J. exp. Biol. (1982), 101, 171–186
With 9 figures
Printed in Great Britain

ACTIVE HCO_3^- SECRETION IN THE RECTAL SALT GLAND OF A MOSQUITO LARVA INHABITING $NaHCO_3$–CO_3 LAKES

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(*Received* 24 *February* 1982 – *Accepted* 19 *April* 1982)

SUMMARY

1. *Aedes dorsalis* larvae are one of the few organisms capable of inhabiting hypersaline $NaHCO_3$-CO_3 lakes. Under laboratory conditions larvae can survive and develop normally in saline media with pH values of 10·5, HCO_3^- concentrations of 250 mM, or CO_3^{2-} concentrations of 100 mM.

2. Despite ingestion of alkaline media at a rate equal to 130% of larval body wt/day, these insects regulated haemolymph pH (7·55–7·70) and HCO_3^- concentration (8·0–18·5 mM) within narrow limits.

3. Analysis of fluid obtained by micropuncture or microcannulation indicated that rectal secretion is an important mechanism of pH and HCO_3^- regulation.

4. The lumen-to-haemocoel HCO_3^- and CO_3^{2-} gradients generated by isolated, microcannulated recta were 21 : 1 and 241 : 1, respectively.

5. Transepithelial potential in cannulated recta was −14·2 to −25·3 mV (lumen negative), demonstrating that net HCO_3^- secretion occurs against a large electrochemical gradient.

INTRODUCTION

Hypersaline lakes found in arid regions of the world are among the most extreme natural aquatic environments known. The ionic composition of these lakes varies widely and can range from saturated brines with ion ratios similar to sea water to athalassohaline $MgSO_4$, Na_2SO_4 and $NaHCO_3$–CO_3 lakes (Topping & Scudder, 1977). The latter group of lakes often have pH values exceeding 10 and HCO_3^- and CO_3^{2-} concentrations as high as 1–2·4 M (Blinn, 1969; Topping & Scudder, 1977). Only a few organisms are capable of surviving under these severe alkaline conditions, the most prominent of which are insects, crustaceans, halophilic bacteria and algae (Blinn, 1969; Scudder, 1969).

Little is known about the physiological adaptations which allow organisms to survive in these alkaline environments with the exception of recent work on the fish *Tilapia grahami* (Reite, Maloiy & Aasehaug, 1974; Johansen, Maloiy & Lykkeboe, 1975, Lykkeboe & Johansen, 1975; Maetz & DeRenzis, 1978; Maloiy *et al.* 1978). Previous studies in our laboratory (reviewed by Phillips & Bradley, 1977; Phillips, Bradley & Maddrell, 1978) have described the mechanisms responsible for regulating

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haemolymph Na^+, K^+, Cl^-, Mg^{2+}, SO_4^{2-} and osmotic concentration in mosquito larvae inhabiting salt lakes. However, the problems of H^+ and HCO_3^- regulation in saltwater mosquito larvae, or indeed any other insect, have not been examined. The few previous studies of insect acid-base physiology were mainly concerned with determining haemolymph pH, buffering capacity and CO_2 content under a single physiological condition (Craig & Clark, 1938; Hastings & Pepper, 1943; Levenbook, 1950*a*,*b*).

We have begun to investigate the mechanisms of insect pH and HCO_3^- regulation using *Aedes dorsalis* larvae which are normally found in alkaline salt lakes of western North America. In the present paper we determined the extremes of alkalinity under which larvae can develop normally and the extent of haemolymph pH and HCO_3^- regulation in these environments. We also show that the rectum is an important site of HCO_3^- excretion, as originally suggested by Bradley & Phillips (1977*a*), and we demonstrate that rectal HCO_3^- secretion occurs by an energy-requiring mechanism.

MATERIALS AND METHODS

Aedes dorsalis eggs were obtained from the Department of Biomedical and Environmental Health Sciences, University of California, Berkeley. Eggs were hatched as described previously (Bradley, 1976) and larvae were reared at 25 °C in an alkaline rearing medium (Table 1) resembling natural lake waters (Topping & Scudder, 1977). Larvae were fed daily with dry fish food (Tetramin Staple Food) and the rearing media were aerated gently and changed periodically to prevent stagnation. Maintenance of the colony was otherwise similar to that described by Bradley (1976).

Survival studies were conducted by abruptly transferring third and fourth instar larvae 4 days after hatching to media with high HCO_3^- and CO_3^{2-}/Cl_3^- ratios. Mortality and development of larvae were observed for four days after transfer. Based on these results three experimental media were chosen for use in the remainder of this study and are referred to as Rearing, 250 mM-HCO_3^-, and 100 mM-CO_3^{2-} medium (Table 1). All experiments were conducted on fourth-instar larvae acclimated to these artificial lake waters for at least 3 days.

Larval drinking rate was estimated at 25 °C using the [^{14}C]inulin uptake method of Bradley & Phillips (1975). Samples of larval haemolymph were collected and Na^+, K^+, Mg^{2+}, Ca^{2+}, Cl^- and osmotic concentrations were measured as described by Bradley & Phillips (1975, 1977*b*).

Physiological salines (Table 2) used to bathe isolated recta were based on measured ionic and osmotic concentrations of natural haemolymph. Free amino acid concentrations in larval haemolymph were determined using a Beckman 118C automatic amino acid analyzer and the four major haemolymph amino acids were included in the salines at physiological concentrations. The large anion deficit observed in larval haemolymph was simulated by including sodium cyclamate in the salines. All salines were gassed with 98% O_2–2% CO_2 to give a CO_2 concentration similar to that measured in haemolymph (see Results).

Capillary pH electrodes. To minimize CO_2 loss from samples of haemolymph and rectal secretion during pH measurements, ultramicro internal capillary pH electrodes

Table 1. *Composition of artificial lake waters in which larvae were reared or acclimated*

Constituent (mM)	Rearing medium	250 mM-HCO_3^- medium	100 mM-CO_3^{2-} medium
Na^+	361·50	361·50	398·00
K^+	2·50	2·50	2·50
Ca^{2+}	0·03	0·03	0·03
Mg^{2+}	0·50	0·50	0·50
Cl^-	246·06	39·56	97·06
SO_4^{2-}	10·00	10·00	10·00
HCO_3^-	43·50	250·00	87·00
CO_3^{2-}	29·00	29·00	100·00
pH	9·50	8·85	9·75
m-osmol	638·00	597·00	576·00

Table 2. *Composition of haemolymph* (mean ± S.E., n = 6–10) *and physiological salines for larvae acclimated to different artificial lake waters*

(All three salines also contained the following (in mM): proline 20, alanine 5, glycine 3, glutamine 4, succinate 7·4, citrate 2·5, glucose 10.)

Constituent (mM)	Larval acclimation medium*					
	Rearing medium		250 mM-HCO_3^- medium		100 mM-CO_3^{2-} medium	
	Haemolymph	Saline	Haemolymph	Saline	Haemolymph	Saline
Na^+	163 ± 2·4	164·5	191 ± 7·2	189·5	182 ± 2·0	180
K^+	9·5 ± 0·7	9	9·4 ± 0·9	9	11·7 ± 0·5	9
Mg^{2+}	3·8 ± 0·2	4	4·8 ± 0·4	4	5·5 ± 0·4	4
Ca^{2+}	8·7 ± 0·5	4	12·7 ± 0·8	4	14·2 ± 0·9	4
SO_4^{2-}	—	5	—	5	—	5
Cl^-	56 ± 1·8	56	39·1 ± 2·3	39	49 ± 1·6	49
Cyclamate	—	89	—	125	—	108·5
HCO_3^-	8·1 ± 0·5	12·5	18·5 ± 0·6	18·5	12·1 ± 0·3	15·5
pH	7·55 ± 0·03	7·51	7·70 ± 0·02	7·70	7·70 ± 0·02	7·60
m-osmol	359 ± 1·5	380	432 ± 3	427	401 ± 12	420

* See Table 1 for composition of artificial lake waters.

were used (Fig. 1). These electrodes were modified from Khuri, Agulian & Harik (1968) and required a total sample volume of 10–30 nl.

Electrodes were calibrated at 25 °C in four buffers (pH 6·4–9·0) of constant ionic strength similar to that of natural haemolymph or rectal secretion. The pH of these buffers was determined before each experiment using a Radiometer Model 27 pH meter and Radiometer pH electrode calibrated with Radiometer buffers at 25 °C. Only electrodes with full response times of less than 60 s and a calibration curve slope of 55–61 mV/pH unit were used. The mean (± S.E.) calibration curve slope and correlation coefficient for all electrodes used throughout this study were 58·32 ± 0·19 and 1·00 ± 0·00 (n = 34), respectively.

One difficulty experienced with these electrodes was a small downward drift of the voltage recorded in any calibration buffer after pH measurements on biological fluids. The cause of this drift was uncertain and could not be corrected by rinsing with chromic acid or by repeatedly 'exercising' the electrode in two buffers. To monitor

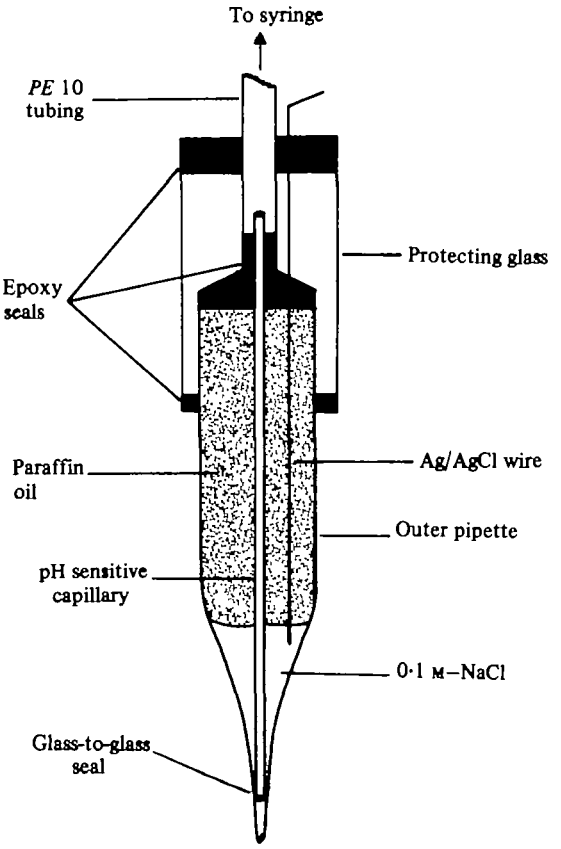

Fig. 1. Ultramicro internal capillary pH electrode.

this problem, electrodes were recalibrated in two buffers following each pH measurement. Subsequent pH measurements on haemolymph or rectal secretion samples were then corrected for this drift using the new calibration curve. Electrodes showing deviations in calibration slope of more than ± 2 mV/pH unit were discarded.

Haemolymph pH measurements. Regulation of haemolymph pH was examined by transferring third and fourth instar larvae to high HCO_3^- and CO_3^{2-} media 4 days after hatching. Haemolymph samples were collected by briefly rinsing larvae in distilled water, blotting them dry and placing them under heavy paraffin oil (MCB Reagents; Saybolt viscosity 340–355). The cuticle was then torn with forceps and the drop of exuded haemolymph collected immediately in a mercury filled micropipette. This sample was transferred rapidly to a heavy paraffin oil bath (25 °C) and a capillary pH and 3 M-KCl microelectrode were lowered into the haemolymph drop visualized under a dissecting microscope. The potential difference between the electrodes was measured with a Keithley Model 602 electrometer and Model 6013 pH electrode adaptor and the voltage recorded on a Fisher Series 5000 Recordall.

Haemolymph HCO_3^- measurements. Haemolymph HCO_3^- was measured using the ultramicro Astrup method of Karlmark & Sohtell (1973). To equilibrate paraffin oil

baths with gases of different CO_2 content, we used high gas flow rates (2–3 l.min^{-1}) and an equilibration time of at least 1 h before samples were added. Karlmark & Sohtell (1973) found that a minimum gas flow rate of 450 ml.min^{-1} and a 30 min equilibration time were necessary for complete gas to oil equilibration. We confirmed this using an Orion P_{CO_2} electrode, but chose the higher flow rates to assure complete equilibration under all conditions. In addition, we found that complete gas to oil equilibration at 25 °C was only obtained when light paraffin oil (MCB Reagents; Saybolt viscosity 90) was used.

Samples of haemolymph were collected for determination of HCO_3^-, as described above, and allowed to equilibrate with CO_2 under oil for 20–30 min before pH measurements were made with capillary electrodes at 25 °C. Gas flow was turned off briefly (less than 1 min) during sample loading and pH measurements. Different haemolymph samples were equilibrated with four gases of varying CO_2 content (0·5–10% CO_2, balance N_2; Canadian Liquid Air Company, certified analysis). Resulting pH versus log P_{CO_2} titration curves were plotted using the least squares method of linear regression.

Rectal micropuncture studies. The micropuncture preparation used in this study was modified considerably from that described by Bradley & Phillips (1975). Briefly, the rectum was isolated by ligating the larvae just anterior to the seventh abdominal segment and also at the terminal anal segment. The portion of the larva anterior to the first ligature, the cuticle over the rectum, and the tracheal connections were then dissected away. Using the terminal ligature, the rectum was suspended in 10–15 ml of the appropriate physiological saline maintained at 25 °C and gassed with 98% O_2–2% CO_2. After 90 min the rectum had swollen noticeably with secretion and was removed gently from the bath, touched lightly to bibulous paper to remove adhering external fluid, and placed under paraffin oil. Ten to 40 nl of rectal secretion were collected by puncturing recta with sharpened and bevelled (30°, tip diameter of 10–15 μ) acid-washed micropipettes filled with Sudan black-stained paraffin oil.

Cannulated in vitro *rectal preparation.* To examine rectal HCO_3^- secretion in greater detail, a microcannulated preparation was developed which permitted rapid and complete collection of secreted rectal fluid. Diagrams of the bathing chamber made from Sylgard 184 resin (Dow Corning) and the cannulated rectum are shown in Fig. 2(*a*) and (*b*). All cannulation experiments were conducted at room temperature (21–23 °C) using larvae acclimated to 250 mM-HCO_3^- medium.

Microcannulae were pulled on a small flame from glass tubing to tip diameters of 35–50 μm. The tips of the pipettes were broken to the appropriate length with forceps and the broken end fire-polished with a microforge. Pipettes were siliconized with Dow Corning 1107 fluid, filled with Sudan black-stained paraffin oil and mounted on a Narishige micromanipulator using a micropipette holder (Leitz, Wetzlar, Germany) attached to a 50 ml syringe.

Recta were isolated by placing a larva in the bathing chamber and tying a fine ligature around the terminal anal segment. The bathing chamber was filled and perfused continuously with physiological saline and the rectum, ileum and a portion of the midgut were dissected free from the larva. Approximately 20–30 nl of saline were then drawn up into a microcannula and the pipette inserted down the midgut

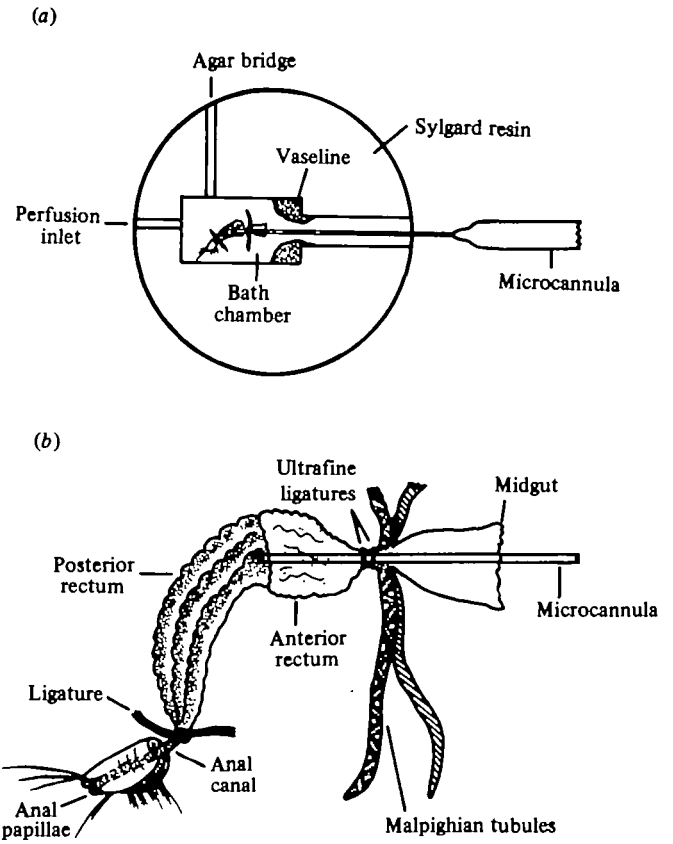

Fig. 2. Microcannulated rectal preparation. (*a*) Sylgard resin bathing chamber. (*b*) Cannulated rectal salt gland.

and into the rectum. The saline was injected into the rectum and then withdrawn completely and expelled into the bath. Having assured that the rectum was completely empty the microcannula was reinserted and tied into place using two ultrafine ligatures (diameter *ca.* 10 μm; see Fig. 2*b*).

During the course of these experiments the bathing chamber was perfused with saline gassed with 98% O_2–2% CO_2 and recta were allowed to swell with secretion for 90 min before fluid was drawn into the cannula using the attached syringe. At the end of the experiments the bath was drained, the rectum removed from the pipette and the sample of secretion prepared for analysis.

Analysis of rectal secretions. Rectal secretion volume was determined by measuring drop diameters under paraffin oil as described by Bradley & Phillips (1975). Osmotic concentrations of rectal secretions were measured with a Clifton nanoliter osmometer. Concentrations of Na^+, K^+, Mg^{2+}, Ca^{2+}, Cl^-, total phosphorous and total sulphur were measured in each sample of secretion by electron microprobe analysis using a Cameca model MBX microprobe and methods described previously (Morel & Roinel, 1969; Roinel, 1975). Because of the high ion concentrations in rectal fluid, samples

Table 3. *Values of pK_1', pK_2' and S used for calculation of CO_2, HCO_3^- and CO_3^{2-} concentrations*

Constant	Larval acclimation medium*		
	Rearing medium	250 mM HCO_3^- medium	100 mM-CO_3^{2-} medium
Haemolymph (pK_1')	6·12	6·11	6·11
Haemolymph (S)	0·0340	0·0338	0·0339
Secretion (pK_1')†	6·04	0·64	6·04
Secretion (S)†	0·0329	0·0329	0·0329
Secretion (pK_1')‡	—	6·00	—
Secretion (pK_2')‡	—	9·64	—

* See Table 1 for composition of acclimation media.
† Rectal secretion collected by micropuncture.
‡ Rectal secretion collected by microcannulation.

were diluted two to three times with distilled water before preparation for microprobe analysis. Chloride concentrations were also measured by the electrometric titration procedure of Ramsay, Brown & Croghan (1955) on rectal secretions pooled from four to five animals.

Bicarbonate concentrations in rectal secretions collected by micropuncture were estimated by measuring the pH of samples equilibrated under paraffin oil at 25 °C with 98% O_2–2% CO_2 according to the methods described above. In later experiments, total CO_2 and pH were measured in rectal secretions collected by microcannulation. Secretion pH was measured at 25 °C immediately after collection while total CO_2 concentrations were determined by microcalorimetry (Picapnotherm; Microanalytic Instrumentation, Bethesda, Maryland) as described by Vurek, Warnock & Corsey (1975). Aliquots of rectal secretion were transferred to the Picapnotherm by constant volume nanolitre pipettes (2–3 nl) similar to those of Prager, Bowman & Vurek (1965).

Transepithelial potential. Rectal transepithelial potential (TEP) was measured using a microcannula (see above) with 3 M-KCl–Agar in the tip and the remainder of the pipette filled with 3 M-KCl solution. The microcannula and bath chamber made contact via salt bridges to calomel electrodes and TEP was measured using a Keithley Model 602 electrometer. TEP was recorded continuously for 90 min on a Fisher Series 5000 Recordall with subsequent corrections for junction and asymmetry potentials.

Calculations. Concentrations of CO_2, HCO_3^- and CO_3^{2-} were calculated from the Henderson–Hasselbalch equation after correcting pK_1', pK_2', and CO_2 solubility coefficients (S) for ionic strength using the equations of Hastings & Sendroy (1925) and McGee & Hastings (1948). Values of pK_2', pK_1' and S used throughout this study are shown in Table 3.

RESULTS

In preliminary studies we examined the ability of *A. dorsalis* larvae to survive in saline media buffered with low concentrations of HCO_3^- and CO_3^{2-} at high pH values. Larval survival and development were normal in media with pH values up to 10·5. At

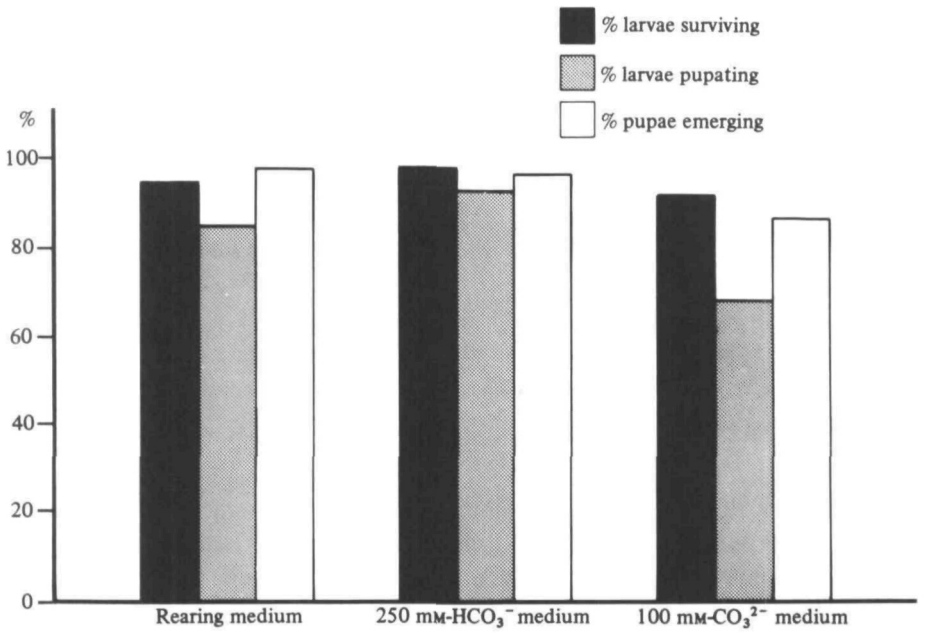

Fig. 3. Survival and development of fourth instar larvae in alkaline environments. Refer to Table 1 for media composition.

pH 11·0 survival was still normal but pupation and pupal-to-adult eclosion were greatly reduced. Since the pH of alkaline lakes rarely if ever rises above 10·5 due to the inherent nature of their buffering systems, factors other than pH *per se* must limit the distribution of larvae in natural alkaline waters. Further survival studies were therefore conducted by replacing NaCl in the Rearing medium with different concentrations of $NaHCO_3$ and/or Na_2CO_3. Fig. 3 demonstrates that survival and development are normal in media containing 250 mM-HCO_3^- or 100 mM-CO_3^{2-}. Slightly higher concentrations of these two ions (300 mM-HCO_3^- and 150 mM-CO_3^{2-}) resulted in greatly reduced survival (32–46% after 4 days) and failure of any of the larvae to develop past the pupal stage.

Ionic and osmotic concentrations of haemolymph from animals acclimated to high HCO_3^- and CO_3^{2-} environments are shown in Table 2. Haemolymph osmotic concentrations increased slightly but significantly ($0{\cdot}005 < P < 0{\cdot}01$) from 359 to 401–432 m-osmol following transfer of larvae from the Rearing medium to either 250 mM-HCO_3^- or 100 mM-CO_3^{2-} medium. In addition, haemolymph Na^+ concentration increased significantly ($P < 0{\cdot}001$) from 163 to 181–190 mM, while Ca^{2+} increased from 8·5 to 12·5–14·0 mM ($P < 0{\cdot}001$). Haemolymph Cl^- concentration decreased slightly but significantly ($0{\cdot}001 < P < 0{\cdot}005$) as external Cl^- was replaced by HCO_3^- and CO_3^{2-}.

Fluid ingestion rate was measured to estimate the load imposed on pH and HCO_3^- regulatory mechanisms in the larvae and to determine whether larvae could reduce this load by reducing drinking rate. Fig. 4 shows that drinking rate (53–56 $nl.mg^{-1}.h^{-1}$) was independent of external HCO_3^- and CO_3^{2-} levels.

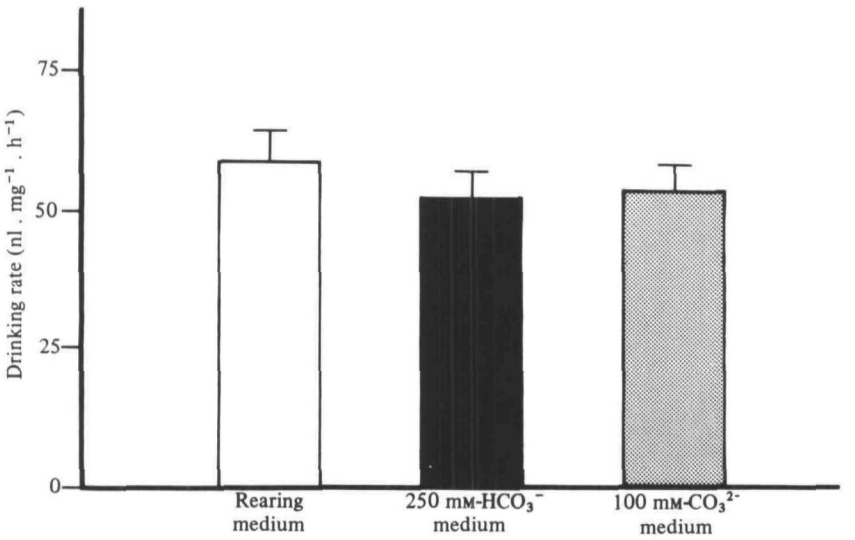

Fig. 4. Drinking rates estimated by [^{14}C]inulin ingestion for fourth instar larvae acclimated to alkaline environments (means ± S.E., n = 10–12). Refer to Table 1 for media composition.

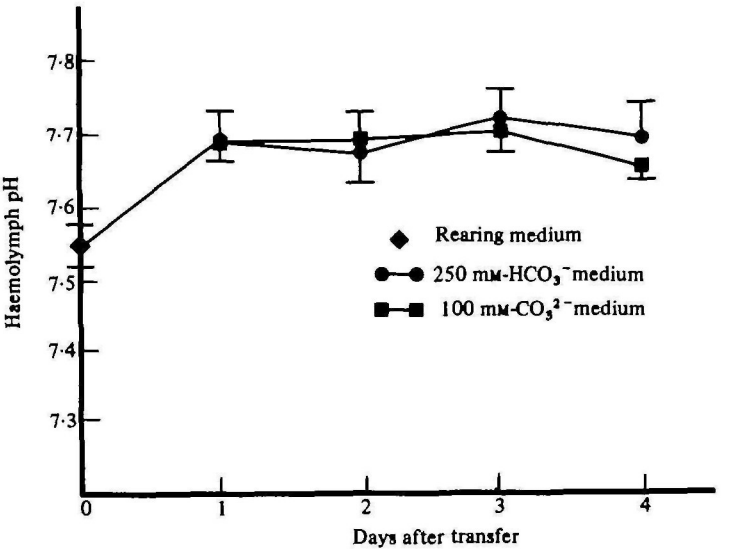

Fig. 5. Haemolymph pH regulation in fourth instar larvae during acclimation to high HCO_3^- and CO_3^{2-} media. Animals were transferred from the Rearing medium to 250 mM-HCO_3^- or 100 mM-CO_3^{2-} media at zero time (means ± S.E., n = 6–9). Refer to Table 1 for media composition.

To determine how well larvae regulate haemolymph acid-base status when faced with such high ingestion rates, we measured haemolymph pH for 4 consecutive days following transfer of larvae from the Rearing medium to either high HCO_3^- or high CO_3^{2-} environments (Fig. 5). Haemolymph pH increased only slightly from 7·55 to 7·70 during the first day and remained constant thereafter.

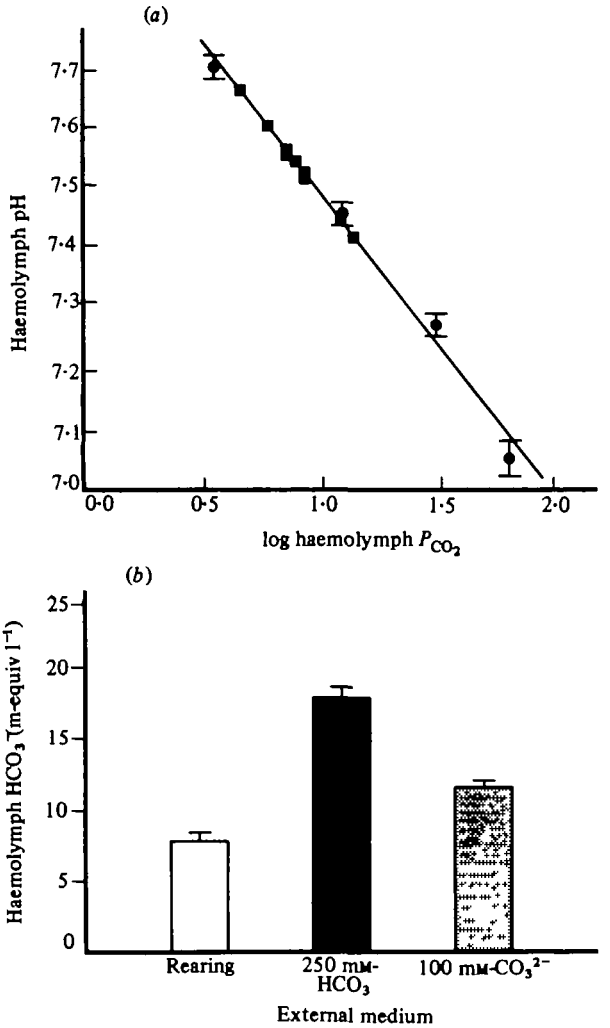

Fig. 6. Haemolymph HCO_3^- concentration. (*a*) pH *v.* log P_{CO_2} titration curve for haemolymph from animals acclimated to the Rearing medium. Solid circles are means ± S.E. (n = 8–9) of haemolymph samples titrated with one of four different CO_2–N_2 gas mixtures. The line is the linear regression calculated for these points ($y = 0{\cdot}51x + 8{\cdot}03$; $r = -0{\cdot}994$). Solid squares are pH values of native haemolymph (see Fig. 5). The values were fitted to the line using the calculated regression equation. (*b*) Concentration of haemolymph HCO_3^- in animals acclimated to alkaline media. P_{CO_2} values were obtained from pH *v.* log P_{CO_2} titration curves and HCO_3^- concentration calculated using the Henderson–Hasselbalch equation (means ± S.E., n = 9–29). Refer to Table 1 for media composition.

Haemolymph HCO_3^- concentration was determined in larvae acclimated to all three artificial waters (Fig. 6). A typical titration curve of haemolymph pH versus log haemolymph P_{CO_2} is shown in Fig. 6(*a*). Titration curves for the three groups of larvae were linear with correlation coefficients varying between −0·98 and −0·99. Haemolymph P_{CO_2} was determined using these titration curves and the measured haemolymph pH values in Fig. 5. P_{CO_2} values were (in mm Hg, mean ± S.E., n = 9–29)

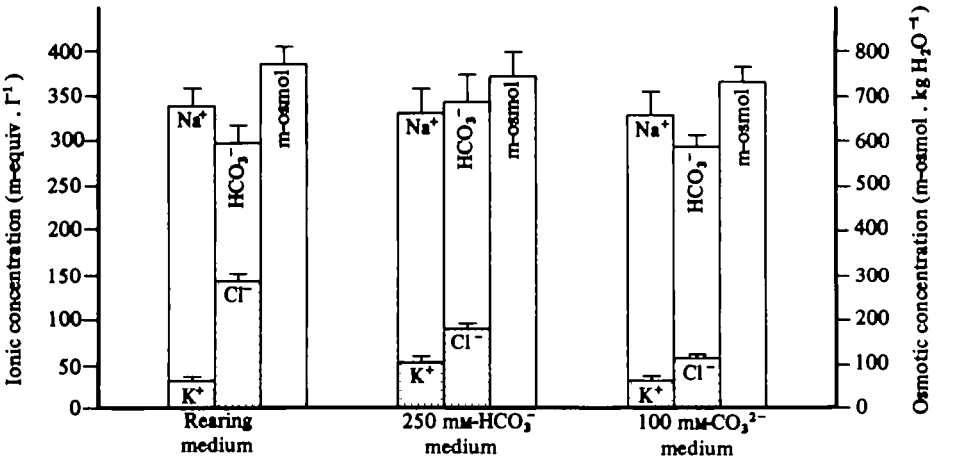

Fig. 7. Osmolarity and concentrations of major ions in rectal secretion collected by micropuncture from larvae acclimated to three different alkaline media (means ± S.E., n = 5–12). See Tables 1 and 2 for composition of acclimation media and physiological salines.

9·05 ± 1·10, 14·79 ± 1·33 and 9·44 ± 0·50 for animals acclimated to the Rearing, 250 mM-HCO_3^- and 100 mM-CO_3^{2-} media, respectively. Haemolymph P_{CO_2} was significantly higher ($P < 0{\cdot}001$) in animals acclimated to 250 mM-HCO_3^- medium. Bicarbonate concentrations calculated from these data are shown in Fig. 6(*b*). Despite large increases in external HCO_3^- and CO_3^{2-} levels, haemolymph HCO_3^- concentration remains low and only increase from 8·0 to 18·5 mM.

As previously described by Bradley & Phillips (1975, 1977*a*) for *Aedes taeniorhynchus* and *A. campestris* larvae from other hypersaline waters, the ligated rectum of *A. dorsalis* also swells with secretion when bathed in artificial haemolymphs. The osmotic and ionic concentrations of secretions collected by micropuncture of isolated recta are shown in Fig. 7. Mean Na^+ (270–300 mM) and osmotic (700–735 m-osmol) concentrations were essentially the same for larvae acclimated to the three external media. Mean chloride concentrations in rectal secretions increased from 50 mM to 135 mM, being lowest in animals acclimated to high HCO_3^- and CO_3^{2-} media. Concentrations of K^+ in rectal secretions were between 27 and 46·5 mM. Ca^{2+} and Mg^{2+} concentrations in secretions were very low (0·3–1·0 mM) while total phosphorus varied from 1·5 to 4·6 mM and total sulphur was *ca.* 8·0 mM (Table 4).

Osmotic and ionic concentrations of rectal secretions collected from microcannulated recta of larvae acclimated to 250 mM-HCO_3^- medium are shown in Fig. 8 and were significantly higher than those of secretions collected by micropuncture (cf. Fig. 7). Mean osmotic concentration was 1030 m-osmol, while mean Na^+, K^+ and Cl^- concentrations were 413 mM, 31·4 mM and 44·9 mM, respectively. Concentrations of Ca^{2+} and Mg^{2+} were again very low (0·5–0·8 mM) while total phosphorus levels were 2·4 mM and total sulphur was 13·4 mM (data not shown).

The major objective of our micropuncture studies was simply to determine whether the rectum was an important site of HCO_3^- excretion and regulation. Bicarbonate concentrations were thus initially estimated by measuring the pH at a known CO_2

Table 4. *Concentrations of Ca^{2+}, Mg^{2+}, total sulphur and total phosphorus and pH in rectal secretions collected by micropuncture*

(pH values were determined on secretions equilibrated with 2% CO_2; mean ± S.E., n = 7–11).

	Larval acclimation medium*		
Constituent (mM)	Rearing medium	250 mM-HCO_3^- medium	100 mM-CO_3^{2-} medium
Mg^{2+}	0·77 ± 0·11	1·04 ± 0·26	—
Ca^{2+}	0·34 ± 0·04	0·76 ± 0·22	—
Total sulfur	7·85 ± 0·81	8·90 ± 1·84	—
Total phosphorus	4·63 ± 1·03	3·61 ± 1·64	1·49 ± 0·30
pH	8·43 ± 0·04	8·72 ± 0·05	8·72 ± 0·03

* See Table 1 for composition of acclimation media.

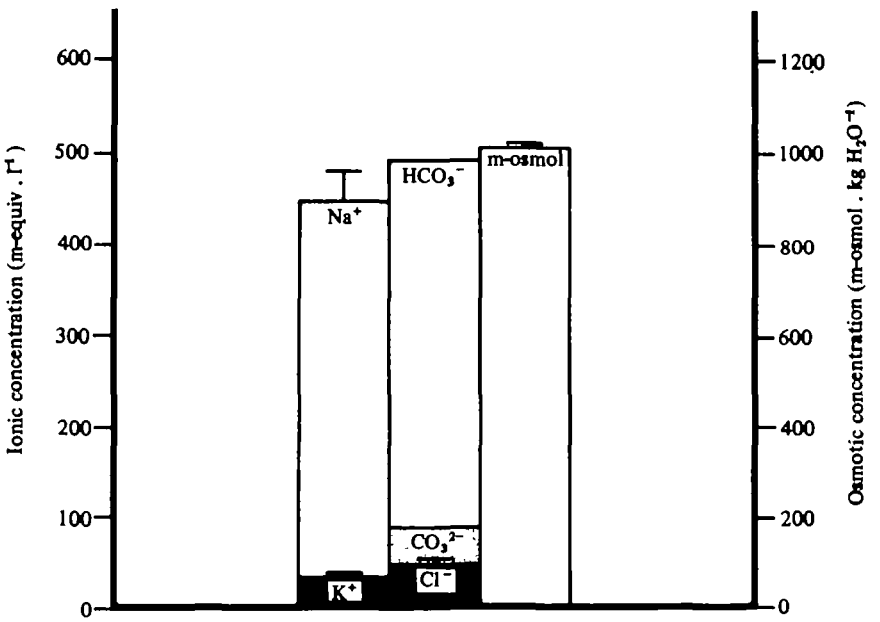

Fig. 8. Osmolarity and concentrations of major ions in rectal secretion collected from cannulated recta of animals acclimated to 250 mM-HCO_3^- medium (see Table 1). Values are means ± S.E., n = 8–9. Concentrations of HCO_3^- and CO_3^{2-} are calculated from measurements of total CO_2 and pH as described in the text.

tension of rectal secretions collected by micropuncture. This method is the same as that used in early work on the kidney (Gottschalk, Lassiter & Mylle, 1960; Viera & Malnic, 1968) and pancreas (Swanson & Solomon, 1973) and is based on the assumption that luminal P_{CO_2} is the same as that in the haemolymph (i.e. 2·0% CO_2). The concentrations of HCO_3^- calculated from the Henderson–Hasselbalch equation using the measured pH and known P_{CO_2} are shown in Fig. 7 and ranged between 150 and 240 mM.

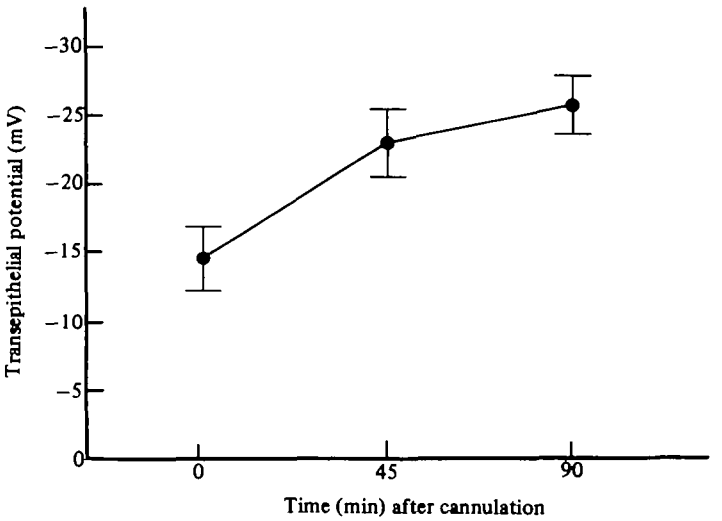

Fig. 9. Transepithelial potential (lumen relative to haemocoel side) across cannulated recta from animals acclimated to 250 mM-HCO_3^- medium (see Table 1). Values are means ±S.E., $n = 6$.

It must be stressed here that the calculated HCO_3^- levels shown in Fig. 7 are slight overestimates of actual concentrations. At the pH values of the rectal secretions (8·4–8·7; Table 4) a significant fraction of the total CO_2 pool is present as CO_3^{2-} which cannot be calculated directly from the Henderson–Hasselbalch equation using only known pH and P_{CO_2} values. To determine actual HCO_3^- and CO_3^{2-} concentrations in rectal secretion and to calculate transepithelial HCO_3^- and CO_3^{2-} gradients across the rectal wall, we subsequently measured total CO_2 in secretions collected from cannulated recta of animals acclimated to 250 mM-HCO_3^- medium. The total CO_2 concentration in these secretions was 444 ± 25 mM (mean ± S.E., $n = 6$) while pH was 8·65 ± 0·05 (mean ± S.E., $n = 7$). Using these measured values, concentrations of CO_2, HCO_3^- and CO_3^{2-} were calculated from the Henderson–Hasselbalch equation and were 0·90 mM, 402 mM and 41 mM, respectively (see Fig. 8).

Secretion rate was estimated in cannulated recta by measuring the volume of collected fluid and was 37·6 ± 6·4 nl.h^{-1}.$rectum^{-1}$ (mean ± S.E., $n = 6$). The calculated total CO_2 secretion rate in cannulated recta was 16·7 nmoles total CO_2.h^{-1}.$rectum^{-1}$.

Transepithelial potentials across cannulated recta are shown in Fig. 9. Immediately after ligation, mean TEP was −14·2 mV (lumen negative). During the course of the experiment, TEP gradually increased as the rectum swelled with secretion and reached a value of −25·3 mV (lumen negative) after 90 min.

DISCUSSION

The results of survival studies indicate that *A. dorsalis* larvae survive and develop normally in concentrated $NaHCO_3$–CO_3 media and that the distribution of this species in alkaline lakes is limited not by high pH *per se*, but instead by the concentrations of

HCO_3^- and CO_3^{2-} in these environments. Larvae inhabiting alkaline waters ingest the external medium at a rate equivalent to *ca.* 130% of their body wt/day. The degree of haemolymph pH and HCO_3^- regulation exhibited by these insects (Figs. 5, 6) is remarkable considering the acid-base regulatory problems which must be imposed by such high rates of fluid ingestion.

The elevated haemolymph P_{CO_2} observed in animals acclimated to 250 mM-HCO_3^- medium (see Results) is difficult to explain at present, but may represent an attempt of the larvae to retain CO_2 so as to lower haemolymph pH. This could be a valuable regulatory response, especially if the excretory system is not capable of eliminating HCO_3^- rapidly enough to maintain haemolymph pH within physiological limits. Alternatively, this elevated P_{CO_2} value could arise from passive processes and largely be a result of a leftward shift of the CO_2–HCO_3^- equilibrium as ingested HCO_3^- rapidly enters the haemolymph.

Larval haemolymph exhibits a large inorganic anion deficit (Table 2), which is characteristic of dipteran haemolymph and is due to the presence of large concentrations of organic acids (Florkin & Jeuniaux, 1964). This inorganic anion deficit increased as haemolymph Na^+ concentrations increased and Cl^- levels dropped when larvae were acclimated to high HCO_3^- and CO_3^{2-} media (Table 2). If organic acids account for this deficit, then an increase in these compounds would not only maintain haemolymph electroneutrality, but may also enhance haemolymph buffering capacity necessary to counter short-term alkaline shifts in haemolymph pH.

Haemolymph Ca^{2+} concentrations (Table 2) also increased when larvae were acclimated to 250 mM-HCO_3^- or 100 mM-CO_3^{2-} media. The rise in Ca^{2+} levels may reflect changes in the amount of Ca^{2+} bound to organic molecules. In addition, elevated haemolymph pH may increase Ca^{2+} complexation and precipitation, making it necessary for larvae to increase total haemolymph Ca^{2+} concentration as a means of regulating Ca^{2+} activity.

Rectal HCO_3^- secretion

The function of the rectum of saline water mosquito larvae in osmoregulation has recently been described in detail (Bradley & Phillips, 1975, 1977*a–c*). Briefly, the rectum is composed of two ultrastructurally distinct segments (Meredith & Phillips, 1973; cf. Fig. 2(*b*) and is functionally analogous to avian and reptilian salt glands. When larvae are acclimated to hypersaline media, the posterior rectal segment produces a strongly hyperosmotic secretion with ion ratios and total osmotic concentrations reflecting those found in the environment (Bradley & Phillips, 1975, 1977*a*,*b*). This segment is the major site of Na^+, Mg^{2+} and Cl^- regulation in saline waters (Bradley & Phillips, 1977*a*). Bradley & Phillips (1977*c*) suggest that the anterior rectum is involved in selective reabsorption of solutes and water.

Data from micropuncture studies (Fig. 7 and Table 4) demonstrate clearly that the rectum, presumably the posterior segment, is also an important site of HCO_3^- excretion and regulation as originally suggested by Bradley & Phillips (1977*a*). Larvae acclimated to 250 mM-HCO_3^- and 100 mM-CO^{2-} media produce rectal secretions with significantly higher concentrations of HCO_3^- than animals acclimated to the Rearing medium.

The total osmotic and ionic concentrations of secretions collected by micro-

cannulation were considerably higher than those of secretions collected by micropuncture (cf. Figs. 7 and 8). The most likely explanation for this discrepancy is related to the differences between the two isolated rectal preparations used. With the micropuncture preparation it is possible that small amounts of relatively dilute midgut and Malpighian tubule fluid remain in the rectum after ligation, even though the larvae normally empty the rectum when handled (Bradley & Phillips, 1975; K. Strange, unpublished observations). When using the cannulated preparation, however, it was always possible to rinse and then empty the rectum completely before the start of any experiment. This presumably results in the collection of pure rectal secretion.

Data from microcannulation studies allowed determination of transrectal HCO_3^- and CO_3^{2-} gradients. Lumen-to-haemocoel side HCO_3^- and CO_3^{2-} ratios of 21 : 1 and 241 : 1, respectively, were calculated using results shown in Fig. 8. In addition, the calculated luminal CO_2 concentration (see Results) was slightly higher than that in the bath, giving a lumen-to-haemocoel CO_2 ratio of 1·8 : 1.

To generate the observed HCO_3^- and CO_3^{2-} gradients by passive mechanisms, a transepithelial potential of +69 mV to +76 mV (lumen positive) would be required as calculated from the Nernst equation. The measured rectal TEP (Fig. 9) varied between −14 and −25 mV (lumen negative) demonstrating clearly that HCO_3^- transport occurs by an energy-dependent process.

In conclusion, it is clear from these studies that the rectal salt gland is an important site of HCO_3^- excretion and regulation in mosquito larvae inhabiting $NaHCO_3$–CO_3 waters. An *in vitro* microperfusion system has recently been developed (K. Strange, in preparation) and is currently being used to characterize the cellular mechanisms of rectal HCO_3^- secretion.

We thank Dr William Milsom for critically reviewing the manuscript and Ms Joan Martin and Dr Mary Chamberlin for assistance with the art work. We also thank Mr Michael Turell for the gift of A. *dorsalis* eggs. The invaluable technical assistance of Ms Christine McCaffrey and Margaret Muhlert in electron microprobe analyses is gratefully acknowledged. Supported by NSERC (Canada).

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