and the radioactivities due to ^{22}Na were measured. The radioactivities due to ^{36}Cl were then obtained by subtraction. The fluxes were calculated as described in (3) but without the use of the correction factor, the measurement of specific counting rates being made with $559\ \mu\text{M}$ of dextrose on the planchettes.

Chemical measurements of chloride

These were made as described earlier (Stobbart, 1965) or with the Aminco-Cotlove chloride titrator. For measurement of chloride in the haemolymph each sample consisted of 5 lots of pooled haemolymph $4\text{--}7\ \mu\text{l}$. in volume, and collected from up to 10 larvae. For measurement of total chloride each sample consisted of 5 groups of 10 larvae macerated and extracted with a suitable volume of de-ionized water for about 1 hr. For each sample the mean and its standard deviation were calculated. Evidence was given earlier (Stobbart, 1965) showing that the extraction of the macerates was adequate, and this is supported by the quantitative recovery, with the Aminco-Cotlove titrator, of small amounts of chloride added to aliquots of pooled macerate.

Chemical measurements of sodium and potassium

These were made with the E.E.L. flame-photometer. Haemolymph sodium and potassium were measured in samples similar to those used for chloride measurements, but diluted with 2 ml. of de-ionized water. In the case of total sodium and potassium the groups of 10 larvae were ashed, and the ash was converted to chlorides, dissolved in 2 ml. of de-ionized water and then analysed for sodium and potassium. For each sample the mean and its standard deviation were calculated.

Miscellaneous techniques

Larvae were washed with de-ionized water before transference to the various solutions and before analyses were made on them. Drying and weighing of the larvae were carried out as described earlier (Stobbart, 1965) and as before all the experiments were carried out at 28°C . In some experiments the pH of the external medium was

measured (at 28° C.) using a pH meter and small samples of the medium. The lines through the points in the figures were drawn in by eye and the usual convention of significance was used in the statistical tests.

Preliminary experiments

III. RESULTS

As a preliminary to more extensive investigation of chloride movements it was shown that (1) about 90 % of the steady-state exchange of chloride occurs through the anal papillae, and that starvation reduces the fluxes by a factor of 6–7; (2) chloride-deficient starved larvae are able to take up chloride only when the papillae are intact.

In view of the very great similarity of these results to earlier ones for sodium (Stobart, 1959, 1960) it would seem that the sodium and chloride pumps are structurally and functionally closely related.

Salt balance

In earlier work (Stobart, 1965, fig. 1) it was shown that starved L larvae placed at a density of 1 larva/2 ml. in de-ionized water could, after about 20 hr., bring themselves into sodium balance at the very low external concentration of about 5 $\mu\text{M/l}$. This has been confirmed for OSU larvae. This 'balancing' treatment, which involves a relatively small loss of sodium potassium and chloride (10, 6 and 26 % respectively; Stobart, 1965), stimulates the sodium pump maximally; it was used exclusively in the earlier work, and has been so used here on the grounds that it is also likely to so stimulate the chloride pump. All the experiments reported subsequently therefore have used larvae 'balanced' in this way for at least 24 hr. after the period of starvation. The larvae (of both stocks) cannot achieve true potassium balance, but the potassium loss is very small (Stobart, 1965), for after 100 hr. the external concentration has risen to only 10 $\mu\text{M/l}$. The chloride concentration in the external medium after balancing is complete is equivalent to the combined concentration of sodium and potassium. The larvae therefore lose a mixture of sodium and potassium chlorides during the balancing process.

Flux measurements, I

The effect of the external concentration of different chlorides upon the chloride fluxes

Sodium chloride. Measurements of chloride influx and outflux at different external concentrations of sodium chloride are shown in Fig. 1 (L larvae) and Fig. 2 (OSU larvae). The sodium fluxes are also shown. The stippled and cross-hatched areas indicate the net uptakes of sodium and chloride, and the areas between the outflux lines and the abscissae indicate the overall exchange components in the sodium and chloride fluxes. At the higher concentrations some 90 % or more of the exchange component must be due to something similar to 'exchange diffusion', as the passive losses of sodium and chloride into de-ionized water are very small (see also Fig. 7). The sodium fluxes are in general very similar to those found earlier (Stobart, 1965) and approach a maximum value at the higher external concentrations. The data for both influx and outflux follow roughly the Michaelis equation, $M = Kc/(K_m + c)$ where M = flux, K = maximum flux, K_m = external concentration for half-maximum flux, c = external concentration. The relationship between the chloride fluxes and external concentration is similar.

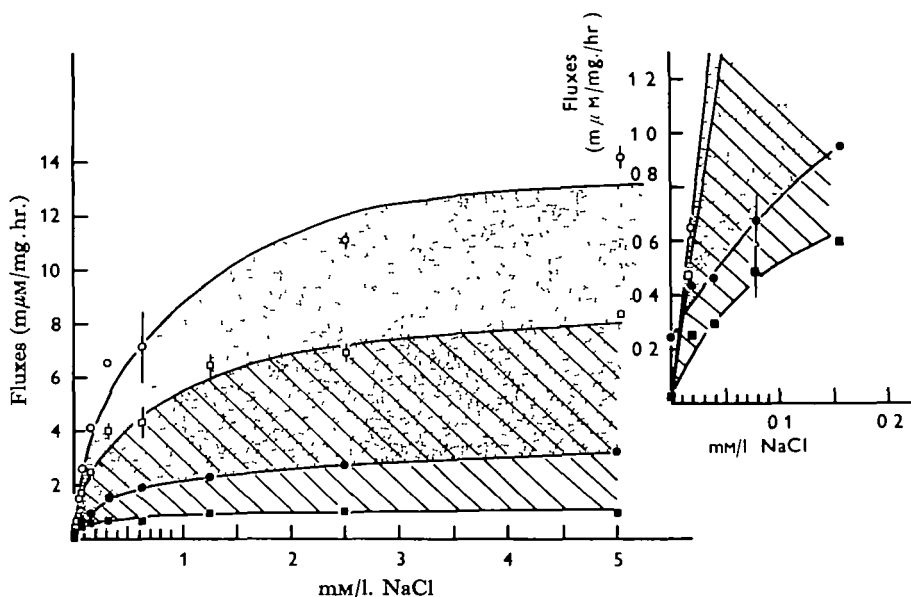


Fig. 1. The relationships between external concentrations of NaCl, and Na and Cl influx and outflux (measured simultaneously) in starved balanced larvae (stock L). The fluxes at very dilute external concentrations are shown in the inset. \circ — \circ , Na influx; \bullet — \bullet , Na outflux; \square — \square , Cl influx; \blacksquare — \blacksquare , Cl outflux; stippled area, net Na uptake, cross-hatched area, net Cl uptake. In this and all figures except Figs. 3–5 and 11 each point represents information from 40–50 larvae, and in all except Figs. 3 and 11 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 outflux measurements shown in Figs. 1, 2 and 7 the standard deviation was found for only one point in each series. See Stobbart (1965) for details.

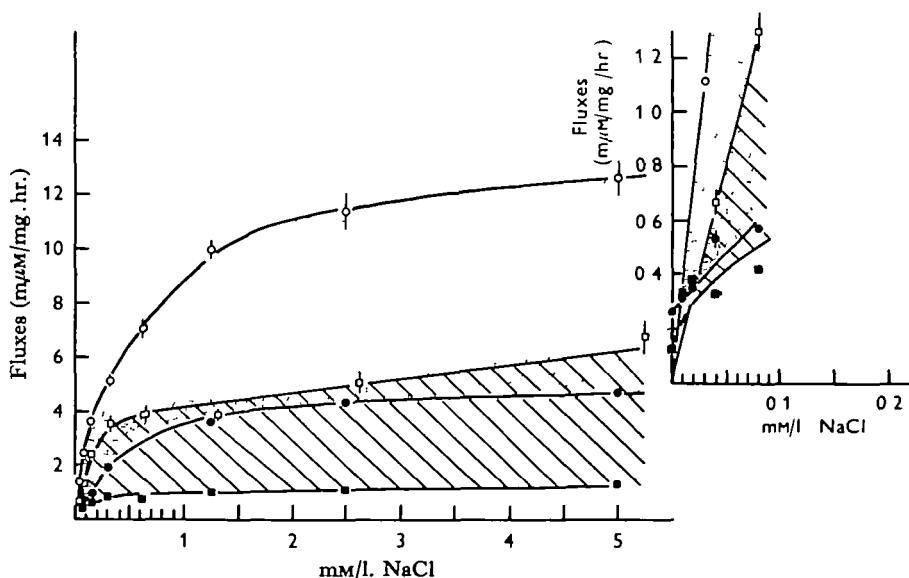


Fig. 2. The relationships between external concentrations of NaCl and Na and Cl influx and outflux (measured separately) in starved balanced larvae (stock OSU). Symbols and format as for Fig. 1.

The K_m values are as follows: for Na about 0.55 mM/l. , the same in both stocks and the same as found earlier (Stobbart, 1965); for Cl 0.5 mM/l. (L larvae) and 0.2 mM/l. (OSU larvae), here there seems to be a definite difference between the stocks. The intersections between the influx and outflux lines in the insets of the figures give *rough* estimates of the external concentrations at which sodium and chloride balance can be maintained. The results indicate a balance point of about $10 \mu\text{M/l.}$ for sodium, in reasonable agreement with the balancing data described above. In the case of the OSU larvae the chloride balance point is about $20 \mu\text{M/l.}$, while for the L larvae it is much lower, about $3 \mu\text{M/l.}$ In view of the fact that the larvae do not achieve potassium balance at external concentrations of about $10 \mu\text{M/l.}$, and that losses of sodium and potassium are accompanied by equivalent losses of chloride, it seems likely that this low chloride balance point is due to experimental error.

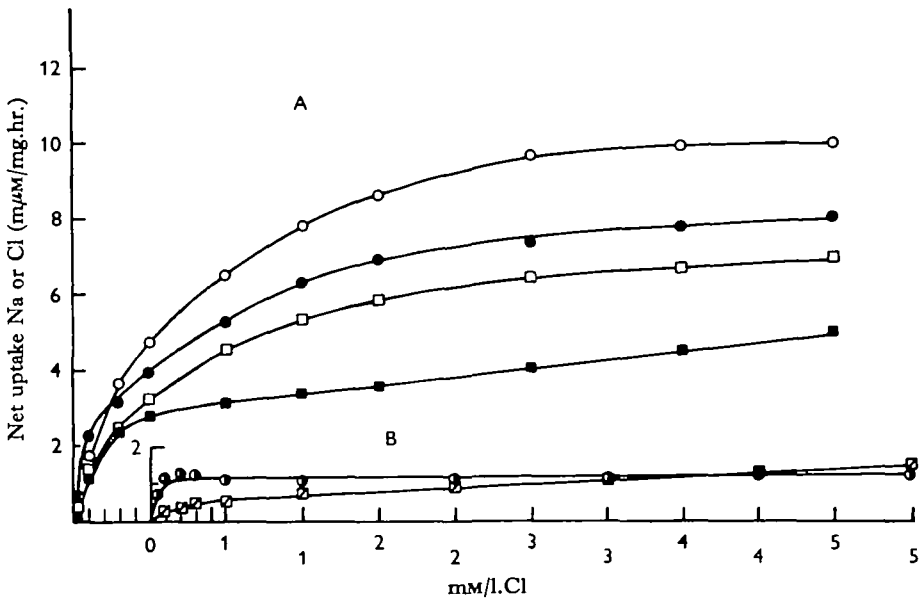


Fig. 3. A, The relationship between external concentrations of NaCl and rates of net uptake of Na and Cl (calculated from Figs. 1 and 2) in starved balanced larvae. ○—○, Na uptake; □—□, Cl uptake; L larvae. ●—●, Na uptake; ■—■, Cl uptake; OSU larvae. B, The relationship between external concentrations of Cl and rates (calculated from Fig. 7) of net uptake of Cl in starved balanced OSU larvae. ●—●, Cl uptake from KCl; □—□, Cl uptake from CaCl_2 .

The relationships between external concentration and rates of net uptake of sodium and chloride (calculated from Figs. 1 and 2) are shown in Fig. 3 A. The similarity in both cases to the Michaelis-type relationship is obvious, and is in agreement with earlier data for sodium (Stobbart, 1965, fig. 6). The rates are somewhat higher in L larvae and in both stocks at all the external concentrations tested the rate of net sodium uptake is greater than that of chloride. The rate of the net chloride uptake does not appear to be a rigidly fixed proportion of that for sodium. In L larvae chloride values range between 66% and 77% of sodium values over the range of external concentrations tested. In OSU larvae the range is from 20% to 78%. The greater variation in OSU larvae, however, is probably due to the fact that here the sodium and chloride measurements were made on different samples of larvae.

Independent uptake of sodium and chloride (OSU and L larvae)

To find out whether larvae were capable of independent sodium and chloride uptake they were first balanced, or treated more extensively with de-ionized water, and then transferred to solutions of various salts. After a suitable time they were removed and analysed to detect any net uptake. Fig. 4 shows that balanced larvae can largely or completely restore the chloride concentration in the haemolymph after a 5 hr. stay in KCl 2 mM/l. + CaCl₂ 1 mM/l. When they are moved from this to NaCl 2 mM/l. for 5 hr. the sodium concentration is restored and there is some evidence for

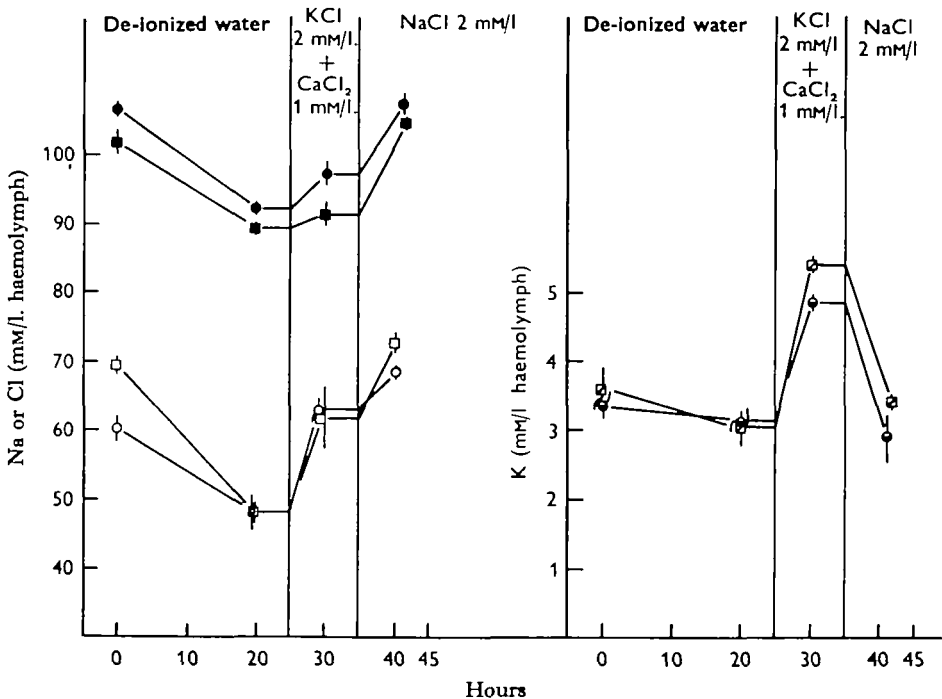


Fig. 4. The restoration of haemolymph Na, Cl and K concentrations in starved balanced larvae. Here and in Fig. 5 each point is the mean of five haemolymph samples, each of which consists of pooled haemolymph from up to ten larvae. ●—●, haemolymph Na concentrations; ○—○, Cl; ●—●, K; stock L. ■—■, Na; □—□, Cl; □—□, K; stock OSU.

an overshoot in the chloride concentration. With respect to potassium the picture is different. Balancing results in no significant fall in haemolymph concentration, but upon transference to the KCl+CaCl₂ solution there is a pronounced rise in the concentration, which then falls to the original level after 5 hr. in NaCl 2 mM/l. A similar experiment is shown in Fig. 5, which demonstrates effective restoration of haemolymph potassium and chloride concentrations in KCl 2.41 mM/l., and restoration of the sodium concentration in Na₂SO₄ 1.23 mM/l. Now the haemolymph of the larvae makes up at least 62.5 % of the body weight, which means that about 90 % of the sodium is in the haemolymph compartment (Stobbart, 1965). In contrast, data for haemolymph and total potassium and chloride concentrations (this paper) show that about 75 % of the chloride, and about 6.4 % of the potassium, is in the haemolymph

compartment. It is possible therefore that some of the changes in haemolymph concentrations shown in Figs. 4 and 5 are due to ionic shifts between tissues and haemolymph and not to uptake from the medium; e.g. the increase in chloride in larvae in Na_2SO_4 (Fig. 5). So in order to get more definite data a similar experiment was carried out (Fig. 6) in which the total concentrations of sodium potassium and chloride were measured. Here the larvae were subjected to a more severe treatment with de-ionized water—5 changes of water over a period of 72 hr. at a density of 1 larva/2 ml. The object was to deplete the larvae of ions more severely so as to

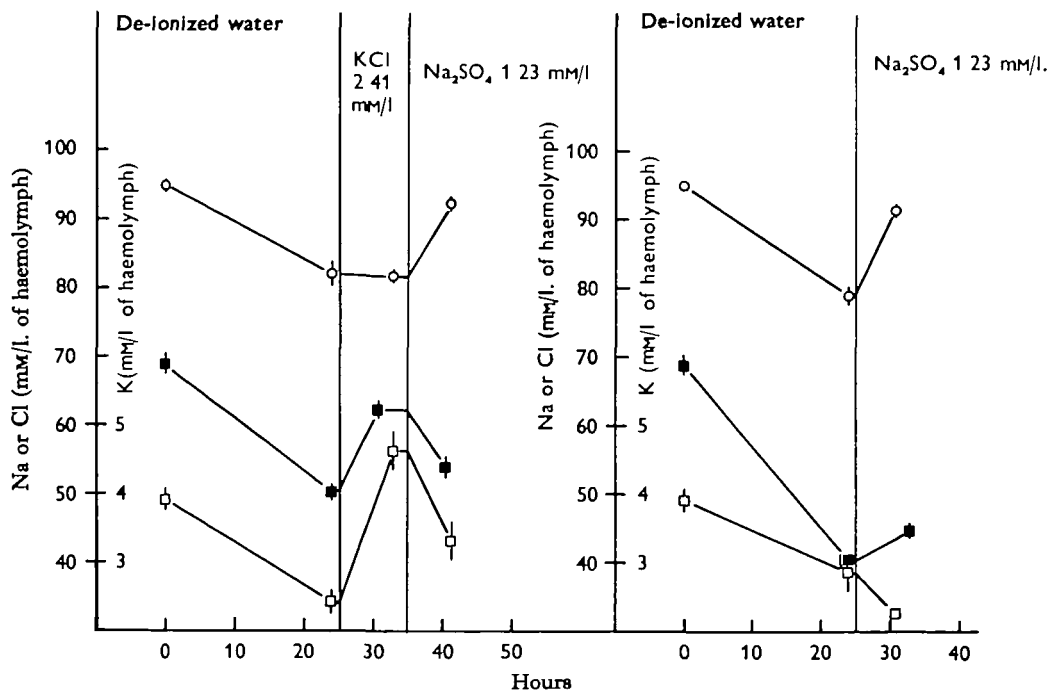


Fig. 5. The restoration of haemolymph Na, Cl and K concentrations in starved balanced OSU larvae. \circ — \circ , haemolymph Na concentrations; \blacksquare — \blacksquare , Cl; \square — \square , K.

emphasize the restorative processes. After depletion the larvae were divided into groups A and B, the fates of which are shown in A and B of Fig. 6. The treatment with de-ionized water causes a drop in sodium of 18.25 mm/kg. wet wt., and in potassium of 6.5 mm/kg., total = 24.75. This is almost exactly balanced by the chloride drop of 24.5 mm/kg. After 10 hr. in Na_2SO_4 1 mm/l. + K_2SO_4 1 mm/l. group A larvae have restored their sodium completely. Their chloride continues to decline very slowly as would be expected, but, surprisingly, so does their potassium. Upon transference to the three chloride solutions shown (2 mm/l. with respect to chloride) chloride is taken up: uptake is more rapid from NaCl than from NH_4Cl and CaCl_2 , with, in the former solution, a significant overshoot after some 10 hr. Potassium continues to decline as expected and sodium in NH_4Cl and CaCl_2 stays effectively the same or declines very slowly. However, in NaCl there is a very pronounced overshoot associated with the uptake of Cl. Group B larvae show the situation when chloride uptake is allowed as the first step in the restorative process. After 10 hr. in KCl 2 mm/l. some

61 % of the chloride drop has been made good and there has also been a significant though small rise in potassium amounting to 43 % of the potassium drop. The sodium remains effectively constant. Upon transference to the sodium solutions shown (2 mM/l. with respect to sodium) sodium is taken up. Uptake is more rapid from NaCl than from Na_2SO_4 and there is some overshoot in the former solution. This is associated with uptake of chloride which also rises slightly above the initial level. In Na_2SO_4 chloride declines slowly, and in NaCl and Na_2SO_4 there is a more rapid decline in potassium. The changes in ionic concentrations are not associated with any significant changes in weight (i.e. water content) of the larvae.

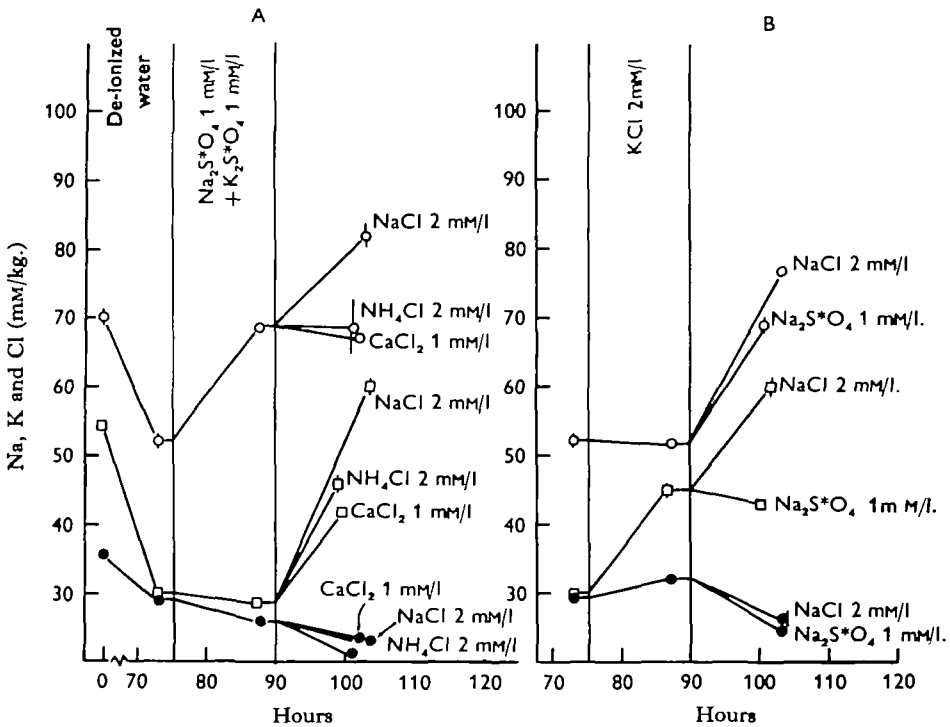


Fig. 6. The restoration of total Na, Cl and K concentrations in starved OSU larvae severely treated with deionized water (see text). Each point is the mean of five groups of ten larvae. $\circ-\circ$, total Na concentration; $\square-\square$, total Cl; $\bullet-\bullet$, total K. After a stay in $\text{Na}_2\text{SO}_4 + \text{K}_2\text{SO}_4$ 1 mM/l. each or KCl 2 mM/l., the larvae were transferred to the various salt solutions which are shown next to the symbols. The sulphate solutions were labelled with ^{35}S .

To summarize: (i) uptake of sodium and chloride can occur independently of one another, but the rates of uptake of the two ions are quicker when they are both taken up together; (ii) potassium uptake occurs from KCl but not from K_2SO_4 ; (iii) larvae are better at retaining sodium and chloride than potassium when these ions are absent from the medium (cf. balancing data).

If sodium and chloride are taken up independently they must either be accompanied by an ion of opposite charge, or be exchanged for one of like charge from inside the larva. Fig. 6 shows that in the case of chloride uptake from KCl 83 % of the uptake is unaccompanied by K^+ . The sulphate solutions used in this experiment were labelled

with ^{35}S , and the radioactivity data show that in the case of Na^+ uptake from Na_2SO_4 1 mM/l. + K_2SO_4 1 mM/l. at least 71 % of the Na^+ uptake is unaccompanied by SO_4^{2-} , and in the case of uptake from Na_2SO_4 1 mM/l. the percentage is 85. Exchange for an ion of like charge must therefore be of great importance during independent uptake of Na^+ and Cl^- , and must also occur to a considerable extent during Na^+ uptake from NaCl (Fig. 3 A). I have no direct evidence about the identity of the ions exchanged for Na^+ and Cl^- .

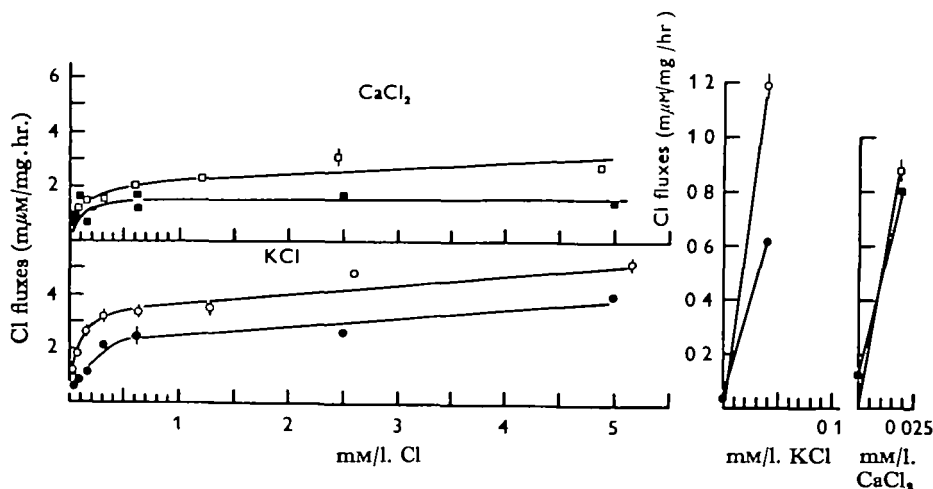


Fig. 7. The relationships between external concentrations of Cl^- and influx and outflux of Cl^- in starved balanced OSU larvae. Format as for Fig. 1. \square — \square , influx; \blacksquare — \blacksquare , outflux; larvae in CaCl_2 . \circ — \circ , influx; \bullet — \bullet , outflux; larvae in KCl .

Flux measurements II (OSU larvae)

The effect of the external concentrations of different chlorides upon the chloride fluxes (continued)

Potassium chloride and calcium chloride. Chloride influxes and outfluxes at different concentrations of these two salts are shown in Fig. 7. Any net uptake of chloride occurring here is obviously independent of sodium. As in Figs. 1 and 2 the relationships between flux rates and external concentrations can be roughly described by the Michaelis equation—the outflux data for CaCl_2 are, however, poor in quality. The K_m value for influx from KCl is roughly 0.2 mM/l., in good agreement with the chloride data from NaCl (Fig. 2). If we compare the flux data for sodium chloride with those for potassium chloride (Figs. 1, 2) we find that generally speaking the influx is lower and the outflux some 2–3 times higher in the potassium chloride solutions. The net chloride uptakes occurring in potassium chloride and calcium chloride are shown in Fig. 3 B. At about 1 mμM/mg./hr. the maximum rates achieved are much lower than when sodium is also taken up. Much significance cannot be given to the shape of the curve for calcium chloride because of the poor quality of the outflux data, but I have included the curve because it does give a rough estimate of the maximum rate of uptake. Allowing for experimental error these rates of uptake account reasonably well for the uptake from potassium chloride and calcium chloride shown in Fig. 6.

The effect of different ions upon the influx of chloride from 0.1 mM/l. potassium chloride

The effects of different concentrations of potassium and sodium salts are shown in Fig. 8A and B. K_2SO_4 has a marked stimulatory effect, the flux being increased to 180% of the normal value, while KNO_3 has no very marked effect though the data suggest a stimulation. $KHCO_3$ in contrast has a strong inhibitory effect, the flux dropping to 32% of the normal value. With respect to sodium salts the stimulatory effect of the sulphate is very much greater (640% of normal), the stimulatory effect of the nitrate is greater, and the inhibitory effect of the bicarbonate is very much less (practically absent) than the corresponding effects of the potassium salts. The effects

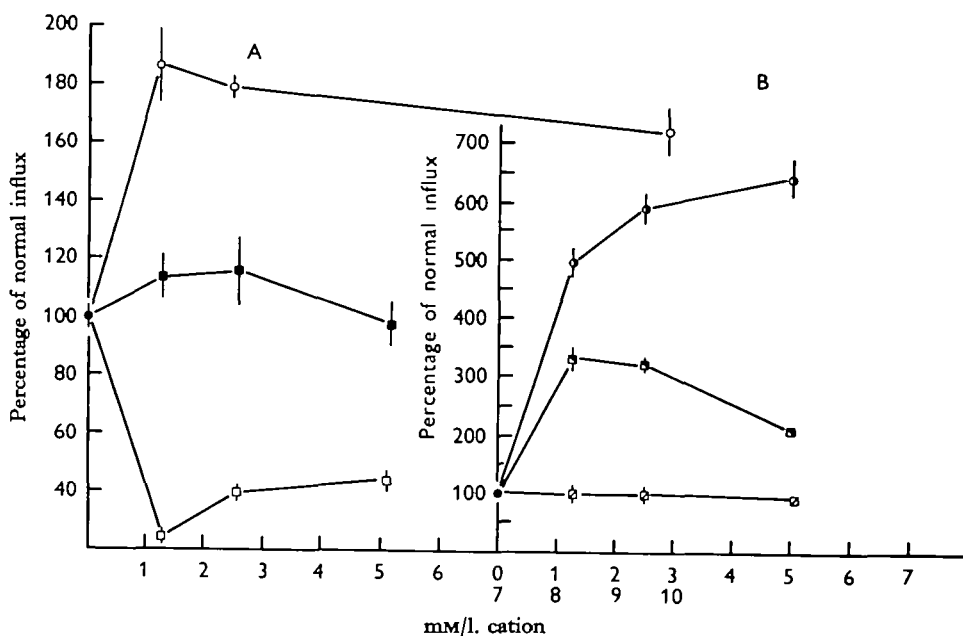


Fig. 8. The effect of various concentrations of different salts upon Cl influx from 0.1 mM/l (OSU larvae starved and balanced). Here and in Figs. 9 and 10 each point is the mean of five groups of ten larvae. A, Data for K salts: ○—○, K_2SO_4 ; ■—■, KNO_3 ; □—□, $KHCO_3$. B, Data for Na salts: ●—●, Na_2SO_4 ; ■—■, $NaNO_3$; □—□, $NaHCO_3$.

of ammonium and calcium and magnesium salts are shown in Fig. 9. Comparing the effects of ammonium salts (Fig. 9A) with the corresponding effects of potassium salts, we see that the stimulatory effect of the sulphate is less, being appreciable only at about 10 m-equiv./l., the slightly stimulatory effect of the nitrate is absent, and is replaced by a strong inhibition (flux down to about 40%), and the inhibitory effect of the bicarbonate is stronger. Fig. 9B shows the effects of magnesium and calcium sulphates and nitrates. The nitrates have a strong inhibitory effect, $CaSO_4$ has a weaker one and $MgSO_4$ at the higher concentrations a marked stimulatory effect.

The high pH of the bicarbonate solutions (about 9 at 5 mM/l.) suggests that the inhibitory effects of the bicarbonates may be partly due to a pH effect and not entirely to a specific inhibition by the bicarbonate ion. The effect of pH on the influx of chloride from 0.1 mM/l. KCl is shown in Fig. 10A, and data for influx of sodium

from 0.1 mM/l. NaCl are also shown. The solutions were acidified with H_2SO_4 and made alkaline with NH_4OH , because SO_4^{2-} and NH_4^+ have only small effects on sodium and chloride fluxes (see Discussion and Stobbart, 1965). Clearly there are separate pH-dependent peaks for the influxes of the two ions occurring at pH 3–5 for chloride, and at pH 9–10 for sodium, the peak flux values being some 250% of the normal fluxes at pH 7. As we should expect from the data of Figs. 1 and 2, at pH 7 the chloride influx is less than the sodium influx. At pH 2 the papillae seem to be directly affected by the hydrogen ion and become extremely fragile. Although the majority of the larvae are still swimming actively, a few may become shrunken due to destruction of the papillae and consequent loss of haemolymph. Microscopical examination of the

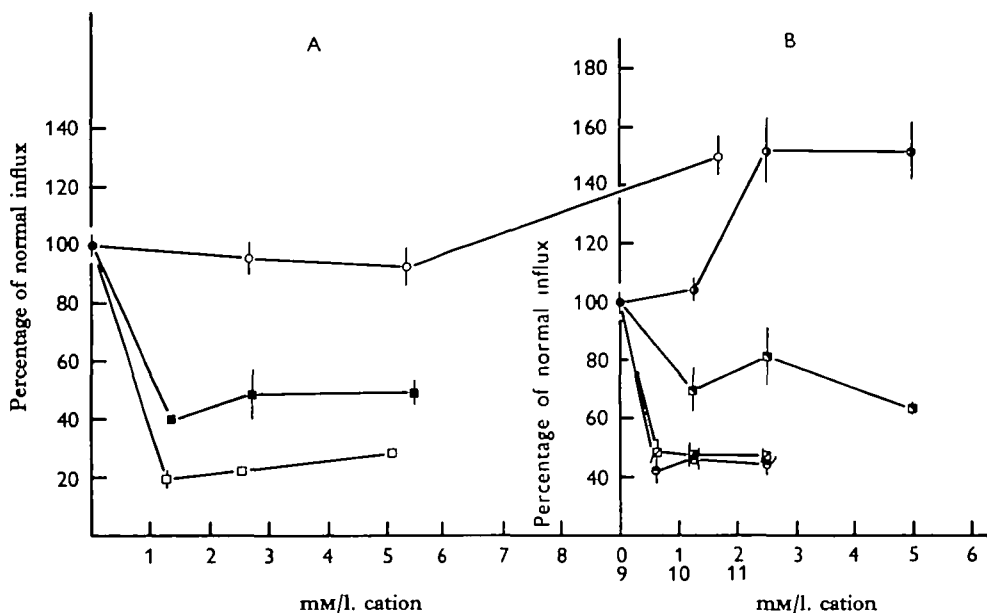


Fig. 9. The effect of various concentrations of different salts upon Cl influx from 0.1 mM/l KCl (OSU larvae starved and balanced). A, Data for NH_4 salts: $\circ-\circ$, $(\text{NH}_4)_2\text{SO}_4$; $\blacksquare-\blacksquare$, NH_4NO_3 ; $\square-\square$, NH_4HCO_3 . B, Data for Mg and Ca salts. $\bullet-\bullet$, MgSO_4 ; $\blacksquare-\blacksquare$, CaSO_4 ; $\square-\square$, $\text{Ca}(\text{NO}_3)_2$; $\bullet-\bullet$, $\text{Mg}(\text{NO}_3)_2$.

papillae shows no gross effect in many cases, in others a shrinkage of the cytoplasm away from the cuticle. This direct effect must be at least a contributory cause of the low fluxes at low pH. At pH 10.8 the larvae lie motionless on the bottom of the beaker apparently unconscious. This anaesthesia must be due to the NH_3 molecule, as ammonium salts lack this effect (Fig. 9A) and it may be a contributory cause of the low sodium flux at this pH.

Fig. 10B shows an experiment designed to give more detailed information about pH effects. Chloride influxes were measured from 0.1 mM/l. NaCl^* and 0.1 mM/l. KCl^* (* denotes radioactive labelling) so that comparison of the two sets of data would show any effect on the chloride influx of the simultaneous sodium influx. The solutions were acidified with H_2SO_4 (see above) and made alkaline with $(\text{CH}_3)_4\text{NOH}$. Sodium influxes were measured from 0.1 mM/l. Na^*Cl and from 0.05 mM/l. Na_2^*SO_4 , so that comparison of the data would show any effect on the sodium influx of the

simultaneous chloride influx. The Na^*Cl solution was acidified with H_2SO_4 , the Na_2^*SO_4 solution with HNO_3 (HNO_3 was used here so that the pH could be altered without altering the concentration of SO_4^{2-} , and because NO_3^- has only a small effect on sodium influx; Stobbart, 1965). The solutions were all made alkaline with $(\text{CH}_3)_4\text{NOH}$. The general picture of two separate pH-dependent peaks in the flux values is very similar to that of Fig. 10A. In spite of considerable scatter the results suggest that over much of the pH range the influxes of sodium and chloride are greater if they occur together. The sodium fluxes shown in Fig. 10A and B are very similar in size which suggests that the tetramethylammonium ion does not appreciably affect the sodium influx. This ion does not anaesthetize the larvae at the higher concentrations, and apparently maximal sodium influxes are shown at pH 11.8. However, the $(\text{CH}_3)_4\text{NOH}$ solution used

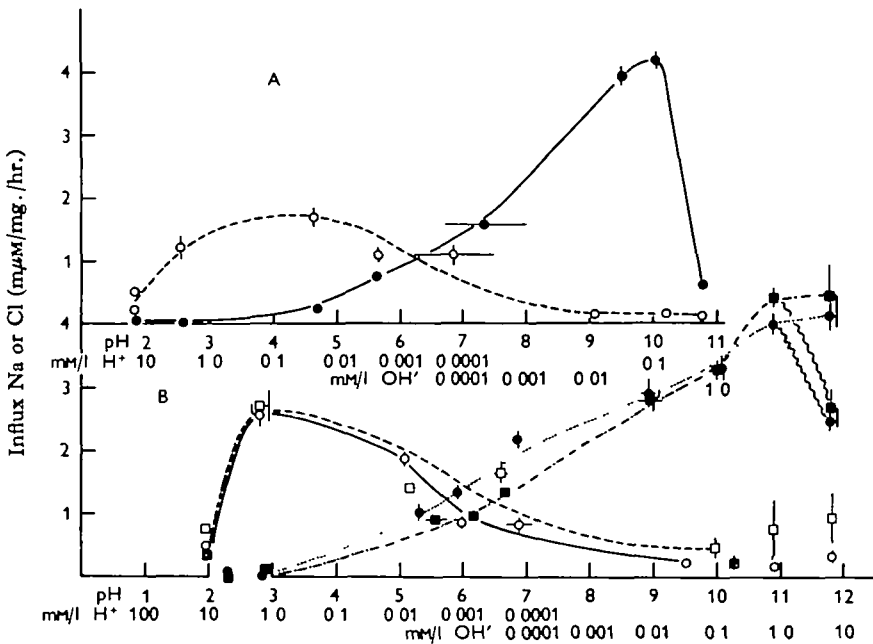


Fig. 10. A, The effect of pH upon the influx of Cl from 0.1 mM/l. KCl^* (* denotes radioactive labelling) and the influx of Na from 0.1 mM/l. Na^*Cl (OSU larvae starved and balanced). $\bigcirc-\bigcirc$, Cl influx; $\bullet-\bullet$, Na influx. The solutions were acidified with H_2SO_4 and made alkaline with NH_4OH . The horizontal lines emerging from some of the symbols indicate the pH drift which occurred during the measurements in spite of the beakers' being sealed with a sheet of 'parafilm'. At pH 2 (approx.) the anal papillae become extremely fragile and it was difficult to dry the larvae for weighing without tearing the papillae. The influx points at this pH therefore are for individual groups of 10 larvae, and not the means of five such groups as at other pH values. This also applies to Fig. 10B. B, The effect of pH upon the influx of Cl from 0.1 mM/l. NaCl^* ($\square-\square$) and from 0.1 mM/l. KCl^* ($\bigcirc-\bigcirc$); and the effect upon the influx of Na from 0.1 mM/l. Na^*Cl ($\bullet-\bullet$) and from Na^*SO_4 0.05 mM/l. ($\blacksquare-\blacksquare$). KCl^* and NaCl^* were acidified with H_2SO_4 , NaCl was acidified with H_2SO_4 , Na_2^*SO_4 with HNO_3 . All solutions were made alkaline with $(\text{CH}_3)_4\text{NOH}$. $\sim\bullet$, $\sim\blacksquare$, Na influx data at pH 11.8 corrected for effect of increased NaCl added as an impurity with $(\text{CH}_3)_4\text{NOH}$ (see text).

contained appreciable amounts of NaCl as an impurity. The amounts of NaCl added were appreciable only at pH 11.8 where the sodium concentration had been increased to 0.2 mM/l. The influxes at this pH (and at pH 10.9) were calculated with the decreased specific activity of the external solution being taken into account. Now Fig. 2

shows that increasing the external sodium concentration from 0.1 to 0.2 mM/l. roughly doubles the influx, and assuming that this would happen at high pH we can find a correction factor to apply to the Na^+Cl data of Fig. 10B. Similar considerations also apply to influx from Na_2^+SO_4 at high pH (Stobart, 1965; fig. 5), for although 0.1 mM/l. Cl is added (with the $(\text{CH}_3)_4\text{NOH}$) to 0.05 mM/l. Na_2^+SO_4 the stimulatory effect of this amount of chloride is likely to be small (Stobart, 1965; fig. 9). The corrected data are shown in Fig. 10B. Obviously the correction is only approximate, but it seems likely that at pH 11.8 the fluxes have passed their peak. I have not applied the corresponding correction to the chloride data as the fluxes at pH 11.8 are small, but if corrected they would be about half their indicated values.

Finally we may note that the pH data reported here are not in conflict with those of earlier work (Stobart, 1959) as there the concentrations of H^+ and OH' were smaller (pH 5.2–7.2) and the concentration of sodium was 20 times greater.

IV. DISCUSSION

The implications of the Michaelis-type relationship between ionic fluxes and external concentration have been discussed earlier for the case of sodium in crustacea (Shaw, 1959, 1961) and for the case of influx and outflux of sodium in *Aedes* (Stobart, 1965). The Michaelis-type data presented here for chloride are very similar to those for sodium, and are clearly compatible with the model of an ionic pump coupled to an 'exchange-diffusion mechanism' suggested earlier (Stobart, 1959, 1960). The K_m values for chloride are 0.2 or 0.5 mM/l. (OSU and L larvae respectively), for Na $K_m = 0.5$ –0.6 mM/l. Compared to other aquatic animals the affinities for their ions of the uptake mechanisms in *Aedes* are rather low. In *Astacus pallipes* $K_{m\text{Na}} = 0.25$ mM/l., and $K_{m\text{Cl}} = < 0.1$ mM/l. (Shaw, 1959, 1960c; figs. 3, 4), and in *Gammarus pulex pulex* $K_{m\text{Na}} = 0.15$ mM/l. (Shaw & Sutcliffe, 1961). In spite of this rather low affinity and the considerable outflux *Aedes* larvae can balance in very dilute external media which they can tolerate well. This is because much of the outflux is due to exchange of ions and the fluxes equalize at low external concentrations. Earlier (Stobart, 1965) it was shown that the passive loss of sodium through the anal papillae (i.e. loss into de-ionized water) was reduced as the larvae adapted to dilute media—in view of the similarities between the mechanisms (Michaelis relationships, low loss from balanced larvae into de-ionized water, siting of fluxes and net uptake in the anal papillae, stimulation of the mechanisms by feeding) it seems likely that this may also apply to the chloride loss.

A consistent feature of the present set of data and the similar one for sodium (Stobart, 1965) is the interaction of sodium and chloride as they pass through the papillae. This shows itself in these ways.

(1) The most obvious is the strong stimulatory effect of Na^+ upon Cl' influx (Fig. 8B) and of Cl' upon Na^+ influx (Stobart, 1965; fig. 9). Here the interfering ion is present in much greater concentration than the other.

(2) Net uptake of Na^+ and Cl' is most rapid when both are taken up together (Figs. 6, 3 and Stobart, 1965; fig. 6). Here Na^+ and Cl' when present together are at the same concentration. This increase in rate of net uptake is in general caused by both an increase in influx and a reduction in outflux (Fig. 10B for Na^+ and Cl' ; and compare Figs. 2 and 7 for Cl' ; and for Na^+ , Fig. 1 (this paper) and figs. 2–5 (Stobart, 1965)).

There are also interactions between other ions and Na^+ and Cl' fluxes, and it will be convenient to deal with these now (together with Na^+ and Cl') before considering Na^+ and Cl' interactions in more detail. The data concern interactions of large concentrations of interfering ions upon influxes from 0.1 mM/l. Na^+ or Cl' . Bringing together the information given in Figs. 8–10 and Stobbart (1965) figs. 7–9 we can, by comparing the effects of the different salts, arrange the various ions in an inhibitory and a stimulatory series for both Na^+ and Cl' according to the magnitude of their effects:

For Na^+ :

stimulatory,

$\text{OH}' \cong \text{Cl}' > \text{SO}_4''$ (effect small or absent) $> \text{NO}_3'$ (slightly inhibitory),

inhibitory,

$\text{H}^+ > \text{K}^+ > \text{Mg}^{2+} \cong \text{NH}_4^+$ (effect small or absent).

For Cl' :

stimulatory,

$\text{H}^+? = \text{Na}^+ > \text{K}^+ > \text{NH}_4^+$ (effect small or absent) $> \text{Ca}^{2+}$ (inhibitory);

the position of Mg_2^+ is equivocal, being stimulatory as MgSO_4 and inhibitory as $\text{Mg}(\text{NO}_3)_2$:

inhibitory, $\text{OH}' > \text{HCO}_3' > \text{NO}_3' > \text{SO}_4''$ (effect small or absent);

it seems likely that the inhibitory effects of bicarbonate solutions may in part be due to their high pH.

Clearly the effect of the anions tested is in general to stimulate sodium influx while cations inhibit it, and the reverse holds true for chloride influx. It seems to me reasonable to include the pH data in this treatment because (a) the larvae appear to be normal in all the solutions used except the most acid and those containing about 1 mM/l. NH_4OH ; (b) the pH effects fit nicely into the general picture of stimulation and inhibition. However, the possibility must be kept in mind of direct effects of pH on the sodium and chloride pumps. To anticipate, it seems likely that the inhibitory effects are due to competition for available sites on the sodium and chloride carriers, and that the stimulatory effects may either be due to alterations in the activities in the haemolymph (or medium) of the ions presumed to exchange for Na^+ or Cl' , or to electrostatic attraction between Na^+ (or Cl') and the interfering ion.

Na^+ and Cl' also interact when they are present in equal concentrations in the medium. We can demonstrate this interaction by comparing the data of Fig. 1 (influx and outflux of Na^+ and Cl' at different external NaCl concentrations) with those of Fig. 5 (Stobbart, 1965) which give influx and outflux of Na^+ at different external concentrations of Na_2SO_4 . We know that uptake of SO_4'' is small compared to that of Na^+ , so clearly by making the appropriate subtractions† we can, for any external concentration, find the alterations in the sodium fluxes and net uptake caused by the net chloride uptake occurring at that concentration (data for L larvae). The external concentrations which have been chosen are 4, 2, 1, 0.5 and 0.1 mM/l. The changes in

† Subtraction of the chloride outflux curve from the chloride influx curve at the appropriate external concentrations gives the net chloride uptake at these concentrations (Fig. 1) and similar subtraction of the sodium influx curves (Fig. 1; and Stobbart, 1965, fig. 5) and the sodium outflux curves gives the changes in sodium fluxes due to the net chloride uptake.

sodium fluxes caused by the net chloride uptake are shown in Fig. 11 A, from which the effect of net chloride uptake upon net sodium uptake may be obtained by subtracting the outflux from the influx data. Similarly we may compare the data of Fig. 2 with those of Fig. 7 (KCl) in order to obtain (for OSU larvae) the effects of net sodium uptake upon the fluxes and net uptake of chloride, it being known that K^+ uptake from KCl is small compared to Cl^- uptake. These effects are shown in Fig. 11 B. Figs 11 A and B demonstrate the increase in influx and decrease in outflux mentioned earlier, and also that a given amount of net chloride uptake is more effective at increasing the sodium uptake than sodium is in the converse case. However, this comparison is made between the two stocks, so too much importance should not be attached to it. Although K^+ uptake from KCl is small compared to Cl^- uptake, significant amounts do

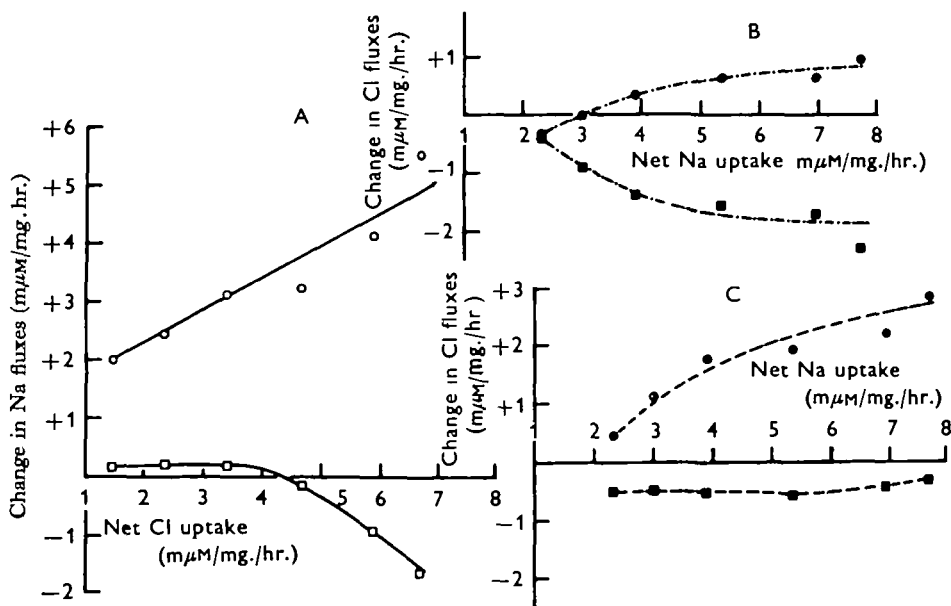


Fig. 11. A, The interaction between net Cl uptake (abscissa) and Na influx and outflux (ordinates) in starved balanced larvae. Data from Fig. 1. and Stobbart (1965), fig. 5. The effect of net Cl uptake upon net Na uptake may be obtained by subtracting the curve for outflux from that for influx. \circ — \circ , Na influx; \square — \square , Na outflux; L larvae. B, The interaction between net Na uptake and Cl influx and outflux. Data from Fig. 2 and Fig. 7 (KCl). Format as for A. \bullet — \bullet , Cl influx; \blacksquare — \blacksquare , Cl outflux; OSU larvae. C, The interaction between net Na uptake and Cl influx and outflux. Data from Fig. 2 and Fig. 7 ($CaCl_2$). Format as for A. \bullet — \bullet , Cl influx; \blacksquare — \blacksquare , Cl outflux. OSU larvae.

occur and it may be therefore that the $CaCl_2$ data of Fig. 7 (in view of possible interactions between K^+ and Cl^-) are a better 'base-line' to compare against the chloride data of Fig. 2. The effects of sodium uptake (worked out from this comparison) upon chloride fluxes and uptake in OSU larvae are shown in Fig. 11 C. Again there is an increase in influx and a decrease in outflux, and the changes in net uptake are not very different from those found by the previous comparison. Comparison of the $CaCl_2$ data and the KCl data of Fig. 7 shows that the interaction between K^+ fluxes (which have not been measured) and the Cl^- ones differ from the Na/ Cl interaction in that K^+ uptake causes *both* the Cl^- influx and outflux to increase over much of the range

of the external concentrations, and that Cl' uptake is appreciably greater from KCl solutions only at the more dilute end of the range.

As several generations of larvae have necessarily been used in this and the earlier work, the comparisons involved in obtaining the data of Fig. 11 are comparisons in some cases between different generations: it is therefore difficult to know what significance to attach to the shapes of the curves in this figure. In view of this I think it best to use these data merely to demonstrate a positive interaction between sodium and chloride uptake, resulting, in general, from an increase in influx and a decrease in outflux.

We saw earlier that the evidence indicates an exchange of Na^+ for some other cation, and of Cl' for some other anion, during chloride-independent net sodium uptake and sodium-independent net chloride uptake. No direct evidence is yet available for *Aedes* about the identity of these exchanged ions. Similar exchanges occur in *Astacus* (Shaw, 1960*a-c*) and here the evidence suggests that Na^+ is exchanged for NH_4^+ (or H^+) and Cl' for HCO_3' , and this is also the case for *Carassius auratus* (Garcia Romeu & Maetz, 1964; and Maetz & Garcia Romeu, 1964). In *Astacus* NH_4^+ and H^+ both depress sodium influx strongly when applied to the outside of the animal, while substituted ammonium ions do not. The situation in *Aedes* is similar except that NH_4^+ has practically no effect. With respect to anions we find in *Aedes* that both OH' and HCO_3' depress the chloride influx strongly. On the basis of their affinities for the ionic carriers therefore, the ions in *Aedes* most likely to participate in exchanges for Na^+ and Cl' are H^+ and OH' (or HCO_3'); any OH' entering the haemolymph is of course likely to give rise to HCO_3' there. This is reasonable; the larvae develop in small volumes of stagnant water where it is likely to be disadvantageous for NH_4^+ to have a strong affinity for the Na^+ carrier, and in any case depletion of the larvae in either Na^+ or Cl' is likely to be closely tied to the pH of the body fluids (cf. Shaw, 1964).

We may now bring together the results of this and previous work (Treherne, 1954; Stobbs, 1959, 1960, 1965) and suggest some models to account for the observed sodium and chloride movements, the stimulation of these movements by feeding and salt loss, and the interaction between sodium and chloride. The models are depicted in Fig. 12. In Fig. 12A and B separate cationic and anionic carrier molecules are supposed to be situated close to each other in the anal papillae and dispersed throughout some (presumably lipid) osmotic barrier between the external medium and the haemolymph. The carriers have ion-exchange properties, their affinities for some ions being as follows: cationic carrier, $\text{H}^+ > \text{Na}^+ > \text{K}^+$ (Figs. 10A, B; Stobbs, 1965, figs. 7, 8); anionic carrier, $\text{OH}' > \text{Cl}' > \text{HCO}_3'$ (Figs. 10A, B, 8A, 9A). The carriers alternate, probably as a result of thermal agitation, between the two surfaces of the barrier—this is indicated by the circular arrows inside the barrier. Net uptake of Na^+ and Cl' are supposed to take place in exchange for H^+ and HCO_3' respectively, which are available in the haemolymph. The situation for chloride-independent sodium transport is shown in Fig. 12A. At the inner surface of the barrier some process requiring energy removes Na^+ from the carrier allowing H^+ to take its place. This presumably enzymic process bears a Michaelis-type relationship to the amount of Na^+ on the carrier, from which it is not capable of removing all the sodium (Figs. 1-3; Stobbs, 1965, figs. 2-6). The process may be envisaged as a reduction, according to requirements, of the carrier's affinity for one of the ions, the affinity for the others

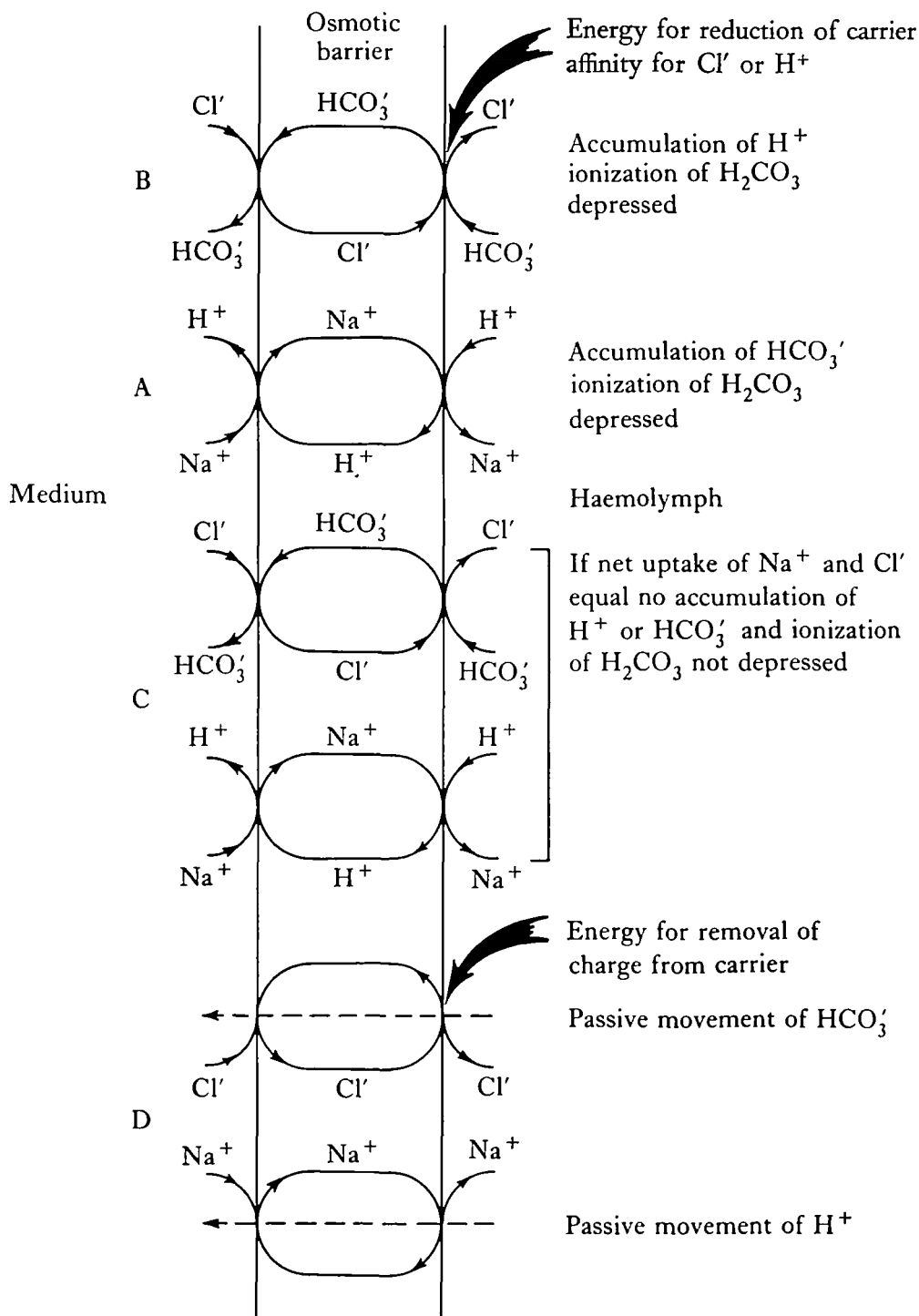


Fig. 12. Models proposed to account for the fluxes and net uptake of sodium and chloride. The curved thin arrows outside the osmotic barrier represent movement of the ions on to or away from the carriers. The circular arrows within the barrier represent the alternation of the carriers between the two surfaces of the barrier. The arrows in broken line represent passive ion movements not associated with the carriers. See text for explanation.

being left unaltered. In normal larvae only enough sodium is removed to balance losses with the urine and passive losses through the body surface. The carrier H^+ complex eventually reaches the outer surface of the barrier where H^+ is displaced from the carrier by Na^+ because of the much higher activity of Na^+ in the (neutral) medium. The carrier- Na^+ complex will later reach the inner surface where the whole process will be repeated. Some Na^+ always remains on the carrier, and this Na^+ can participate in exchange reactions with that in the medium and haemolymph and so give rise to the exchange component in the fluxes (the passive loss into deionized water is very small). The activities of H^+ and HCO_3' in the haemolymph are likely to be very small as the pH values of most insect haemolymphs lie between 6.0 and 7.5 (Buck, 1953). As chloride-independent net sodium uptake proceeds HCO_3' will accumulate in the haemolymph depressing the ionization of H_2CO_3 and hence the availability of H^+ for the exchange with Na^+ . The increased exchange caused by feeding (Stobbart, 1959) is considered to be due to the synthesis of more carriers, and the increase in net uptake and fluxes of Na^+ caused by salt depletion and by feeding (Stobbart, 1960) to the supplying of more energy for the removal of Na^+ from the carrier in addition to the synthesis of more carriers. Unpublished work of mine suggests that the larvae cannot increase the *affinity* of the carriers for Na^+ . A similar mechanism is supposed to exist for the sodium-independent transport of chloride (Fig. 12B). The model for simultaneous net transport of sodium and chloride is shown in Fig. 12C. Here a large proportion of the sodium movement occurs simultaneously with chloride movement. The larger net uptake of both ions (due to increased influx and reduced outflux) presumably results from the greater availability of H^+ and HCO_3' , the ionization of H_2CO_3 being now less liable to depression due to accumulation of either H^+ or HCO_3' . As before the Na^+ and Cl' remaining on the carriers can participate in exchange reactions. The model accounts for the stimulatory effects (discussed earlier) of Na^+ and K^+ on Cl' influx, and of Cl' on Na^+ influx. The stimulatory effect of Na^+ on Cl' influx for example would be caused by the accumulation of HCO_3' (resulting from Na^+/H^+ exchange) which would augment the Cl'/HCO_3' exchange and so increase the Cl' influx. The model will also account for the strong stimulation by H^+ of Cl' influx from KCl , and for that by OH' of Na^+ influx from Na_2SO_4 . Consider the latter case of larvae in 0.05 mM/l. Na_2SO_4 of pH about 11 (Fig. 10B). Here we should expect the anionic carrier to be occupied by OH' at the external surface of the barrier, but it is supposed that OH' is not allowed to leave the carrier at the internal surface in exchange for some other anion, consequently it can only give rise to exchanges of OH' between haemolymph and medium. The cationic carrier at the external surface, however, will be occupied only by Na^+ (external Na^+ concentration = 0.1 mM/l., external H^+ concentration = 10^{-8} mM/l.). As Na^+ is allowed to exchange for H^+ at the internal surface of the barrier the Na^+ influx will be increased because of the removal of competition from external H^+ . Similar arguments apply to the effect of H^+ on the Cl' influx.

An alternative possibility, which should be considered as it cannot be definitely refuted by the evidence available, is that the carriers are capable of transporting charge (Fig. 12D) and that the appropriate ions are removed from them at the inner surface of the barrier without other ions taking their places. As before the Na^+ and Cl' remaining on the carriers participate in exchange reactions. If net uptake of Na^+

and Cl' are equivalent, electrical neutrality is preserved; if one exceeds the other then some other ion of like charge (possibly H^+ or HCO_3^-) will move passively outwards down the electro-chemical gradient. The positive interactions between Na^+ influx and Cl' , and between Cl' influx and Na^+ and K^+ , are now explicable in terms of electrostatic attraction between the ions, and as before the pH effects on Na^+ or Cl' influx are explicable in terms of a reduction in external activity of H^+ or OH' .

The following considerations, however, argue against such an interpretation. Firstly the modification necessary to make a carrier yield up its charge seems likely to be more extensive than that needed to reduce its affinity for one ion species only. Secondly, the passive permeabilities of the papillae of balanced larvae to Na^+ and Cl' are low (outfluxes into de-ionized water 0.1 or less of those into 2 mM/1. NaCl); probably this would apply to other ions, and if so it would render the independent uptake of Na^+ and Cl' a slow process; as we know that the cationic carrier has affinities for H^+ , Na^+ and K^+ (and the anionic one for OH' and HCO_3^-) carrier-mediated exchanges would appear to be the more likely explanation. It will, however, be extremely difficult to come to a definite decision between these alternatives.

In passing we may compare the mechanisms for sodium-independent chloride uptake in *Aedes* and *Astacus* (Shaw, 1960c) and note that in *Aedes* it is not necessary to make the animal deficient in chloride only in order to activate the mechanism for net uptake; balancing the larvae is sufficient (this removes slightly more chloride than sodium) though the resulting net uptake from KCl is small and there is a large exchange component in the fluxes. This difference between the two species may be related to the fact that treatment with de-ionized water removes more sodium than chloride from *Astacus*.

The anal papillae of *Culex quinquefasciatus* and of *Aedes aegypti* have been examined with the electron microscope by Copeland (1964) and Sohal & Copeland (1966) respectively. In both species the syncytia show regular tubular inpushings of the plasma membrane of the cuticular (external) surface. These inpushings are associated with numerous mitochondria, and must correspond to the striated region of Wigglesworth (1933a). In *Aedes* they are known to be the site of an alkaline phosphatase (Sohal & Copeland, 1966). There are also inpushings of the plasma membrane of the inner surface of the syncytia. These inpushings, also associated with many mitochondria, penetrate tortuously far into the interior of the syncytia and may anastomose. In *Aedes* Sohal & Copeland showed that the inpushings and mitochondria were much reduced in larvae reared in 115 mM/l. NaCl (somewhat hypo-osmotic to the haemolymph), compared to those in larvae reared in 6.7 mM/l. NaCl, whereas dystrophic changes appeared in the papillae when larvae were reared in 174 mM/l. NaCl (somewhat hyper-osmotic to the haemolymph). Therefore it seems likely that the greater need for salt uptake in the larvae from the dilute solution is associated with greater development of the membrane-mitochondria associations which seem to be characteristic of secretory cells in general. This is supported by the hypertrophy of the cytoplasm of the papillae (including the striated region) induced by feeding and correlated with greatly increased sodium fluxes (Stobart, 1959).

We may attempt to bring together the cytological and chemical data in one of several possible schemes (illustrated in Fig. 13) by supposing that something similar to the model outlined in Fig. 12A-C resides in the inpushings of the external plasma

membrane, and that the energy for ion uptake is provided by the associated mitochondria. Ions are discharged from the carriers and are moved into the ends of some of the inpushings of the inner plasma membrane by entrainment in a net active transport of water into these tubes by energy derived from their associated mitochondria. The ions are thus flushed out of the cytoplasm into the haemolymph, and the difficulty of a long diffusion path for the ions is avoided. Some haemolymph enters the syncytia by other inpushings of the inner plasma membrane to replace the fluid extruded, so that there is a recycling of fluid through the channels of the cytoplasm which ensures that the carriers on returning to the inner surface of the osmotic barrier are exposed to a

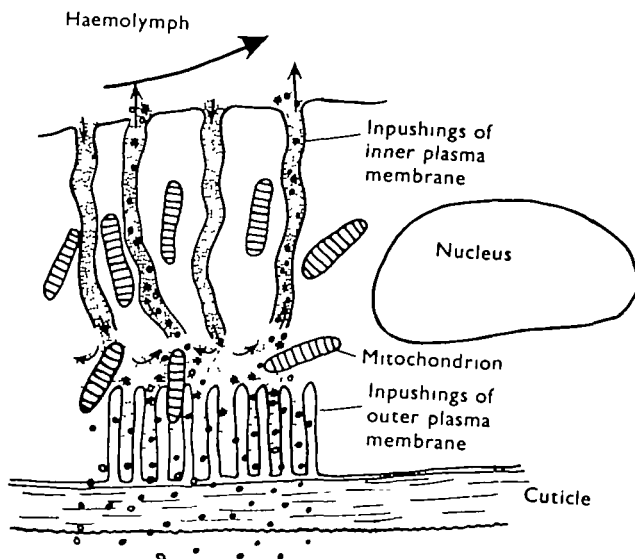


Fig. 13. Scheme suggested for combining the physiological/chemical data with the cytological data of Copeland (1964) and Sohal & Copeland (1966). A simplified diagram is shown of a longitudinal section of a small part of the syncytium of an anal papilla; the widths of the inpushings of the plasma membranes are not to scale. The small circles represent water entering the papilla by osmosis, the stars represent ions liberated from the carriers, and the stippling represents the water and solutes of the haemolymph. The small arrows indicate active movement of water, those entering the haemolymph are larger than those entering the cytoplasm to indicate the net osmotic movement of water through the papilla. The large arrow represents the flow of haemolymph through the papilla. See text for further explanation.

fresh supply of fluid and ions derived from the haemolymph. This entry of haemolymph may also be envisaged as dependent on active water transport. Superimposed on this is an osmotic inflow of water through the papillae into the haemolymph (Wigglesworth, 1933*b*). This inflow is later voided as urine and works out at roughly 33 % of the body weight per day (maximal estimate; Shaw & Stobbart, 1963), or for a 1.66 mg. larva 0.137 μ l. per papilla per day. The active movements of water suggested above would constrain this inflow into the exit channels, so that it would not mix extensively with the recycled haemolymph. Mixing, however, would not matter so long as the carriers when at the internal surface of the barrier were exposed to ionic concentrations which would keep them fully saturated (i.e. not less than about 5 mM/l.). We may note that active transport of water has been shown to occur in the rectum of the locust *Schistocerca* (Phillips, 1964) and that as we are not suggesting active

movement of water against any appreciable concentration gradients the energy needed for it would presumably be slight. It follows that the cuticle of the papillae must be freely permeable to ions, and to some extent permeable to water, in fact rather similar to that of the rectal epithelium of *Schistocera* (Phillips, 1961).

Schemes such as this seem at present rather speculative, and the one just suggested may be over complicated in that diffusion may be enough to cause adequate renewal of ions next to the inner surface of the osmotic barrier. It does, however, have the merit of suggesting a function for the prominent inpushings of the inner plasma membrane; it is also compatible with the demonstration of greatly increased fluxes through much thicker cytoplasm in fed as compared to starved larvae, and is open to testing in so far as a study of sodium and chloride movements between medium and haemolymph, while water is being extracted osmotically at known rates through the papillae, may yield some sort of clue about the importance of any entrainment of ions in water movements.† In so far as they may suggest lines of inquiry leading to better evidence about the relationship between structure and function at the subcellular level in secretory processes, such schemes are probably worth some consideration.

SUMMARY

1. The anal papillae of the aquatic larva of *Aedes aegypti* are responsible for 90 % of the steady-state exchange of chloride.
2. The relationships between chloride flux and external chloride concentration are approximately described by the Michaelis equation.
3. There is net uptake of chloride, independent of uptake of sodium, from KCl, CaCl₂ and NH₄Cl, probably in exchange for OH' or HCO₃', but the rate is much slower than from NaCl. The following ions stimulate influx of chloride from 0.1 mM/l. KCl: H⁺ = Na⁺ > K⁺. The following ions inhibit it: OH' > HCO₃' > NO₃'.
4. Movements of sodium and chloride ions are explicable in terms of an anionic and a cationic carrier located in an osmotic barrier in the papillae, the carriers being functionally coupled to sodium and chloride pumps located at the inner surface of the barrier.
5. An attempt is made to relate these findings to recent electron microscopical studies of the papillae.

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† Recent brief unpublished measurements of mine show that increasing the osmotic pressure of external NaCl solutions with sucrose to twice that of the haemolymph reduces the Na influx into the haemolymph by 50%. The data at present are inadequate to show whether the smaller reductions observed at lower osmotic pressures are significant.

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