

THE EFFECT OF EXTERNAL SALINITY
ON DRINKING RATE AND RECTAL SECRETION IN THE
LARVAE OF THE SALINE-WATER MOSQUITO
*Aedes taeniorhynchus**

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SUMMARY

1. The drinking rate of the saline-water mosquito larva *Aedes taeniorhynchus* ($100 \text{ nl. mg}^{-1} \cdot \text{h}^{-1}$) is unaffected by the salinity of the external medium, but is directly proportional to the surface area of the animal.

2. Haemolymph Na^+ , Mg^{2+} , K^+ , Cl^- , SO_4^{2-} and osmotic concentrations were measured in larvae adapted to 10%, 100% and 200% sea water and were found to be regulated within a narrow range.

3. With the exception of potassium, ionic concentrations in rectal secretion were found to increase with increasing concentrations of the sea water in which larvae were reared.

4. The osmotic concentration of rectal secretion was unaffected by changes in haemolymph osmotic concentration but did rise when sodium or chloride concentrations of the haemolymph were increased. High levels of these ions also stimulated the rate of fluid secretion.

5. Transport of chloride and sodium by the rectum exhibits the kinetics of allosteric rather than classical enzymes.

INTRODUCTION

Hyperosmotic urine is formed in the larval rectum of the saline-water mosquitoes, *A. taeniorhynchus* and *A. campestris*, by the secretion of a hyperosmotic fluid into the lumen (Bradley & Phillips, 1975, 1977). This secretion has an ionic composition closely resembling the hyperosmotic external medium, suggesting that the rectum is the major site of osmoregulation in these larvae. These observations were made on larvae adapted to 100% sea water or, in the case of *A. campestris*, to other saline waters of similar osmolality. We wished to determine how the composition of rectal secretion was affected by changes in external osmolality and how such changes might influence osmoregulation in whole larvae. We therefore measured the drinking rates, haemolymph ion concentrations, and concentrations of rectal secretions in *A. taeniorhynchus* larvae adapted to various concentrations of sea water. We also investigated the effect of various parameters of haemolymph composition on the rate and composition of rectal secretion.

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MATERIALS AND METHODS

The larvae were raised according to the method of Nayar (1966) and starved 1–2 days before use. Four rearing media were used: 100% Vancouver sea water (832 ± 8.8 mOsm; mean \pm S.E.), 10% sea water, 50% sea water, and sea water concentrated to one-half original volume by evaporation at room temperature (200% sea water).

Drinking rates were determined according to the procedure of Bradley & Phillips (1975). In this study, however, larvae were removed and weighed individually after 1 h in the ^{14}C -carboxy-inulin solution. A further difference was that special care was taken not only to mince the larvae before KOH digestion, but also to cut the midgut into several pieces so as to achieve better release of inulin. Drinking rates were determined in the same concentrations of sea water as those in which the larvae were raised.

The length and diameter of larvae, partially immobilized by being placed on moist filter paper, were measured using an eyepiece micrometer. The length was measured from the mouthparts to the posterior edge of the base of the siphon. The diameter of the larvae was measured across the first abdominal segment. The volume of the larvae was calculated from equation (1) and surface area from equation (2). These estimations are based on the assumption that the shape of larvae approximates to that of a cylinder, as found by Nicholson & Leader (1974).

$$\text{Volume (mm}^3\text{)} = \pi r^2 l, \quad (1)$$

$$\text{Surface area (mm}^2\text{)} = 2\pi r(r + l). \quad (2)$$

The symbols r and l refer to radius and length respectively, both in millimetres. The estimated volume and surface area of larvae of known weight were used to generate equations relating these values.

Haemolymph samples were obtained, osmolality was determined, and ion concentrations of Na^+ , Mg^{2+} , K^+ and Cl^- were measured as described previously (Bradley & Phillips, 1975). Calcium concentrations were measured using a 'Techtron AA 120' atomic absorption spectrophotometer in the absorption mode. Determinations were made on pooled $5 \mu\text{l}$ samples of haemolymph diluted in 1 ml of 0.5% LaCl_3 . Sulphate concentrations were determined by measuring the radioactivity of haemolymph samples of known volume from larvae raised in media containing $^{35}\text{SO}_4^{2-}$. Specific activity was determined by measuring the radioactivity of the external medium, the sulphate concentration of which was known (Prosser, 1973).

Samples of rectal fluid were taken either from whole larvae with the anus blocked or from ligated recta bathed in artificial haemolymph and with intact tracheal connexions, as described previously (Bradley & Phillips, 1975, 1976). The artificial haemolymphs used were based on the original formula from Berridge (1966) with modifications as previously described (Bradley & Phillips, 1975). Ionic concentrations of these artificial haemolymphs and any changes in content of organic solutes are shown in Table 1. To examine the effect of haemolymph osmolality, one series of artificial haemolymphs contained all the constituents of the normal artificial haemolymph, but sucrose was varied to achieve various final osmolalities. A second series varied only in chloride and sulphate concentrations. The stock solution contained all

Table 1. The ionic (mM) and osmotic (mOsm) concentrations in the artificial haemolymphs used to study the effects of varying haemolymph parameters

(The variable osmotic and chloride concentration series had the same organic constituents as normal haemolymph (Bradley & Phillips, 1975). The variable sodium concentration series had reduced levels of acids (mg/100 ml): malic acid, 50; citric acid, 25. Na⁺ succinate and Na⁺ glutamate were omitted)

	Variable chloride concentration series	Variable osmotic concentration series	Variable sodium concentration series
Na ⁺	150	150	Variable
K ⁺	18	15	18
Mg ²⁺	5	5	5
Cl ⁻	Variable	100	100
SO ₄ ²⁻	Variable	27	Variable
mOsm	350	Variable	348

constituents except NaCl. Sodium was added as Na₂SO₄, and various levels of chloride were obtained by replacing different amounts of sucrose with choline chloride so that osmotic concentrations did not vary. A third series of artificial haemolymphs was devised to examine the effect of varying sodium levels. NaCl was omitted from the stock solution and Cl⁻ was added as the choline salt. Sodium levels were varied by substituting Na₂SO₄ for differing amounts of sucrose in the artificial haemolymph.

RESULTS

Drinking rates

The drinking rate did not vary significantly when the osmotic concentration of the sea water in which larvae were reared ranged between 10% and 200% sea water (Fig. 1). The average rate was very high indeed (8.4 μ l. larva⁻¹. day⁻¹) for larvae of average weight (3.5 mg). This result was surprising. Larvae living in hyperosmotic waters must drink to replace water lost by osmosis across the body-wall. If such losses were the only factor controlling drinking rate, then drinking should increase as the salinity of external media is raised. Drinking should also cease altogether in hyposmotic media, as observed for freshwater mosquito larvae (Wigglesworth, 1933) where the direction of osmosis across the integument is into the haemolymph. To understand better the factors controlling drinking, the drinking rate of individually weighed larvae was determined and compared with estimates of body surface area.

Nicholson & Leader (1974) obtained two nearly identical estimates of the surface area for the mosquito larva, *Opifex fuscus*, firstly by measuring its diameter and length and assuming it to be a cylinder, and secondly by measuring the total content of cuticular wax. These values agreed to within 3%. We therefore made similar measurements of the dimensions of *Aedes taeniorhynchus* larvae and calculated the body volume and surface area for larvae over a wide range of sizes.

The relationship between the weight of the larvae (x) and the calculated volume of the same individual (y) can be accurately described by the equation $y = -0.19 + 1.07x$ ($r = 0.98$, $P < 0.001$; Bradley, 1976). The y intercept is very close to zero and the slope is nearly one. This result is very close to the one expected if larvae have a

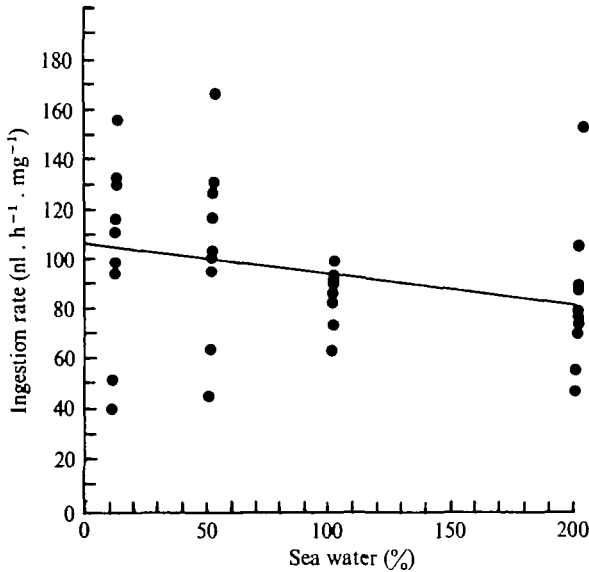


Fig. 1. The drinking rate of larvae in the media in which they were reared. The regression line was fitted by the least squares method.

specific gravity close to one and if the equation used to calculate body volume gives an accurate estimate. The relationship between the calculated surface area (y) and weight (x) of larvae is adequately expressed by the equation $y = 0.13 + 0.75x$ ($r = 0.98$, $P < 0.001$; Bradley, 1976).

The above relationships were used to relate volume and surface areas to drinking rate of individually weighed larvae living in 10%, 50%, 100% and 200% sea water. The empirical relationship between drinking rate (y) and volume (x) is shown in Fig. 2a. The regression line is expressed by the equation: $y = 0.01 + x^{0.72}$. The same relationship exists between drinking rate and the weight of the larvae since weight and volume are linearly related. Clearly, the drinking rate of the larvae in all salinities studied is related to their weight or volume in some complex fashion, for the exponent of x is 0.72, close to the relationship between the drinking rate and surface area of the larvae (Fig. 2b). The equation for the latter relationship is expressed by the equation $y = 0.08 + x^{1.06}$, i.e. very close to linear. The correlation coefficient is high ($r = 0.80$, $P < 0.01$).

In summary, the drinking rate of *A. taeniorhynchus* larvae is not significantly affected by the salinity of the external solutions over the range of the salinities tested. Instead, the size of the larvae determines the rate at which they drink. The change in this rate with increasing size of larvae parallels the rate of change of their surface area.

Haemolymph ion levels

Ion concentrations were measured in haemolymph of larvae raised in 10%, 100% and 200% sea water (Fig. 3a, b). Sodium, magnesium and potassium were strictly regulated in that no statistically significant concentration differences could be detected between larvae reared in these three concentrations of sea water. Haemolymph calcium levels increased significantly ($P < 0.001$), however, with each increase in external

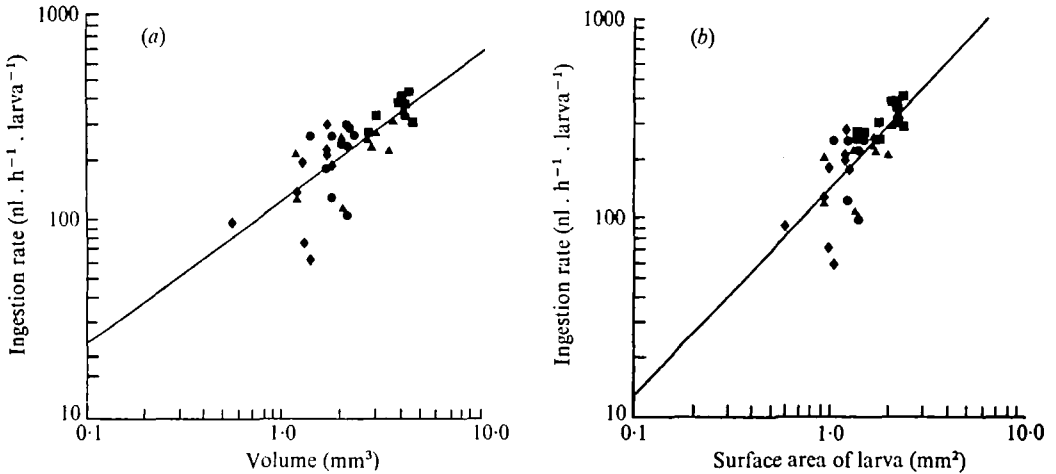


Fig. 2. The relationship between (a) the volume and drinking rate and (b) surface area and drinking rate of larvae in four salinities; \blacklozenge , 10% sea water, \blacktriangle , 50% sea water, \bullet , 100% sea water, \blacksquare , 200% sea water. Drinking rates were measured in the media in which the larvae were raised. The regression lines were fitted by the least squares method: $y = 0.1 + x^{0.73}$ for (a) and $y = 0.98 + x^{1.65}$ for (b), with $r = 0.80$ in both cases.

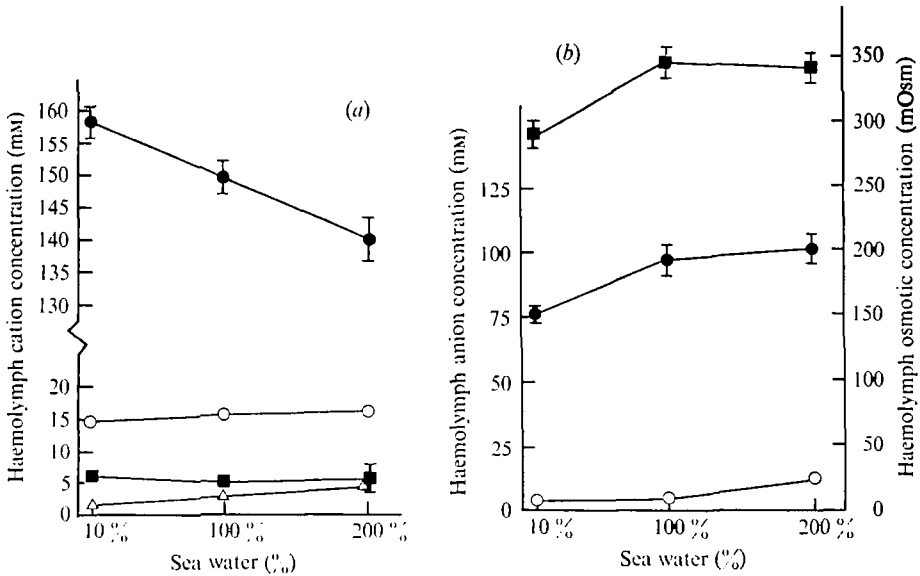


Fig. 3. The mean concentrations of cations (a); sodium \bullet , potassium \circ , magnesium \blacksquare and calcium \triangle ; and anions (b); chloride \bullet , sulphate \circ , and osmotic concentration \blacksquare , in the haemolymph of larvae reared in sea water of differing salinity. Vertical bars denote s.e. of the means unless these are smaller than the symbol. Each point is an average for 10 larvae, except in the case of Ca^{2+} ($n = 5$) and sulphate ($n = 9$).

salinity. The total concentrations of these cations at the three external concentrations were 180mM (10% sea water), 172mM (100% sea water) and 165mM (200% sea water), indicating if anything a slight decrease with increasing external salinity.

Anion regulation was less precise than that of the cations. Chloride concentrations and osmolalities showed parallel trends. Relatively low haemolymph chloride and

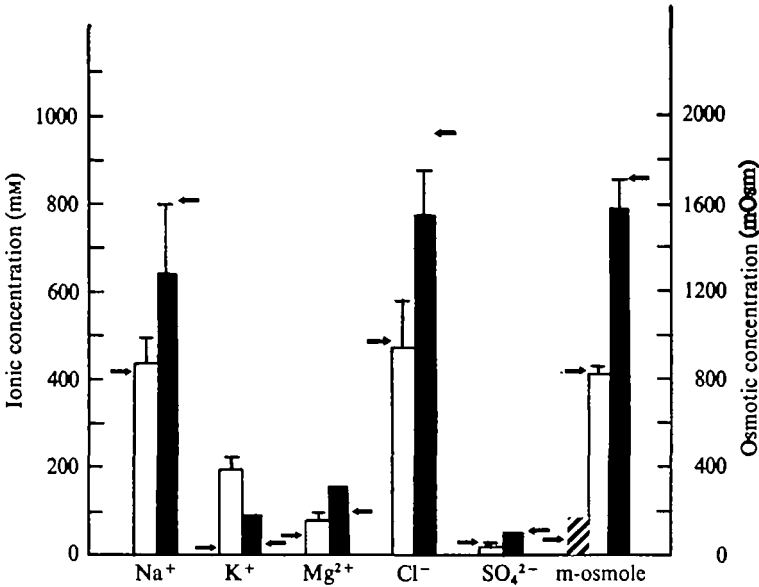


Fig. 4. The ionic and osmotic concentrations (mean \pm s.e., $n = 6$) of rectal fluid from whole larvae living in 10% sea water \square , 100% sea water \square , and 200% sea water \blacksquare . Arrows indicate the respective concentrations in the external medium.

osmotic concentrations were found in animals in 10% sea water, while larvae in 100% and 200% sea water showed somewhat elevated levels (less than 25%), which were not significantly different from each other. Nayar & Sauerman (1975) have measured chloride and osmotic pressure in *A. taeniorhynchus* larvae and found the same pattern of regulation. Sulphate concentrations (Fig. 3b) on the other hand were low in 10% and 100% sea water but increased by a factor of 3.4 in 200% sea water. This suggests that sulphate is closely regulated at lower levels but less so at higher concentrations (discussed by Maddrell & Phillips, 1975). The total anion concentrations were 80 mM (10% sea water), 101 mM (100% sea water), and 113 mM (200% sea water). Minimum estimates of unmeasured anions are 100 mM (10% sea water), 71 mM (100% sea water) and 52 mM (200% sea water).

The total concentration of ions in the haemolymph increases less than does the haemolymph osmolality when larvae living in 10% and 200% sea water are compared. Unless ionic activities undergo considerable changes, which has been shown in some insects (Treherne, Buchan & Bennett, 1975), the proportional contribution of organic solutes to total osmolality of the haemolymph increases in the more saline external media.

Ionic and osmotic concentrations of rectal secretion

(a) In 10% sea water

When ligated recta from larvae raised in 10% sea water were placed in artificial haemolymph, the recta did not swell with secreted fluid as did those from larvae raised in hyperosmotic media. Not only did the recta from 10% sea water larvae not secrete, but the small amount of fluid observed in the rectal lumen initially disappears during the 1.5 h incubation period.

When whole larvae from 10% sea water have their anuses blocked with tissue adhesive (Eastman Chemical Products, Eastman 910), fluid does accumulate in the rectum, presumably due to fluid secretion by the Malpighian tubules. Such small quantities were available that only the osmotic concentration of the fluid was measured (Fig. 4). The average osmolality of this fluid was 170 ± 11 mOsm ($n = 8$) or 50% of the haemolymph value. This supports the original observation by Ramsay (1950) that rectal fluid from *A. detritus* larvae living in distilled water had an osmotic concentration (105 mOsm) one-third that of the haemolymph. Neither saline-water species achieves the low rectal fluid concentrations (35 mOsm) found in *Aedes aegypti* acclimated to distilled water (Ramsay, 1950). These results support the hypothesis that the rectum is the site of salt resorption in larvae acclimated to hyposmotic media (Ramsay, 1950).

(b) *In 100% sea water*

Ion concentrations in rectal fluid from whole larvae reared in 100% sea water were reported by Bradley & Phillips (1975). It was suggested that the apparent anion deficit observed in the rectal fluid (68 mEq) might be due to sulphate ions. The sulphate concentration of rectal fluid was found to be 17 ± 3 mM ($n = 10$), accounting for only one-half of the apparent anion deficit. However, we presented evidence (Bradley & Phillips, 1977) that most of the ingested sulphate is removed from the haemolymph by the Malpighian tubules rather than the rectum and this might be the source of much of the sulphate observed in rectal fluid from whole larvae. Therefore, the anion deficit which is observed in the rectal fluid from isolated recta, which contain little sulphate, must be due to some other unmeasured component (e.g. bicarbonate or negatively charged organic molecules).

The ionic and osmotic concentrations of rectal fluid from larvae adapted to 100% sea water are close to those of sea water (Fig. 4), with the exception of potassium, as previously reported (Bradley & Phillips, 1975). It was of interest to us to determine whether concentrations of rectal secretion are adjusted to match those of more concentrated external media, just as ion ratios in the secreted fluid are regulated to match those in various chemical types of hyperosmotic waters (all 700 mOsm) to which larvae are adapted (Bradley & Phillips, 1977).

(c) *In 200% sea water*

The levels of Na^+ , Mg^{2+} , Cl^- and SO_4^{2-} in the rectal secretion are higher in whole larvae from 200% sea water than in those from 100% sea water (Fig. 4). Sodium and chloride concentrations in the rectal secretion of larvae reared in 200% sea water were slightly but not significantly lower than those in the external medium. Since these larvae must excrete the ions they ingest at concentrations above external levels, because of osmosis across the body-wall, it follows that either some other organ is aiding in the removal of Na^+ and Cl^- from the haemolymph (e.g. anal papillae) or the rectal preparation used in this study does not accurately indicate the maximum concentrations developed in undisturbed larvae.

The levels of magnesium and sulphate observed in the rectal secretion equalled or exceeded those in 200% sea water. Bradley & Phillips (1975) found that the potassium concentration was sixteen times higher in rectal secretion than in 100% sea water. In

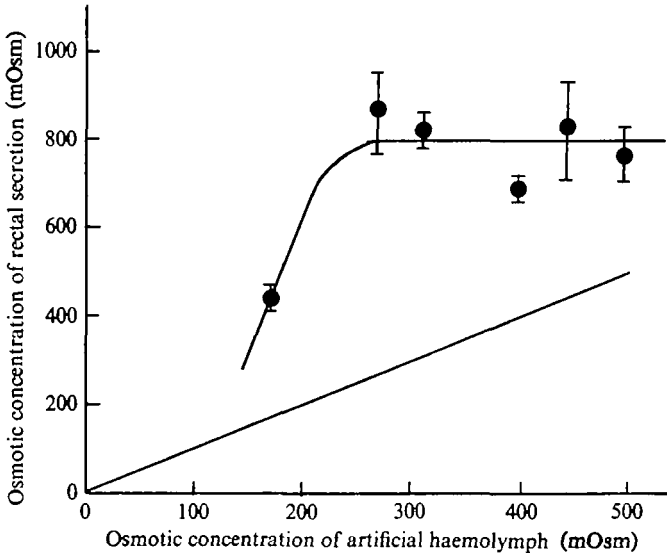


Fig. 5. The effect of varying osmotic (sucrose) concentrations of artificial haemolymphs on the osmotic concentration of rectal secretion (mean \pm S.E., $n = 6$). The line was drawn by eye.

the present study, the level of this cation in the rectal secretion from larvae living in 200% sea water was found to be lower than in larvae from 100% sea water, unlike all other ions measured. However, the level in rectal secretion was still 3.6-fold higher than the external concentration. In summary, larvae respond to more concentrated sea water by secreting rectal fluid in which levels of all inorganic ions except K^+ are substantially elevated.

Factors regulating ionic and osmotic concentration of rectal secretion

Having established that ionic concentrations of rectal secretion are influenced by the concentration of the external medium (this study) and by the relative concentrations of ions in the medium (Bradley & Phillips, 1977), we wished to determine how this regulation is mediated. Since the composition of rectal fluid reflects concentrations in the external medium, larvae obviously monitor changes in external ion levels. The rectum is bathed in haemolymph and the most direct method of regulatory response would be a sensitivity of the rectal secretion to changes in haemolymph ion levels brought about by the ingestion of the external medium. The similarity of haemolymph ion levels in 100% and 200% sea water (Fig. 3 *a, b*) indicate that these levels do not vary appreciably between larvae fully adapted to different hyperosmotic media. However, upon transfer of larvae from one medium to another, substantial transient changes in haemolymph ion levels occur initially and concentrations only return to normal over a 12 to 24 h period (Nayar & Sauerman, 1975; Phillips, unpublished observations). Rapid changes in the rate and concentration of rectal secretion may be one form of short-term response to such abrupt changes in haemolymph concentration. Therefore, the effect on rectal secretion of varying only one parameter of artificial haemolymph composition at a time was investigated. All larvae used in these experiments were raised in 100% sea water.

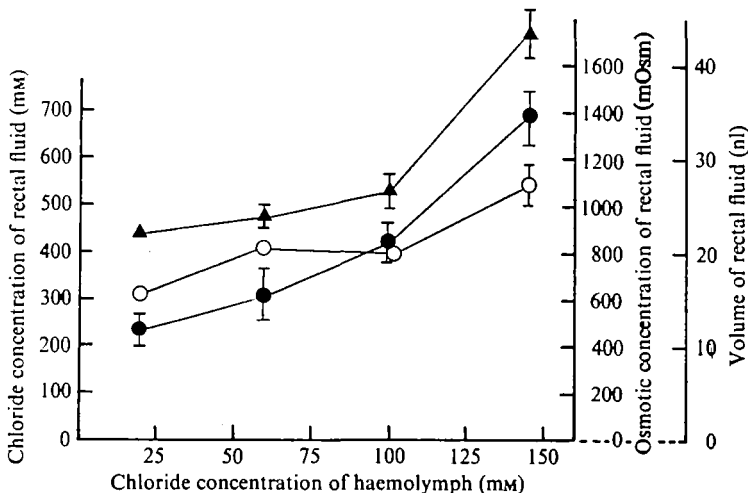


Fig. 6. The effect of varying chloride concentration in artificial haemolymph on the volume of rectal secretion collected after 1.5 h ▲ ($n = 3$), and on the osmotic ○ ($n = 6$) and chloride concentrations ● ($n = 6$) of rectal secretion. Vertical bars denote s.e. of the means, unless these are smaller than the symbols.

Fig. 5 shows the effect of varying haemolymph osmotic concentration, by the addition of sucrose, on the osmotic concentration of rectal fluid produced by ligated recta bathed in artificial haemolymph. In artificial haemolymph of low osmotic concentration (173 mOsm) the rectum is unable to produce a fluid as concentrated as that produced in more concentrated artificial haemolymphs (between 250 and 500 mOsm) or in whole animals. This lower concentration of rectal fluid may be due to tissue swelling in dilute media, with a concomitant reduction in intracellular concentrations, or to increased osmotic permeability. In all artificial haemolymphs of higher osmolality, the rectal fluid had the same osmotic concentration (about 800 mOsm), which was similar to that found in whole animals. This suggests that the osmotic concentration of the haemolymph does not have a significant regulatory effect on the osmotic concentration of rectal secretion. The physiological range of haemolymph osmotic concentrations observed in *A. taeniorhynchus* (303–427 mOsm; Fig. 3*b*) is substantially narrower than the experimental range of Fig. 5. The rate of rectal secretion, as judged by unquantified observations of rectal swelling, did not appear to change substantially even in artificial haemolymph with an osmotic concentration of 500 mOsm.

We considered the influence of changing haemolymph chloride levels when all other parameters were held constant except for sulphate concentration. Response to haemolymph concentration of chloride seemed a likely basis for control of rectal secretion because of the greater changes in haemolymph levels of this ion (Fig. 3*b*). Rectal secretion contained higher levels of chloride than the haemolymph at every haemolymph chloride concentration tested (Fig. 6). However, the chloride concentration in rectal fluid increased more sharply at higher haemolymph chloride levels. The steep slope for the increase in this relationship when haemolymph concentration of chloride rises above 100 mM is of particular interest, since this is just above the normal haemolymph level. Thus when larvae are exposed to media higher in chloride, the

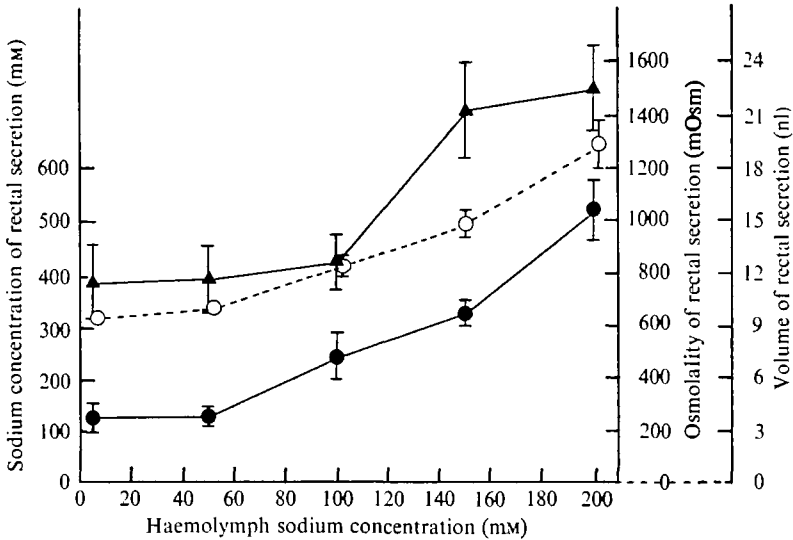


Fig. 7. The effect of varying sodium concentration in artificial haemolymph on the volume of rectal secretion collected after 1.5 h ▲ ($n = 6$), and on the osmotic ○ ($n = 6$) and sodium concentrations ● ($n = 6$) of rectal secretion. Vertical bars denote s.e. of the means, unless these are smaller than the symbol.

haemolymph chloride might initially rise above the average range observed for adapted animals. This would cause the chloride concentration of the rectal secretion to increase substantially, thereby reducing the chloride concentration in the haemolymph. This suggests that there is a degree of intrinsic regulation within the transport process.

Fig. 6 also shows that increasing haemolymph chloride, in the absence of osmotic concentration changes, leads to slightly elevated osmolality of the rectal secretion, but this change is not significant over the physiological range (50–100 mM). Again the osmotic concentration of rectal fluid shows the steepest increase at haemolymph levels above 100 mM. The rate of rectal secretion, as indicated by the largest three samples out of fifteen obtained by micropuncture, shows a positive and significant ($P = 0.01$) correlation with the haemolymph chloride concentration (Fig. 6).

The product of fluid secretion rate and concentration yields an estimate of chloride secretion rate (Fig. 8). The kinetics of the process are not of the Michaelis–Menten type, but rather resemble those of regulatory enzymes. This indicates that the chloride concentration of the rectal secretion in saline-water mosquitoes is influenced and perhaps controlled by changes in haemolymph chloride levels. However, both *A. taeniorhynchus* and *A. campestris* can survive in concentrated waters which are low in chloride (e.g. NaHCO_3 medium; Bradley & Phillips, 1977), and these larvae continue to produce a rectal fluid of appropriate osmotic composition even though haemolymph chloride levels are low. Therefore, parameters other than haemolymph chloride level must also regulate rectal function.

Fig. 7 shows the results of varying haemolymph sodium levels on sodium and osmotic concentrations of rectal secretion. As with chloride, the Na^+ concentration and osmolality of rectal fluid, and its rate of secretion, all increased as haemolymph levels of sodium were raised. The sodium concentration of rectal fluid (128 mM) remained relatively constant when haemolymph levels of this cation were varied

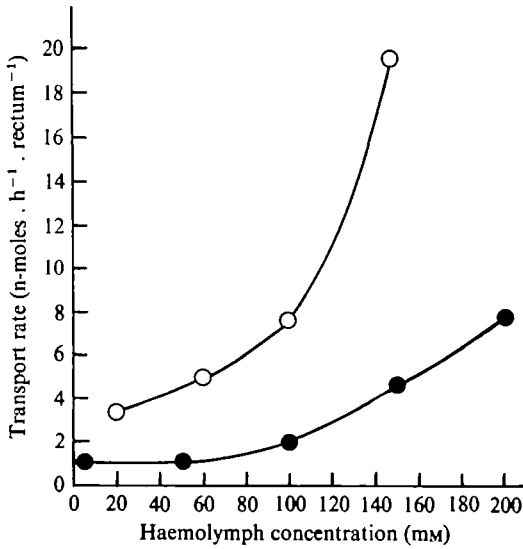


Fig. 8. The relationship between the concentration of chloride \circ or sodium \bullet in artificial haemolymph bathing ligated recta and the rate of transport of that ion (calculated from data on concentrations and volumes of secretion, Figs. 6 and 7).

between 5 and 50 mM. Although the sodium concentration of the rectal secretion was substantially reduced below physiological levels, a hyperosmotic rectal secretion was still produced (634 ± 31 mOsm, $n = 7$). The potassium ion concentration in this fluid was very high (280 ± 70 mM, $n = 7$) suggesting that this cation can partially substitute for sodium ions in the secreted fluid.

The calculated net rate of Na^+ secreted in the rectum during the experimental period is shown in Fig. 8. Since the volume secreted was assumed to equal the volume removed from the recta, and may therefore be subject to slight underestimation, the curves in Fig. 8 are meant to show the relative rather than absolute ion transport rates. As for chloride, sodium transport does not obey Michaelis-Menten kinetics. Rather, the transport rate increases more rapidly when sodium levels in the bathing media are increased within the normal *in vitro* range; i.e. the kinetics resemble those of allosteric enzymes.

DISCUSSION

Beadle (1939) was the first to propose that larvae of saline-water mosquitoes drink the external medium as a means of replacing water lost by osmosis across the cuticle and by excretory processes. We have found that drinking rate is directly proportional to the surface area of larvae (Fig. 2*b*) and not to external salinity (Fig. 1). In 10% sea water, moreover, water enters the larvae both by osmosis across the integument and by drinking. Under these conditions high rates of ingestion are clearly detrimental to osmotic homeostasis and must serve some other function, such as nutrient (i.e. energy) procurement. We suggest that the drinking rate of saline-water mosquito larvae is proportional to the surface area of the larvae because of the commonly observed link between surface area and the metabolic rate of animals (Hemmingsen, 1960).

Aedes taeniorhynchus larvae are able to take up dissolved nutrients by drinking (Nayar, 1966). In the present study, drinking rate experiments were conducted with waters which were filtered prior to use and the inulin was fully dissolved by heating to 90 °C. Therefore, any nutrients and ¹⁴C-inulin taken up had to be in dissolved form. We suggest that these starved larvae drink the external medium, even when particulate food is not available, in order to gain nutrients which might be dissolved in the external medium. The rate of this activity is proportional to the metabolic rate of the larvae and hence proportional to surface area. Such activity may be stimulated by certain organic solutes in natural waters, and variations in concentration of the latter might explain the lower estimate of ingestion at the same salinity reported previously (Bradley & Phillips, 1975); however an improved procedure for extraction of ¹⁴C-activity could explain this difference.

It may be calculated from values observed in this study that larvae drink an amount of fluid equivalent to their total weight every 10 h. The turnover times for total body contents in 100% and 200% sea water are 3.8 h and 1.9 h respectively for sodium, and 4.8 h and 2.4 h respectively for chloride. These animals clearly face a considerable osmoregulatory load due to the rapid ingestion of the external medium. High rates of fluid ingestion could serve a useful function not only in nutrient uptake but also in reducing the rectal fluid concentration necessary to achieve homeostasis in hyperosmotic media (Phillips & Bradley, 1976). This is because the greater the ratio of fluid ingested to water lost across the integument by osmosis, the closer the rectal fluid concentration required to achieve osmotic balance approaches that of the external medium. High rates of ingestion would be advantageous if capacity to transport fluid against increasing osmotic concentration differences were more limited than the potential to increase total ion transport. If the permeability of the external cuticle of *A. taeniorhynchus* is similar to that of *Opifex fucus* (Nicholson & Leader, 1974), then the calculated osmotic water loss for a 3 mg larva is 38 nl.h⁻¹ in 100% sea water (Bradley & Phillips, 1975). In this study, the drinking rate of such larvae was 300 nl.h⁻¹. This necessitates a rectal secretion only 14% more concentrated than the external medium for osmotic balance. In fact, the average value observed for whole larvae was 11% higher (Bradley & Phillips, 1975); i.e. very close to the predicted value necessary for osmotic homeostasis.

Aedes taeniorhynchus larvae show impressive abilities to regulate haemolymph ion concentrations in external media varying from 10% to 200% sea water. In particular, the levels of Na⁺, K⁺ and Mg²⁺ in the haemolymph show no significant variations over this wide range of external concentrations. Only calcium increases significantly with increasing external concentration. Sulphate ions show a different pattern of regulation. Low haemolymph levels are found in animals reared in 10% and 100% sea water, while higher levels are found in larvae from 200% sea water. This ion is excreted largely by the Malpighian tubules (Bradley & Phillips, 1977) and this pattern of regulation reflects the fact that the Malpighian tubules can only produce a secretion isosmotic to the haemolymph. A similar pattern of sulphate regulation was observed in *A. campestris* by Maddrell & Phillips (1975).

The results cited in this paper show that the concentrations of all but one ion were higher in the rectal fluid from larvae reared in 200% sea water than in that from animals living in 100% sea water. If larvae are raised in a medium with increased levels of

either Na^+ , K^+ , Cl^- or Mg^{2+} , then the rectal secretion contains elevated levels of the ion in question (Bradley & Phillips, 1977). It is therefore clear that the secretion of rectal fluid is a major mechanism of osmoregulation in saline-water mosquito larvae. Only potassium secretion is less in larvae from 200% sea water than in those from 100% sea water. In both media, the potassium concentration of the rectal secretion is several times higher than that in the external medium. Bradley & Phillips (1975) suggest that this potassium loss from the haemolymph through secretion may be replaced by potassium uptake from the medium by the anal papillae.

Since normal rectal fluid was analysed from larvae which had been reared from hatching in 100% and 200% sea water, the high levels of K^+ in the secretion were unlikely to be the result of tissue volume adjustments, as described by Schmidt-Nielsen (1975). A more likely explanation is that Na^+ and K^+ compete for the same transport mechanism in the rectum. This explanation gains some support from the observation that rectal secretion contains very high levels of potassium (280 ± 70 mM, $n = 5$), when recta are incubated in artificial haemolymph of low Na^+ concentration.

In 200% sea water, magnesium levels in the rectal secretion were higher than in the external medium but sodium chloride and osmotic concentrations were on the average slightly lower. There are two possible explanations for this. There is a considerable amount of evidence that the anal papillae are a site of chloride and possibly sodium excretion in larvae living in sea water (Phillips & Meredith, 1969; Bradley & Phillips, 1975; Phillips & Bradley, 1976). Alternatively, blockage of the anus in whole larvae (Bradley & Phillips, 1975) could lead to a back flux of ions from the rectal lumen to the haemolymph and increased osmotic flow of water in the reverse direction due to excessive swelling of the rectum under these experimental conditions.

Some of the factors influencing the ionic and osmotic concentrations of the rectal secretion have become clear during this study. *Aedes taeniorhynchus* larvae can survive rapid transfer from 10% to 200% sea water and vice-versa at any time during their development. In 10% sea water the rectum does not secrete, but rather seems to remove fluid from the lumen. In hyperosmotic media, rectal secretion is a major means of ion excretion. Obviously, some neural or hormonal control exists which dictates whether the rectum will function as a net resorptive or secretory site. Long-term changes in rectal function may be initiated by external factors (e.g. via external osmoreceptors or salt receptors) or control mechanisms which respond to haemolymph ion levels over a time course longer than our experimental period (1.5–2 h). Although the relatively constant levels of the ions observed in haemolymph of adapted larvae (Fig. 3a, b) might seem inconsistent with internal receptors, it is reasonable to assume that abrupt changes in external salinity do in fact result in sizeable transient changes in haemolymph ion levels. Variations in total haemolymph osmotic concentration within physiological levels have no effect on the osmotic concentration of the rectal secretion. The haemolymph levels of at least two ions, sodium and chloride, have a profound influence not only on the secretion concentration of these two ions but also on the osmotic concentration of the rectal fluid and its rate of secretion.

The slopes of the curves in Fig. 8 indicate that sodium and chloride transport obey allosteric rather than Michaelis-Menten type kinetics. Whether this is a property of the transport mechanisms themselves, or is a function of changing rates of access of the ions to these mechanisms (e.g. due to variable membrane permeabilities), or is

the result of opposing transport activities in the two rectal segments, is not known at present. Regardless of the actual mechanism involved, the normal levels of sodium and chloride in the haemolymph fall on the steeply rising part of the curve for the transport kinetics (Fig. 8). Clearly, any transient rise in haemolymph sodium or chloride levels will automatically result in increased secretion of these ions by the rectum.

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