

THE SECRETION OF HYPEROSMOTIC FLUID BY THE RECTUM OF A SALINE-WATER MOSQUITO LARVA, *Aedes taeniorhynchus*

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SUMMARY

1. Fourth-instar larvae of the mosquito *A. taeniorhynchus* (Wiedemann), when living in sea water, drink at a rate of $100 \text{ nl h}^{-1} \text{ larva}^{-1}$ and maintain ionic and osmotic levels in the haemolymph at about one-third those of the external medium.

2. Hyperosmotic urine is produced in the rectum by secretion of fluid having an osmotic concentration and ionic composition similar to that of sea water, with the exception that potassium levels are elevated 18-fold in the secretion. The average rate of fluid secretion observed was $19 \text{ nl h}^{-1} \text{ larva}^{-1}$ with a maximum of $92 \text{ nl h}^{-1} \text{ larva}^{-1}$.

3. The concentration and volume of rectal secretion may be too low to account completely for osmotic balance. The possible role of anal papillae is discussed in this regard.

INTRODUCTION

The rectum of insects is generally considered to be the site of selective reabsorption from the primary excretory fluid produced by the Malpighian tubules. This activity has been shown in terrestrial (Phillips, 1964*a-c*, 1970) and freshwater insects (reviewed by Stobbart & Shaw, 1964) and is thought to be ultimately responsible for osmotic regulation in most insects.

A similar mechanism has been postulated for saline-water insects. Several euryhaline insects are known to produce strongly hyperosmotic urine (reviewed by Stobbart & Shaw, 1964; Leader, 1972). Ramsay (1950) showed that slightly hyperosmotic fluid enters the rectum of *Aedes detritus* larvae adapted to sea water but that the rectal fluid leaving the anus had a concentration similar to sea water and up to three times the osmotic concentration of the haemolymph. It has since been suggested that the fluid became concentrated in the rectum in the same manner as in terrestrial insects (Stobbart & Shaw, 1964), that is by selective reabsorption of water without proportional amounts of solute (reviewed by Phillips, 1970; Maddrell, 1971). Ramsay associated the ability of this saline-water larva to produce hyperosmotic excreta with an additional rectal segment absent in freshwater species of mosquito larvae.

However, Phillips & Meredith (1969*a*) and Meredith & Phillips (1973) found that the ultrastructural features associated with the production of hyperosmotic urine in the rectum of most terrestrial insects, namely elaborate development of the lateral membranes which are associated with most of the mitochondria of the cells, were

absent in the rectal epithelium of another saline-water mosquito larva, *Aedes campestris*. Instead, the apical membrane facing the lumen was most highly developed. Since these larvae were observed to drink saline water at high rates and thereby gain water, these authors suggested that the larvae need only rid themselves of the excess ions so ingested to achieve osmotic balance. They proposed, therefore, that hyperosmotic urine was produced by active secretion of these excess ions across the elaborately developed apical membranes of the posterior rectum.

This proposal gained support from the observation of Prusch (1971-4) that ammonium ions are secreted into the hindgut of *Sarcophaga bullata* to produce a hyperosmotic fluid. However, these larvae have no rectum and the secretion of ammonium ions might represent an isolated adaptation to an environment rich in this ion, and thus might be inapplicable to saline-water insect larvae.

The work described in this paper was undertaken to obtain direct physiological evidence for or against the suggestion of Meredith & Phillips (1973) that mosquito larvae living in saline water produce hyperosmotic urine by secretion of ions into the rectum. *Aedes taeniorhynchus*, a mosquito native to coastal sea-water swamps in North America, was used as the experimental animal because of the ease with which it can be reared year-round in the laboratory (Nayar, 1967). Nayar & Sauerman (1974) have shown that this euryhaline species is capable of maintaining haemolymph levels of sodium (173-218 mM) and chloride (48-78 mM) within narrow limits in the face of a wide range of external concentrations (0-300% sea water).

MATERIALS AND METHODS

A culture of *Aedes taeniorhynchus* was obtained from the Entomological Research Center, Vero Beach, Florida, and maintained according to the method of Nayar (1967) in sea water obtained in the Vancouver area (832 ± 8.8 mOsm). Fourth-instar larvae were starved for 1-2 days before being used in experiments. The larvae were reared at 27 °C and all experiments were carried out at that temperature.

Drinking rate was determined by measuring the initial linear increase in whole-body activity following transfer of larvae to sea water containing [¹⁴C]inulin (New England Nuclear Corp.). Larvae were removed in groups of 5 after various time intervals, weighed, placed in 1 ml of 10% KOH, macerated, incubated for 1 h at 90 °C and allowed to cool. The solution was then neutralized with 1 ml of appropriate strength H₂SO₄, and 1 ml aliquots were added to 10 ml of 'Scintiverse' (Fisher Scientific Co.) and counted in a Nuclear Chicago 'Isocap 300' liquid scintillation system.

Haemolymph was obtained from larvae blotted dry on filter paper and torn open so as to allow the haemolymph to flow on to a sheet of 'Parafilm'. No difference in estimates of haemolymph composition was observed between larvae removed directly from sea water and those first rinsed in distilled water. The haemolymph was immediately taken up in a 1 µl Drummond pipette and its volume estimated from the length of the fluid column (Kaufman & Phillips, 1973). Samples of rectal fluid were obtained by tearing open the integument of the larvae on filter paper and thus allowing the haemolymph to be absorbed. A sharp glass micropipette filled with liquid paraffin was used to puncture the rectum and suck up the contents. The resultant drop was

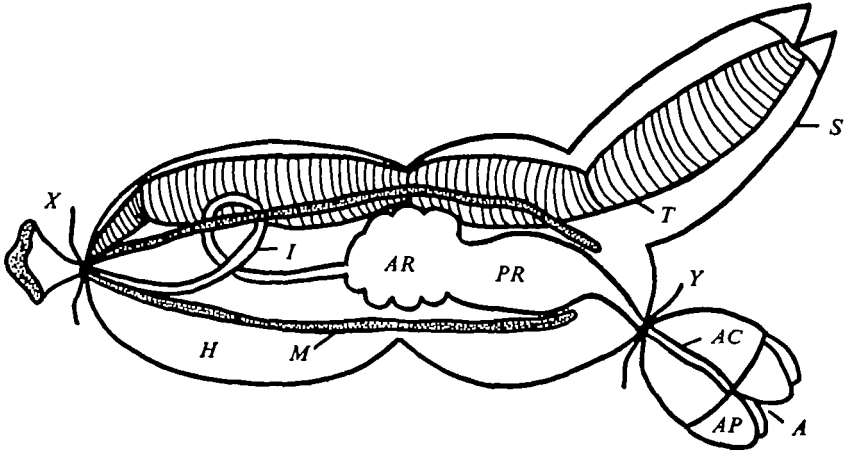


Fig. 1. A schematic diagram showing the three most posterior segments of the mosquito larva with the position of ligatures used to isolate the rectum. *A*, Anus; *AC*, anal canal; *AP*, anal papillae; *AR*, anterior rectum; *H*, haemolymph; *I*, intestine; *M*, Malpighian tubules; *PR*, posterior rectum; *S*, siphon; *T*, tracheae; *X*, anterior ligature; *Y*, posterior ligature.

immediately expelled into mineral oil in a petri dish coated with paraffin wax. The volume of the drop was estimated by measuring its diameter. This volumetric technique was calibrated by estimating radioactivity in drops of a standard [^{14}C]inulin solution.

Ionic concentrations of haemolymph and rectal fluid were measured by transferring the samples to vials containing 1 ml of the following: distilled water for sodium, 500 μM -NaCl for potassium, and 1.5% NaEDTA for magnesium. Concentrations were determined using a 'Techtron AA120' atomic absorption spectrophotometer in the transmission (Na^+ , K^+) or absorption (Mg^{2+}) mode. Chloride concentrations were measured according to the first electrometric method of Ramsay, Brown & Croghan (1955). Osmotic concentrations were determined using a nanolitre osmometer (Clifton Technical Physics Ltd) or the cryoscopic method of Ramsay (1949).

To measure changes in rectal fluid concentration with time, larvae were ligatured with fine silk thread behind the midgut, approximately between the sixth and seventh abdominal segments, thus eliminating delivery of fluid from the midgut and Malpighian tubules. A second ligature around the anal segment prevented any fluid from entering or leaving the rectum other than by passing through the wall of the rectum and a short portion of the intestine. The larva was severed in front of the anterior ligature to produce the experimental preparation shown in Fig. 1. This posterior portion of the animal was suspended from the surface of a solution by means of the respiratory siphon, thereby assuring a constant supply of air to the rectum via the tracheae. Phillips (unpublished observation) found that chloride was actively absorbed by the anal papillae of *Aedes campestris* adapted to fresh water at comparable rates in intact larvae and in posterior segments prepared in the manner outlined above. The above preparation was suspended in sea water, and haemolymph and rectal samples were taken after varying periods of time.

Initial studies indicated that the concentration of the limited volume of haemolymph in the above preparation increased drastically with time. To eliminate this problem

Table 1. *Ionic concentrations (mM) and osmolality (mOsm) of artificial and natural haemolymph (mean \pm S.E.).*

	Haemolymph	Artificial haemolymph
Na ⁺	149 \pm 3	148
K ⁺	16 \pm 1	17
Mg ²⁺	5 \pm 1	12
Cl ⁻	98 \pm 5	97
Osmolality	348 \pm 17	315

in later experiments, the same preparation was floated in an artificial haemolymph solution and the integument was torn open. Under these circumstances the rectum was bathed in a relatively large volume of artificial medium but was still connected to its normal tracheal supply. The artificial haemolymph was a modified form of that used by Berridge (1966) and contained the following in mg/100 ml of water: NaCl 175.3, Na citrate 69.7, KCl 44.8, CaCl₂.2H₂O 29.0, MgCl₂.6H₂O 264, 'yeast extract' (BBL Division, Becton, Dickinson & Co., Cockeysville, Md.) 400, Penicillin 'G' 10, Streptomycin 20, Na succinate 200, Malic acid 200, Na glutamate 235, glucose 200, maltose 200, trehalose 200, glycine 20, proline 53, glutamine 40. The concentrations of the major inorganic ions are given in Table 1.

The preparation described above was used to measure changes in the volume of rectal contents in two ways. Photographs were taken of the recta of larvae immediately after, or 2 h after, ligation. In the second method, after the anterior ligature was in place but before the posterior one had been applied, a known volume of [¹⁴C]inulin in 10% sea water was injected into the rectum by means of a micropipette inserted through the anus. After 20 sec the rectum was emptied using the same micropipette, and the volume and radioactivity of the resultant drop was measured for comparison with the known values for the fluid injected. Since the percentage of the injected radioactivity recovered from the rectum corresponds to the percentage of the rectal fluid removed, and since the volume recovered could be measured, an estimate of the fluid volume remaining in the rectum could be made. The anal segment of the larva was then ligatured to isolate the rectum, the exterior cuticle torn, and the preparation floated in artificial haemolymph for 2 h. At that time the rectum was emptied as described above, and the volume and osmotic concentration of the contents recovered were determined. The difference between the initial and final estimates of volume gave a minimum estimate of the increase in the volume of the rectal contents, i.e. total secretion.

We compared the composition of the rectal fluid secreted by ligated posterior segments with that from intact animals. Determining the composition of the rectal contents from normal, unligated larvae presented some difficulties. Larvae empty the rectum in response to almost any disturbance, making it impossible to introduce a pipette into the rectum via the anus before the rectal contents have been voided. For this reason the anus must be closed to enable the rectal fluid to accumulate and remain in the rectum until sampled. Since a ligature around the anus crushes the tissue and might seriously stress the whole animal, an alternative method, which permitted retention of normal neural and tracheal connexions to the rectum, was used. This

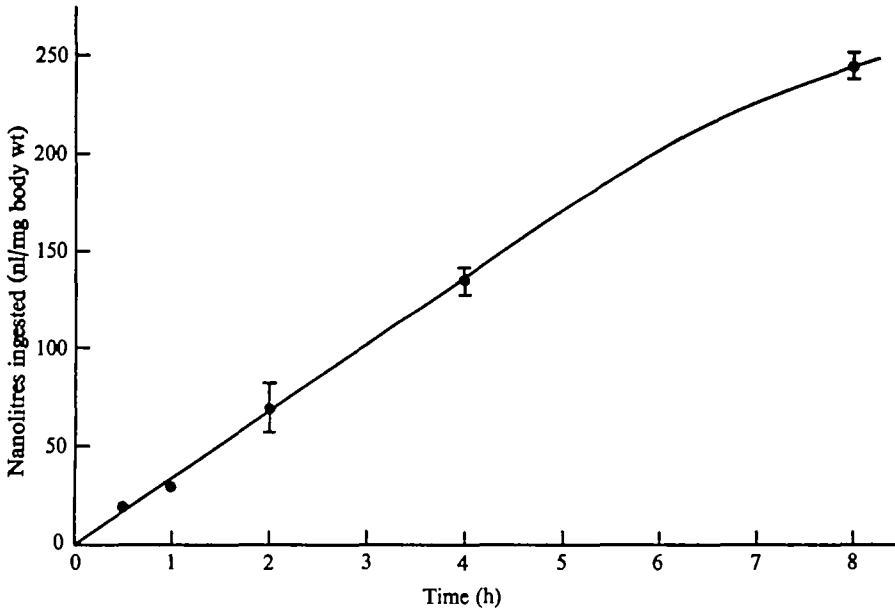


Fig. 2. The amount of external medium consumed in relation to time, as estimated from the increase in whole body radioactivity with time after placing mosquito larvae in sea water containing [^{14}C]inulin. Vertical bars denote S.E. of the means, $N = 6$ for each point.

consisted of blocking the anus of the intact larva with Eastman 910 tissue adhesive. These larvae were placed in sea water for 1 h, at which time the rectal contents were removed as described above.

RESULTS

Estimation of osmotic load

The saline-water mosquito larva *Aedes campestris* ingests large quantities of external medium (Phillips & Meredith, 1969*b*; Kiceniuk & Phillips, 1974). The unavoidable intake of ions associated with this fluid ingestion is the main source of salt entry into this species (Phillips, unpublished observation) and hence largely establishes the rate of ion secretion required to achieve ionic balance in this animal. The drinking rate of *Aedes taeniorhynchus* in sea water was therefore measured to provide a minimum estimate of ion and water turnover.

The initial accumulation of [^{14}C]inulin by whole larvae in sea water (Fig. 2) indicated that they ingested (mean \pm S.E.) $33.5 \text{ nl mg body weight}^{-1} \text{ h}^{-1}$, or $100 \pm 2 \text{ nl h}^{-1} \text{ larva}^{-1}$ for larvae having a mean weight of $3.0 \pm 0.5 \text{ mg}$. Thus, *A. taeniorhynchus* larvae drink their own body weight every 29.6 h. This is comparable to the rates for *A. campestris* in saline media reported by Kiceniuk & Phillips (1974). Since the sodium concentration of sea water is nearly 3 times that of larval haemolymph, the haemolymph content of this ion must be turned over in approximately 5.0–6.6 h, assuming haemolymph to body volume ratios of 1/2 and 2/3 respectively. Chloride must turn over even more rapidly since the blood level of this anion is 2/3 that of sodium. In spite of this rapid turnover, the larvae maintain the ionic and osmotic concentrations of their haemolymph at about one-third that of sea water (Table 1).

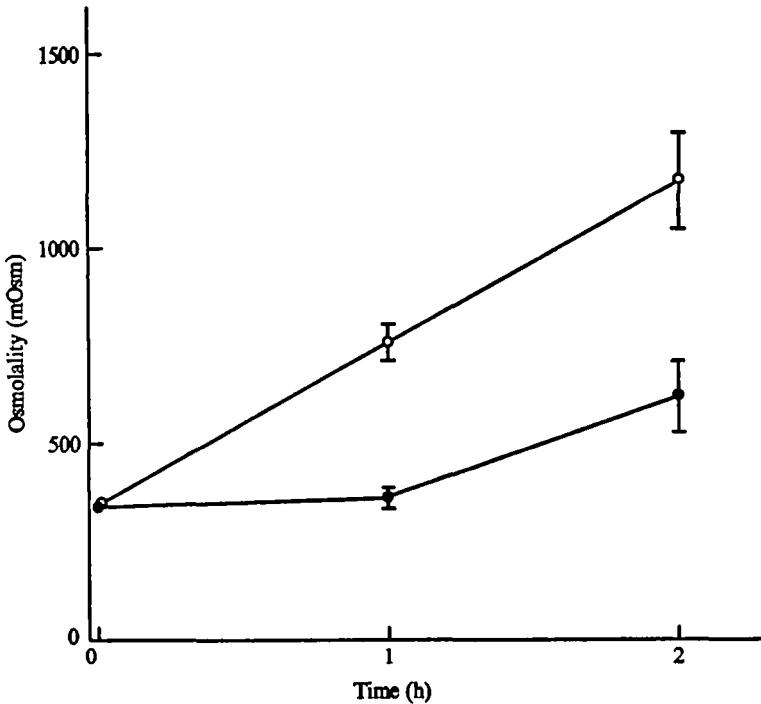


Fig. 3. The change in osmotic concentration of rectal fluid (○) and haemolymph (●) with time after the rectum was ligated as shown in Fig. 1. The preparation was placed in sea water. Vertical bars denote s.e. of the means.

Osmolality of rectal fluid

In order to demonstrate that the urine of *A. taeniorhynchus* becomes hyperosmotic to the haemolymph in the rectum, larvae were ligated as shown in Fig. 1 and placed in sea water. During the manipulations and prior to ligation of the rectum, defecation invariably occurred and the rectum refilled with a very small volume of fluid from the midgut and Malpighian tubules. Not surprisingly, therefore, samples of rectal fluid collected within a few minutes of ligation were isosmotic with the haemolymph (Fig. 3), in agreement with observations on several other insect species (reviewed by Phillips, 1970). The osmotic concentration of the rectal fluid measured after one and two hours showed a continual increase. Haemolymph osmotic concentration did not change significantly over the first hour, but increased significantly during the second. Indeed, over the second hour, the osmotic concentration difference across the rectal wall did not change appreciably. This increase in haemolymph concentration was attributed to passive net exchange of water and ions across the body wall of the larva.

In order to separate the increase in rectal fluid concentration from the influence of the increasing haemolymph concentration the same type of preparation was placed in a large volume of artificial haemolymph (Table 1) and the body wall was torn open to expose the rectum to this external medium. Under these conditions the osmotic pressure of the rectal fluid increased within 0.5 h to a value 2.4 times that of the bathing medium, but did not change significantly over the next 1.5 h (Fig. 4). The osmotic gradients produced by the two types of preparation did not differ significantly at

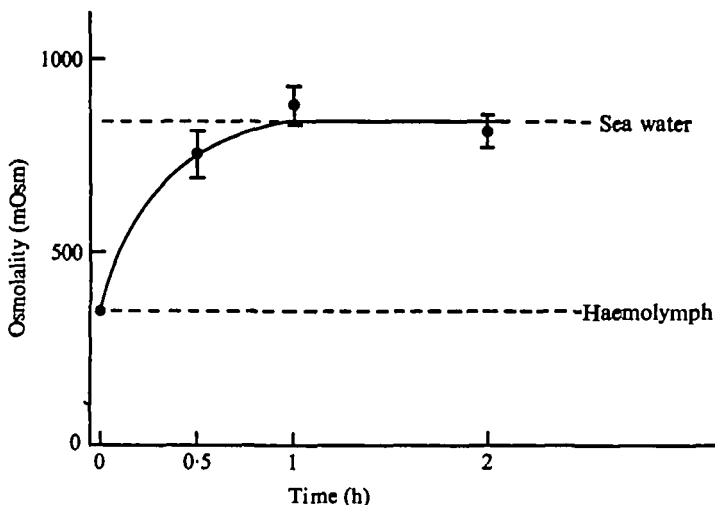


Fig. 4. The increase in osmotic concentration of rectal fluid with time. The rectum was ligated as shown in Fig. 1, the integument was torn, and the preparation was placed in artificial haemolymph. Vertical bars denote s.e. of the means.

1 h ($P > 0.1$), indicating that the rectum was able to function equally well in artificial and natural haemolymph. In summary, the rectal wall of this species is capable of developing large osmotic gradients, thereby producing urine strongly hyperosmotic to the haemolymph. The question remains whether this is achieved by selective water resorption from the small volume of isosmotic fluid initially present in the lumen, or by secretion of a hyperosmotic fluid into the rectum. These possibilities were differentiated by measuring the change in volume of the rectal contents under the experimental conditions described above.

Changes in volume of the rectal contents

Increases in rectal volume during the production of hyperosmotic urine by ligated recta were apparent from the swelling of the rectum and the larger volume of fluid which could be recovered, using micropipettes, at the end of all experiments. This considerable increase in rectal volume was documented by comparing photographs of recta taken within 5 min and at 2 h after isolation of the rectum by ligation (Fig. 5). The rectum, which is nearly empty after being ligated, swells considerably with fluid which can only have been produced by way of rectal secretion. In addition, distinct posterior and anterior segments of the rectum are clearly delineated in these photographs.

A more quantitative estimate of this volume increase was made using [^{14}C]inulin as a volume indicator (Table 2). The initial volume of rectal fluid was estimated from the percentage recovery of injected [^{14}C]inulin, and was found to be very small (2.3 ± 1.2 nl). Within 2 h of ligation, the volume of rectal fluid had increased to a mean value of 38.2 ± 7.8 nl. The largest volume increase was 65 nl after 2 h, and all recta showed an increase. In every case the volume increase was associated with a large increase in osmotic concentration of the rectal fluid, which did not differ significantly ($P > 0.2$) from previous estimates (Fig. 3). Clearly, hyperosmotic urine is formed by

Table 2. *The determination of increase in volume of rectal contents following ligation of larvae, as shown in Fig. 1*

(Recta were injected with a known amount of [^{14}C]inulin by means of a micropipette inserted through the anus before the posterior ligation was applied. The same pipette was used to retrieve as much fluid from the rectum as possible after 20 sec had elapsed (Initial). The percentage of the initial radioactivity recovered was proportional to the percent of rectal contents removed, allowing an estimate of volume of remaining rectal fluid to be made. The anal segment was then ligated and after 2 h the rectal contents were measured for volume and osmotic concentration (Final). The volume of secretion is a minimum estimate because not all the fluid secreted during the 2 h period could be recovered by micropuncture.)

Series	Initial			Final (2h)		
	Volume recovered from rectum (nl)	Radio-activity recovered (%)	Calculated volume remaining in rectum (nl)	Volume of rectal fluid removed (nl)	Minimum volume secreted by rectum (nl)	Osmolality of rectal fluid (mOsm)
1	46	103	0	65	65	834
2	17	92	1.5	34	32.5	651
3	113	103	0	45	45	1039
4	17	73	6.3	24	17.7	794
5	36	91	3.6	23	19.4	1106
Mean	45.8	92.4	2.3	38.2	35.9	884
S.E.	17.7	5.5	1.2	7.8	8.8	186

secretion of a hyperosmotic fluid into the rectal lumen rather than by selective absorption of water without solute.

Composition of rectal secretion

If the rectum is solely responsible for ionic regulation and if all ingested ions are absorbed in the midgut (suggested by Kiceniuk & Phillips, 1974; Maddrell & Phillips, 1975) then the relative ionic composition of the rectal secretion should reflect that of sea water, the total concentration of the former being higher to compensate for osmosis across the body wall. The small volume of fluid secreted by individual recta made it necessary to pool the fluid from 7-10 preparations for a single chemical determination. The average ion concentrations in rectal fluid 2 h after ligation of recta, which were bathed in artificial haemolymph, were: 285 mM- Na^+ ($n = 11$), 158 mM- K^+ ($n = 5$), 25 mM- Mg^{2+} ($n = 5$), 425 mM- Cl^- ($n = 5$), 818 mOsm ($n = 18$) (Fig. 6).

Rectal secretion would seem inadequate to explain completely the maintenance of ionic balance. The fluid is not more concentrated than sea water and the potassium excretion is many times higher than can be accounted for by drinking. The larvae were starved prior to the experimental period, therefore the excess potassium might have been due to autolysis of tissue but probably was not the result of ions present in the food. We felt that the unexpected Na:K ratio may have been due to decreased activity of the preparation, or lack of hormonal or neural stimulation of secretion. With these possibilities in mind, rectal fluid was collected from intact, whole larvae reared in sea water, 1 h after the anus had been blocked with tissue adhesive to allow the accumulation of sufficient rectal fluid for analysis. The mean concentrations of all

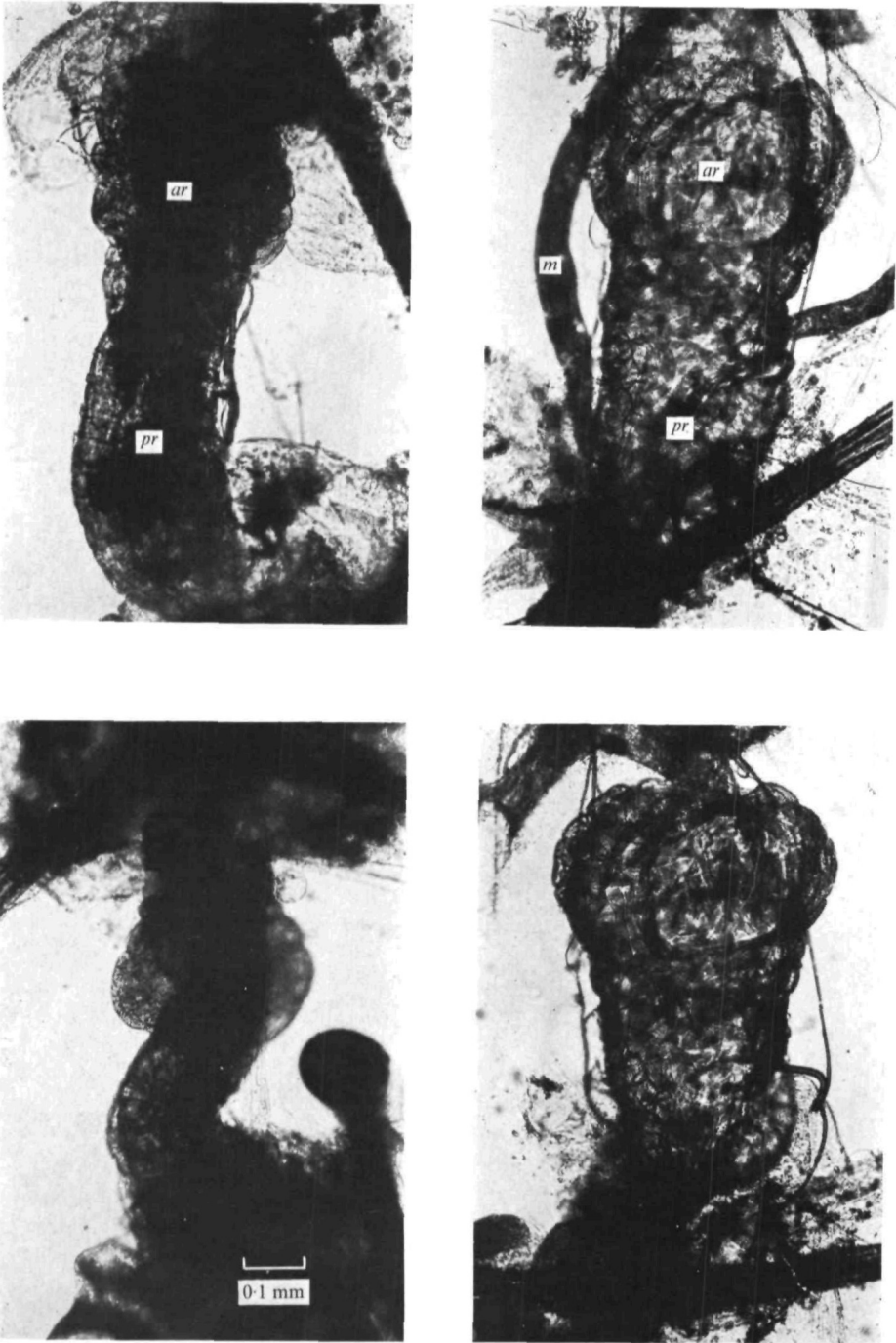


Fig. 5. Photographs of recta 5 min (left) and 2 h (right) after ligation. At 2 h the recta are swollen with fluid secreted into the lumen by the rectum. Exposing the rectum to view destroys the preparation, therefore different recta are shown in each photograph at the same magnification. *ar*, anterior rectum; *m*, Malpighian tubules; *pr*, posterior rectum.

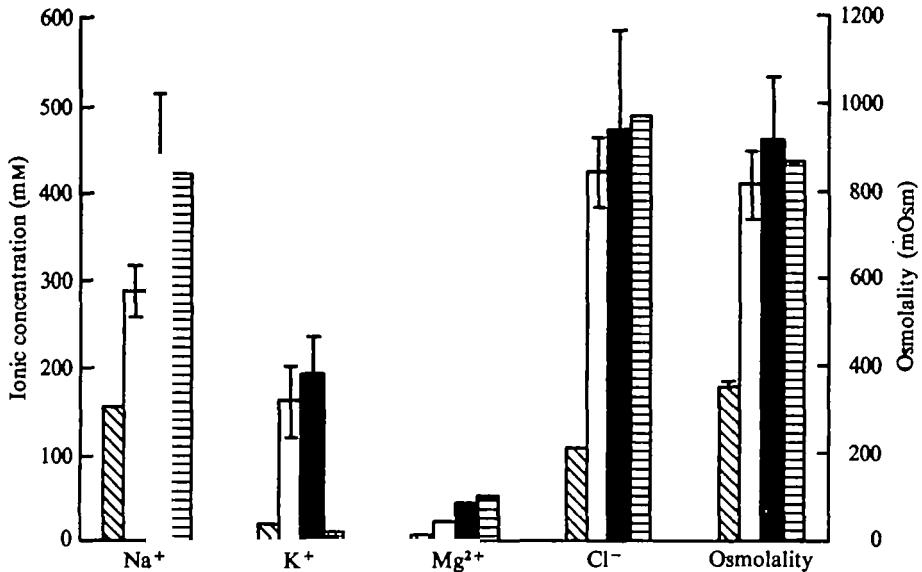


Fig. 6. A comparison of rectal fluid with haemolymph and sea water. The ionic and osmotic concentrations of natural haemolymph (▨), secretion from ligated rectal preparations (Fig. 1) with a torn cuticle, in artificial haemolymph (□), rectal fluid from intact larvae with the anus plugged and placed in sea water (■) and Vancouver sea water (▤). Vertical bars denote the S.E. of the means.

ions in this rectal fluid were slightly higher (Fig. 6), but not significantly so ($P < 0.05$ for all ions measured), and the ion concentration ratios remained unchanged: 435 mM- Na^+ ($n = 5$), 192 mM- K^+ ($n = 5$), 36 mM- Mg^{2+} ($n = 5$), 468 mM- Cl^- ($n = 6$), 920 mOsm ($n = 6$). Occasionally during the sampling of the rectal contents the rectum was punctured in such a way that no fluid could be seen to leak out and the rectum was observed to empty completely into the pipette. This occurred three times during the sampling of the recta of the larvae with the anus blocked, and yielded volumes of 21, 21 and 92 nl.

In whole larvae with the anus blocked, recta are exposed to normal haemolymph and natural hormones, have intact innervations, and receive fluid from the midgut. The fluid derived under these conditions is very similar to that secreted by the isolated, ligated rectal preparation. Both preparations demonstrate that the larval rectum of *Aedes taeniorhynchus* in sea water secretes a fluid which, though 16–19 times higher in potassium than sea water, is otherwise nearly identical to sea water in its ionic and osmotic characteristics (Fig. 6).

DISCUSSION

The results clearly confirm the hypothesis (Phillips & Meredith, 1969*a*) that saline-water mosquito larvae produce hyperosmotic urine by ion secretion into the lumen rather than by water reabsorption, as occurs in terrestrial insects. This appears to be the first example of an insect rectum which functions in this way, although Prusch (1971–4) has shown similar activity in the hind gut of another dipteran larva, *Sarcophaga bullata*. Since the anterior and posterior rectum were not isolated from one another in the present experiments it is not yet possible to assign the secretory activity

to a specific segment of the rectum, although Meredith & Phillips (1973) have presented ultrastructural evidence which implicates the posterior rectum in this activity. They have suggested that the anterior rectum is involved in selective reabsorption when larvae are in hyposmotic environments.

The observed concentrations of ions in the secretion from ligated recta were not significantly different from those of sea water, except that the potassium concentration was much higher in the secretion. Using cryoscopic coefficients for pure monovalent salt solutions, the mean levels of K^+ , Na^+ and Cl^- will account for 98% of the observed osmotic pressure of the rectal fluid (818 ± 37 mOsm, $n = 18$). However, when the magnesium concentration is included, there is an excess of cations over anions totalling 68 mEquiv. l^{-1} , suggesting that other anions prevalent in sea water (e.g. SO_4^{2-}) may be present (suggested by Maddrell & Phillips, 1975). If, to simplify calculations, one assumes that the entire anion deficit is sulphate ions associated with magnesium and sodium ions, the calculated osmotic concentration of the rectal fluid using cryoscopic coefficients is 856 mOsm l^{-1} , which is within the standard error of the observed osmotic pressure. When the same calculations are made using the ionic concentrations in the rectal fluid from intact larvae with blocked anuses, the anion deficit is 231 mEquiv. l^{-1} and the total calculated osmotic pressure is 1144 mOsm l^{-1} , which exceeds the standard error of the observed osmotic concentration (i.e. equal to 137% sea water). It is therefore necessary to postulate some ion binding to polyvalent anions, e.g. macromolecules of faecal material, as reported for magnesium in *Aedes campestris* (Kiceniuk & Phillips, 1974). In summary, the major components of the rectal secretion have been identified with the exception of a small anion deficit. The secretion of all of these ions (Na^+ , K^+ , Mg^{2+} , Cl^-) occurs against large concentration differences of 2- to 18-fold. Electrical potentials across the rectal wall must be measured to determine which of these transport processes are active.

Aedes taeniorhynchus larvae, in 100% sea water, drink and probably assimilate 100 nl of external fluid per larva per hour. Some of this water is presumably lost by osmosis across the body wall. Nicholson & Leader (1974) have estimated this loss for another saline-water mosquito (*Opifex fuscus*) of similar size, ligatured at the neck and anus, at $0.0026 \mu l mm^{-2} h^{-1}$. Assuming a similar osmotic permeability for *Aedes taeniorhynchus* and an approximation of body surface area of $14.6 mm^2$, the calculated net loss of water across the body wall is 38 nl h^{-1} . Subtracting this value from the drinking rate gives a value of 62 nl h^{-1} for water loss through the excretory system. The largest volume of rectal secretion observed was 96 nl h^{-1} but the mean was 19 nl h^{-1} . While secretion of Malpighian tubule fluid might account for the balance of excreted fluid, such fluid is isosmotic to the haemolymph and would not contribute to osmotic regulation.

It would be premature to conclude that rectal secretion is inadequate to account for osmotic regulation, since the calculations described above for larvae with the anus blocked suggest that the effective concentration of the secretion can reach 137% sea water. In order to collect enough fluid for analysis, the anus was blocked in all experiments. The accumulation of fluid in the rectum might lead to underestimation of both ion concentrations and volume of the rectal secretion for various reasons: (1) stretching of the rectal wall might lead either directly, or indirectly through stretch receptors, to increased passive permeability of the rectal wall; (2) the build-up of hydrostatic

pressure in the rectum might oppose further fluid secretion; (3) reabsorption of ions or water might occur in the anterior rectum when distension leads to direct continuity between the two rectal segments.

Ramsay (1950) observed that the rectal fluid of *A. detritus* was also only isosmotic or slightly hyposmotic to the seawater medium. *A. taeniorhynchus* can survive in 300% sea water. If the rectal fluid is isosmotic to the external environment in this situation, then the rectum is clearly capable of creating larger gradients than have been observed. If Ramsay's observations with intact larvae and the present values for larvae with the anus ligated indicate the true upper limit for hyperosmoticity of rectal secretion, then other sites of salt secretion, such as the anal papillae, must be invoked.

Phillips & Meredith (1969*b*) have presented preliminary evidence that the anal papillae of *A. campestris* larvae living in hyperosmotic media might actively secrete chloride to the external medium. If sodium is also secreted by anal papillae in exchange for an inward movement of potassium (e.g. as in the gills of salt water teleosts; Maetz, 1971), this might balance the loss of K^+ ions by rectal secretion (Fig. 6). Leadem (unpublished observation) in our laboratory has obtained some preliminary evidence for such a mechanism.

A. campestris larvae that live in waters of high $NaHCO_3$ or high $MgSO_4$ content are also known to produce hyperosmotic urine, although fluid leaving their Malpighian tubules is isosmotic to the haemolymph (Phillips & Meredith, 1969*a*; Kiceniuk & Phillips, 1974; Phillips & Maddrell, 1974; Maddrell & Phillips, 1975). Presumably the dominant ions in the rectal secretion of this species, which can also live in sea water, can be varied as dictated by the environment in which the larvae develop. Otherwise, it is necessary to postulate distinct physiological races of this species in saline waters having different dominant ions.

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