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THE LOCATION AND MECHANISM OF HYPEROSMOTIC FLUID SECRETION IN THE RECTUM OF THE SALINE-WATER MOSQUITO LARVAE *AEDES TAENIORHYNCHUS**

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SUMMARY

1. Ligation between the anterior and posterior segments of the rectum *in vitro* was used to demonstrate that the posterior rectum is the site of hyperosmotic secretion to the lumen side. Observations were consistent with a reabsorptive function for the anterior rectum. These results support predictions from ultrastructural studies of these two segments.

2. The initial potential of the rectal lumen, relative to the haemocoel side, was of opposite polarity in the anterior (−10 mV) and posterior (+10 mV) segments and these values decreased to −2 and +6 mV respectively in ligated recta which had secreted for 2 h.

3. A comparison of these potential difference measurements with concentration differences developed across the rectal epithelium under the same experimental conditions indicates that Na^+, K^+, Mg^{2+}, and Cl^- are all actively transported by the posterior segment to the lumen side.

4. The influence of different haemolymph concentrations of Na^+, K^+, and Cl^- on the potential differences across the basal cell border and across the whole rectal epithelium are reported. Based on this and previous data, we propose a model for the organization of transport processes within the single cell-type present in the posterior rectal epithelium.

INTRODUCTION

The production of a concentrated urine in saline-water mosquito larvae is achieved by the secretion of a hyperosmotic fluid into the rectal lumen (Bradley & Phillips, 1975, 1977*a*). The recta of all saline-water mosquito larvae examined to date have two morphologically distinct segments of the rectum (*Aedes detritus*, Ramsey, 1950; *A. campestris*, Meredith & Phillips, 1973; *A. taeniorhynchus*, Bradley & Phillips, 1975). Ultrastructural evidence suggests that the posterior rectal segment is the site of hyperosmotic fluid secretion (Meredith & Phillips, 1973). We therefore studied changes in fluid volume and osmotic concentration in the lumen of isolated anterior

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and posterior rectal segments in an attempt to distinguish the physiological processes occurring in these two parts of the rectum, particularly with regard to the site of hyperosmotic fluid secretion.

The latter fluid contains Na^+, Mg^{2+}, Cl^- and probably HCO_3^- in ratios and concentrations resembling the external medium to which the larvae are acclimated (Bradley & Phillips, 1977*a*, *b*). However, the rectal fluid contains much higher levels of K^+ than the haemolymph and the external medium under every experimental condition which has been studied. We wished to determine which of these ions are actively transported during the process of rectal secretion. *Aedes taeniorhynchus* larvae raised in 100% sea water were chosen for this study because the ionic concentration differences across the rectal epithelium were known (Bradley & Phillips, 1975). In the present study, electropotential differences across the rectal epithelium were determined under the same experimental conditions in order to determine whether the distribution of any of the ions could be explained by passive forces alone (i.e. by electrochemical potential difference). A model for trans-epithelial transport of ions is proposed, based on these observations and those from Bradley & Phillips (1975, 1977*a*, *b*).

MATERIAL AND METHODS

Aedes taeniorhynchus larvae were raised in 100% sea water as previously described (Bradley & Phillips, 1975) and starved for 1–2 days prior to use. An *in vitro* preparation of the rectum was prepared by dissecting larvae in normal artificial haemolymph (Bradley & Phillips, 1975). The gut was removed from the larvae and ligated with fine silk thread at any two of the following three points as appropriate: the posterior part of the ileum, the junctional region between the anterior and posterior rectal segments, or the anal canal near the anus. These ligatures isolated either the entire rectum, or the posterior and anterior segments individually. The regions of the gut not isolated between the ligatures were removed. These ligated recta were placed in hanging drops (*ca.* 10 μl) of normal artificial haemolymph suspended from the cover of a 60 × 15 mm glass Petri dish (Marks & Holman, 1974). The bottom of the Petri dish was filled with distilled water to retard evaporation. The osmotic concentration of the hanging drops increased by less than 15 mOsm during the 2 h experimental period. After 2 h, recta were removed from such drops with an eyedropper and placed on a microscope slide, where the adhering fluid was blotted up with filter paper. The rectum was punctured using a glass micropipette and a sample of rectal fluid was removed. The osmotic concentration of the sample was measured immediately using a nanoliter osmometer (Clifton Technical Physics, Ltd).

The electropotential difference across the rectal epithelium was measured using two separate methods. In one case (hereafter referred to as electrical preparation (1)), larvae were ligated between the sixth and seventh abdominal segments and the portion of the larvae anterior to the ligature was removed. The larva was placed on a Petri dish, the bottom of which was lined with paraffin wax into which a hole 2 mm × 5 mm × 3 mm had been made, such that the siphon extended into this hole. A glass coverslip was placed over the hole, allowing a space for the siphon and the edge was sealed with melted paraffin wax. The siphon was thereby isolated and exposed to the pocket of air under the coverslip. The area immediately adjacent to the larva was

sealed by melting wax with a hot probe so that only a very small amount of melted wax touched the larva to hold it in place. The Petri dish was then filled with paraffin oil which was prevented from entering the siphon by the sealed coverslip. The exposed cuticle of the larvae was torn and a drop of artificial haemolymph was placed on it. Under these circumstances the rectum was bathed in a solution of known composition while supplied with oxygen via the tracheae. Electrical potentials were measured by introducing the recording electrode into the lumen of the rectum through the anus.

Electrical preparation (2) was used to measure both intracellular and trans-epithelial potential differences, by impaling rectal cells from the haemolymph side with glass microelectrodes. To this end, larvae were placed on moist filter paper and ligated between the sixth and seventh abdominal segments and at the anal segment so as to isolate the rectum. The portion of the larva anterior to the first ligature was removed. The remaining larval portion was anchored securely to paraffin wax lining the bottom of a Petri dish by melting the ends of the ligatures into the wax. The larval cuticle was torn and a drop of haemolymph was placed on the preparation. Since the siphon was open to the air, the rectum was bathed in a solution of known composition and had a normal oxygen supply via the tracheae. The artificial haemolymph was removed and replaced every few minutes to avoid changes due to evaporation. This preparation was used only for short-term measurements.

Electrical potentials were measured using a M701 Microprobe System (W-P Instruments, Inc.) with potentials recorded on an expanded scale voltmeter (Radiometer, Copenhagen). The indifferent calomel electrode was connected to the artificial haemolymph bathing the recta in both electrical preparations by means of a salt bridge consisting of polyethylene tubing (P.E. 50, Clay Adams, Inc.) filled with 3 M-KCl in 3% agar. The recording glass electrodes, which contained 3 M-KCl, were inserted into the rectum either through the anus (electrical preparation (1)), in which case the tip resistance was low (1–2 MΩ), or through the rectal wall (electrical preparation (2)) using high resistance tips (10–17 MΩ).

The artificial haemolymphs used in this study were identical to those described by Bradley & Phillips (1977*b*). These various haemolymph solutions differed only in the substitution of a single ion. Thus Na^+ was replaced with choline, chloride was replaced with SO_4^{2-}, and K^+ was added as K_2SO_4 to normal haemolymph.

RESULTS

Secretion by rectal segments

The *in vitro* preparation of the larval rectum was used to differentiate the function of the anterior and posterior segments of the rectum. This was most easily done by placing a ligature between the two segments and monitoring fluid concentrations and volumes in the two portions with time. The results of such an experiment could not be compared with previous observations, *in vivo*, because of interference with neural and tracheal connexions to the rectum. For this reason an *in vitro* preparation of the whole rectum served as a control. In all three preparations, neural connexions had been broken, and oxygen entered the tissue from the surrounding fluid rather than by the tracheae.

Table 1. *The osmotic concentrations (mOsm) of rectal fluid removed from lumina of* in vitro *preparations of the rectum immediately (0 h) or 2 h after ligation*

(In some experiments the artificial haemolymph, which in all experiments had an osmotic concentration of 355 mOsm, contained potential stimulatory agents. Mean ±S.E. (number of observations). Mean values which are significantly different from that of controls are indicated by asterisks (* $P < 0.05$; ** $P < 0.02$))

Preparation	Treatment	Hours of treatment	Osmotic concentration
Whole rectum	Control	0	475 ± 29 (5)
	—	2	480 ± 13 (6)
	$+10^{-3}$M AMP	2	587 ± 80 (6)
	$+10^{-3}$M AMP and theophylline	2	431 ± 36 (4)
	$+10^{-4}$M 5-HT	2	511 ± 40 (6)
Anterior rectum only	—	2	*444 ± 8 (6)
Posterior rectum only	—	2	**711 ± 88 (7)

Fig. 1, A and B compare a single *in vitro* preparation of a whole isolated rectum 5 min and 2 h after dissection respectively. The rectum swells with fluid during the 2 h experimental period as previously observed for *in vivo* preparations of the rectum (Bradley & Phillips, 1975). Similar results were observed for 23 preparations. Photographs C and D of Fig. 1 show the posterior segment of a single rectum at 5 min and 2 h after dissection respectively. When ligatured such that only the posterior rectum fills with fluid, the preparation is not capable of greatly increasing in volume due to a greater tension on the rectal wall. Nevertheless some swelling of this segment is obvious, if less marked than for the whole rectum (11 observations). The conclusions we wish to draw in this case only require that there is no substantial decrease in lumen volume with time.

No change in volume could be discerned in any of the 13 preparations of the anterior rectal segment observed. Because of the small volume of the anterior rectum, it is impossible to conclude absolutely from photographic data alone that the anterior rectal portion either secretes or resorbs any fluid *in vitro*. However, since both the whole rectum and posterior rectal portion did swell with fluid when isolated, we were interested in determining whether the secretion *in vitro* was hyperosmotic to the haemolymph as previously demonstrated *in vivo* (Bradley & Phillips, 1975).

Osmotic concentrations of rectal secretion in vitro

The same procedures as were used to obtain photographic evidence of rectal secretion were followed to obtain fluid from the lumina of whole recta and isolated posterior and anterior rectal segments (Table 1). When whole recta were dissected from larvae, ligated, and immediately sampled by micropuncture, the rectal fluid was found to be hyperosmotic to the artificial haemolymph by an average value of 127 mOsm. This initial rectal fluid concentration was high compared to that previously observed with isolated *in vivo* preparations possibly because of the following difference in procedure. Larvae used for *in vitro* experiments were killed by grasping the thorax with forceps which may not have allowed time for complete defecation of

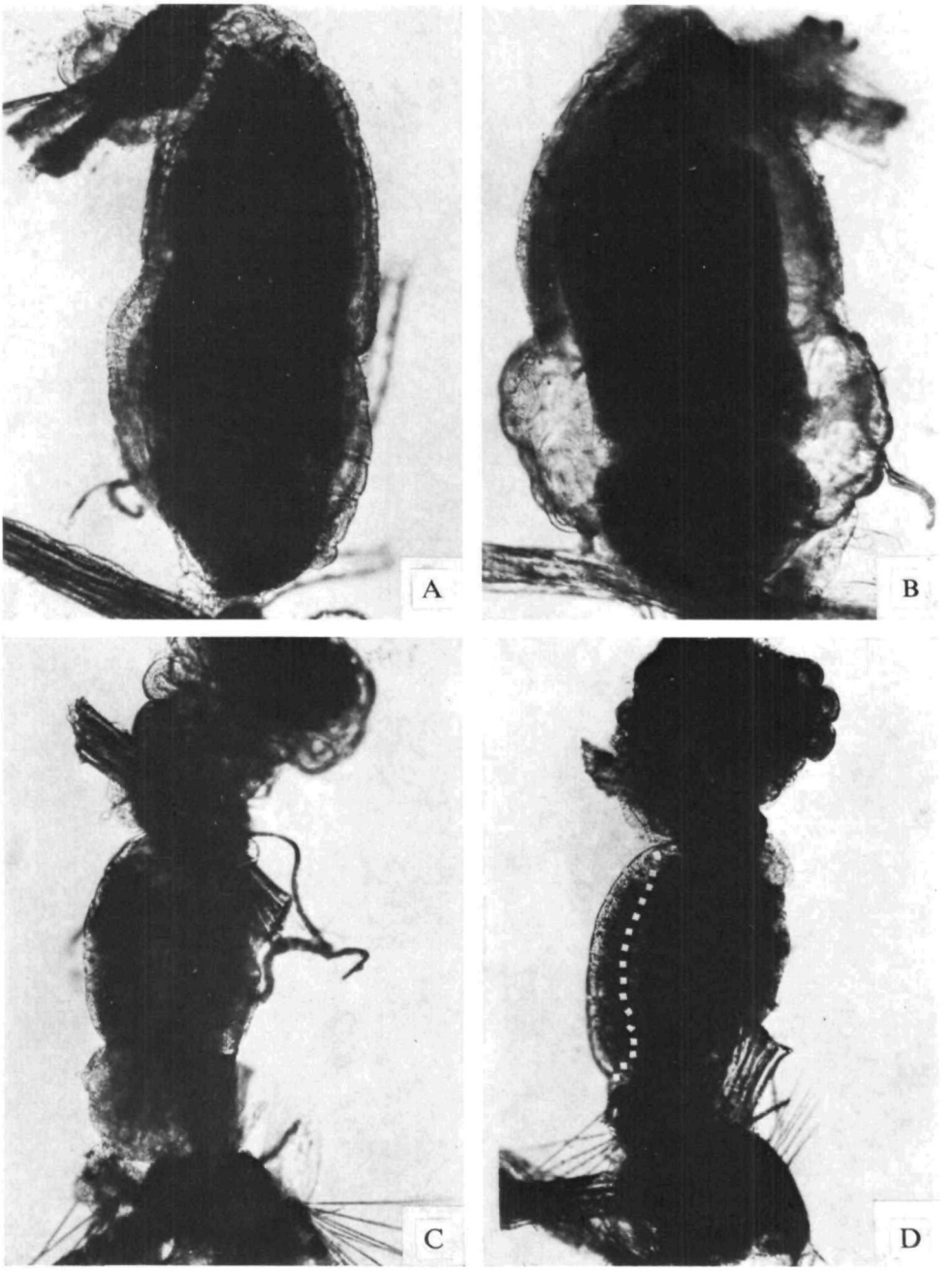

Fig. 1. A and B show a single, whole *in vitro* rectum 5 min and 2 h after ligation respectively. In B the rectum can be seen to be filled with secreted fluid (note border of outer clear area, *not* central dense faecal mass). C and D show a single, isolated posterior rectal preparation 5 min and 2 h after ligation respectively. D, the posterior segment can be seen to be swollen with secreted fluid. The initial shape of the segment is outlined in white. Similar photographs for anterior rectal segments showed no change in volume.

Table 2. *Paired measurements of the trans-rectal potential difference (mV) in the anterior and posterior segments of recta bathed in the normal artificial haemolymph, recorded within* 10 *min of dissection and after* 2 *h (electrical preparation* (2))

(The right column shows the potential difference between the lumina of the two segments of the same rectum. Mean ±S.E., $n = 9$)

Time (min)	Anterior rectal potential (lumen side)	Posterior rectal potential (lumen side)	Posterior relative to anterior potential
< 10	−10·4 ± 3·0	+10·5 ± 4·6	+20·9 ± 3·0
120	−1·8 ± 2·6	+6·4 ± 1·0	±8·2 ± 3·0

rectal contents. During preparation of ligated *in vivo* recta (Bradley & Phillips, 1975), the rectum completely empties and partially refills with midgut fluid while the larva is being blotted dry on filter paper. This results in an initial rectal fluid concentration which is isosmotic to the haemolymph.

While the volume of fluid in whole *in vitro* recta increased over 2 h (photographs A and B of Fig. 1), the final osmotic concentration of the secreted fluid was not significantly different from that initially present (Table 1). The isolated posterior segment of the rectum filled with a fluid which was significantly hyperosmotic to the initial rectal fluid, the artificial haemolymph, and also to the secretion from *in vitro* whole recta after 2 h ($P < 0{\cdot}02$). In isolated anterior rectal segments, where no volume change was observed, a significant reduction ($P < 0{\cdot}05$) in osmotic concentration occurred during the 2 h experimental period. Possibly, the failure of the whole rectum *in vitro* to secrete fluid as concentrated as the posterior rectum alone is due to solute reabsorption in the anterior rectum. This is consistent with the decline in fluid concentration in isolated anterior rectal segments.

The relatively low concentration of the rectal secretion *in vitro* could reflect lack of a natural neural or hormonal stimulus. Compounds known to stimulate secretion in some other insect tissues were therefore added to the artificial haemolymph bathing *in vitro* rectal preparations. No significant increase in rectal fluid concentration was found upon adding cyclic 3′–5′ adenosine monophosphate (10^{-2} M) with or without theophylline (10^{-2} M), or 5-hydroxytryptamine (10^{-4} M; Table 1).

In summary, these experiments indicate that the posterior rectal segment is a site, if not the only one, of hyperosmotic fluid secretion. The anterior rectal segment seems to reduce the concentration of the rectal fluid *in vitro*, which is consistent with the suggestion that this part of the rectum is involved in solute reabsorption (Meredith & Phillips, 1973).

Trans-rectal potential differences

Recta of saline-water mosquito larvae can secrete fluid containing Na^+, K^+, Mg^{2+}, Cl^- and probably HCO_3^- at concentrations 2–18 times those of the haemolymph (Bradley & Phillips, 1975, 1977*a*, *b*). To determine which of these ions are actively transported, a study was undertaken of the potential difference (P.D.) across the whole rectal wall during secretion by *A. taeniorhynchus* larvae adapted to 100% sea water.

When electrical preparation (1) was used, in which the measuring microelectrode was advanced into the rectal lumen through the anus, two quite different P.D. values

Table 3. *Paired measurements of the electrical potentials* (mV) *within the rectal cells and the rectal lumen, relative to the haemolymph, in the anterior and posterior segments*

(Measurements were made on recta bathed in normal artificial haemolymph and were recorded within 10 min of dissection)

	Anterior rectum		Posterior rectum	
Serial	Cell interior	Lumen	Cell interior	Lumen
1	−34	−16	−37	+14
2	−50	−16	−50	+17
3	−40	−12	−41	+6
4	−60	−12	−27	+2
5	−61	−7	−41	+15
Mean	−49	−13	−39	+11
±S.E.	±5	±2	±4	±3

for the lumen relative to the haemolymph were successively and consistently observed. These two regions showed not only different potentials but, in a majority of cases, opposite polarity relative to the haemolymph. In order to locate these regions more precisely, electrical preparation (2) was used, in which the trans-epithelial P.D. was measured by passing glass microelectrodes from the haemolymph side through the rectal wall into the lumen of either the anterior or posterior rectal segment such that the precise location of the microelectrode tip was known (Table 2). The lumen of the anterior segment was always negative and that of the posterior segment was always positive relative to the haemolymph with the exception of one value of −12 mV. The lumen of the posterior segment was always positive with respect to the lumen of the anterior segment of the same rectum by an average of 20·9 mV.

Preparation (2) was used to measure the P.D. across the wall of recta which had been ligated and placed in artificial haemolymph for 2 h, so that conditions were identical to those previously used to determine ionic composition of rectal secretion (Bradley & Phillips, 1975). Under these conditions, when the rectum was swollen with secretion, the anterior rectal lumen showed both small negative and positive values relative to the haemolymph (mean of −1·8 mV) and the posterior rectal lumen was always positive relative to the haemolymph by +6·4 mV (Table 2). The lower potentials for recta filled with secretion compared to those recently ligated reflect the fact that the P.D. between the lumina of anterior and posterior portions was reduced (mean of 8·2 mV). Perhaps a constriction at the junctional region (Meredith & Phillips, 1973) provides a relatively high electrical resistance between the rectal segments in the unswollen condition, while swelling of the rectum after 2 h of secretion leads to increased continuity between the lumina of the anterior and the posterior rectum and hence to a short-circuiting of the opposing P.D.s in these two segments.

Influence of haemolymph ion concentrations on the trans-epithelial P.D.

We were interested in how P.D.s arise across the rectal wall and whether they are associated with active transport of ions responsible for the formation of hyperosmotic

rectal secretion. Potential profiles through the anterior and posterior rectal cells were measured to determine the size and sign of the potential steps across apical and basal plasma membranes (Table 3). Although the trans-epithelial P.D. differed substantially in the anterior and posterior rectal portions, the intracellular potential relative to haemocoel (i.e. P.D. across the basal plasma membrane) was not significantly different in the two segments (-39 and -49 mV respectively). When intracellular potentials reached steady values, it was possible to change the artificial haemolymph such that the electrode remained in the cell during the entire procedure. It was therefore possible to measure the effect of varying ion concentrations in the haemolymph on the P.D. across the basal membrane of the posterior rectal segment. This P.D., which was always negative (cell interior relative to haemolymph), could be made less negative by increasing haemolymph potassium levels, being reduced by an average of $8 \pm 0{\cdot}7$ mV ($n = 4$) in 39 mM-K^+ and 13 mV in 63 mM-K^+ (single observation). Decreasing the Cl^- concentration of the haemolymph to 1·3 mM also reduced the P.D. across the basal membrane by 7 ± 0 mV ($n = 3$). Varying the Na^+ concentration of the haemolymph from 200 mM to 5 mM-Na^+ by substituting choline did not have a significant effect on this P.D.

Since experiments with *in vitro* preparations demonstrated that the posterior rectal segment is a site of hyperosmotic fluid secretion, we examined in detail the effect of varying haemolymph ion concentrations on the electrical potential generated across the entire cell of this segment. The potentials across the two parts of the rectum are usually opposite in polarity and tend to cancel each other out; therefore, it was important to show that changes observed in the posterior rectal portion were not originating in the anterior rectal portion. The potential in the anterior rectal lumen relative to the haemolymph did not vary when the rectum was bathed in haemolymph high in K^+ (63 mM) or Na^+ (200 mM), or low in Cl^- 1·3 mM (eight preparations observed; data available from Bradley, 1976). Of the various haemolymphs used, only that low in Na^+ (5 mM) had any appreciable effect, causing an increased negativity of the lumen (Fig. 2). This change in the P.D. was in the same direction as that observed in the posterior rectal segment. The observation that high K^+ and Na^+, and low Cl^- levels in the haemolymph do not effect the anterior rectal P.D. argues against a substantial electrical short-circuit between the rectal segments in the unswollen rectum, since these parameters have marked effects on the P.D. in the posterior rectal segment.

For studies on the posterior rectum, electrical preparation (1) was used, with the microelectrode placed through the anus into the most positive part of the rectum. Fig. 3 shows the effect on the P.D. across the posterior rectum of various artificial haemolymphs in which the concentration of only one ion and its replacement were varied. This procedure was repeated with twenty different rectal preparations. Individual posterior recta have quite different trans-epithelial potential differences after dissection in normal artificial haemolymph. Therefore, the effects of different artificial haemolymphs are expressed as changes in P.D. relative to the original value observed for the same rectum bathed in normal haemolymph (Figs. 4, 5 and 6).

The lumen of the posterior rectal segment becomes more positive (relative to the haemolymph side) with increasing haemolymph K^+ concentration (Figs. 3 and 4; normal K^+ concentration is 14 mM). The potential increase is proportional to the K^+ concentration increase and is completely reversible upon return to normal haemo-

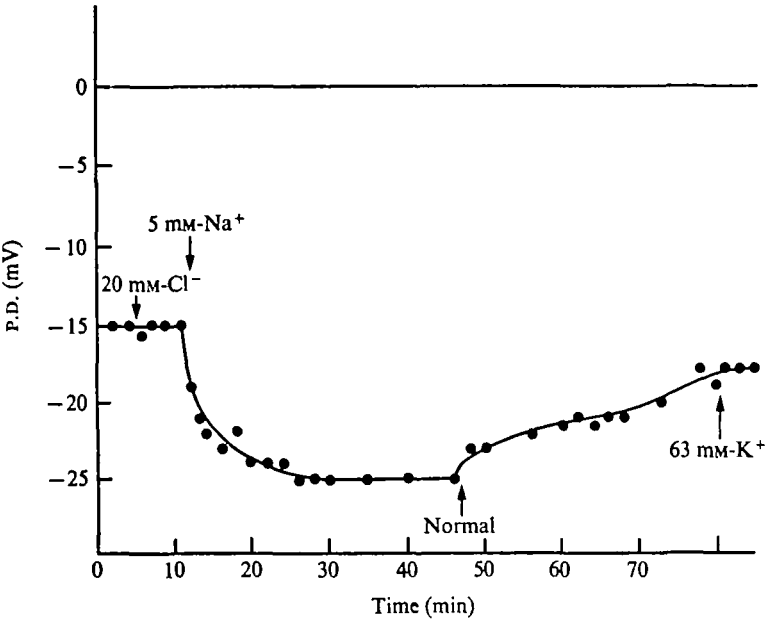

Fig. 2. The trans-rectal P.D. (lumen relative to haemolymph) observed in anterior rectal segments bathed in normal artificial haemolymph or haemolymphs differing in the concentration of only the ion indicated (electrical preparation (1)). Exposure to a new artificial haemolymph differing in the concentration of the ion indicated is shown by arrows. The initial steady P.D. over the first 5 min is for recta exposed to normal artificial haemolymph (i.e. base line).

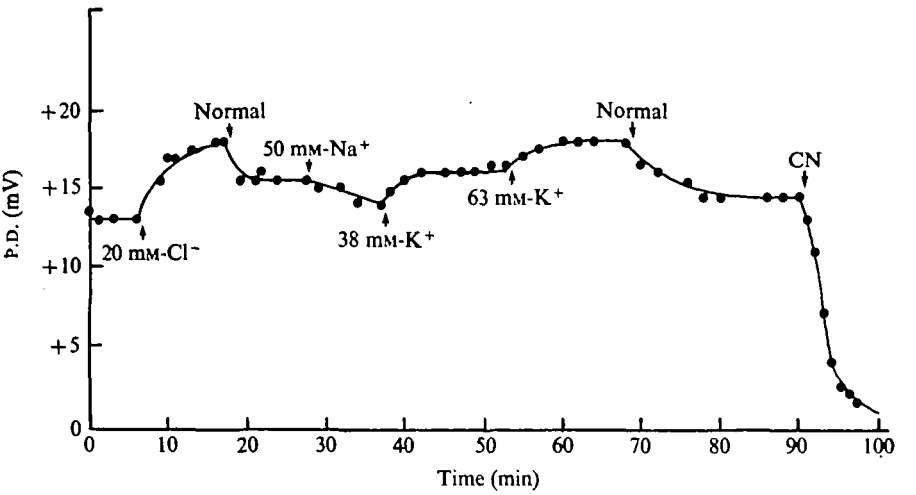

Fig. 3. The trans-rectal P.D. (lumen relative to haemolymph) observed in the posterior rectal segment bathed in normal artificial haemolymph or artificial haemolymphs differing in the concentration of the ion indicated. Changes in artificial haemolymph are indicated by arrows. CN^- indicates normal artificial haemolymph to which 10^{-3} M-KCN was added. The initial steady value for the first 7 min. is for recta exposed to normal artificial haemolymph (i.e. base line).

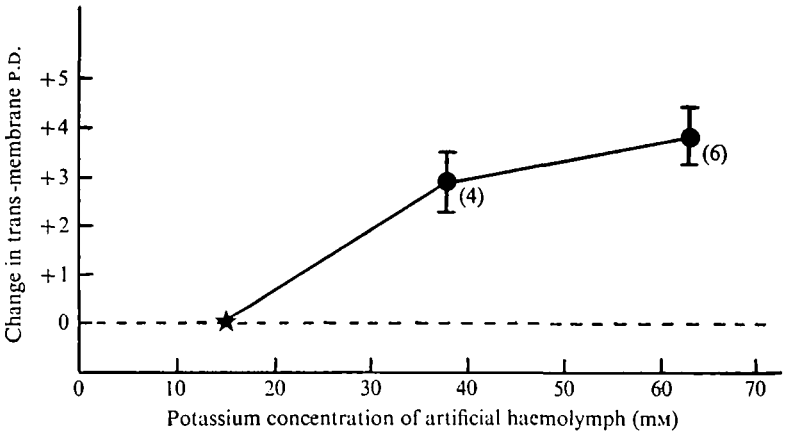

Fig. 4. The effect of increasing the K^+ concentration of the artificial haemolymph on the trans-rectal potential difference of the posterior segment. All values are expressed as the mean change in P.D. ± S.E., relative to that observed for recta bathed in normal artificial haemolymph (★; +10·5 ± 4·6 mV, n = 9). The numbers in brackets indicate the sample size for each point.

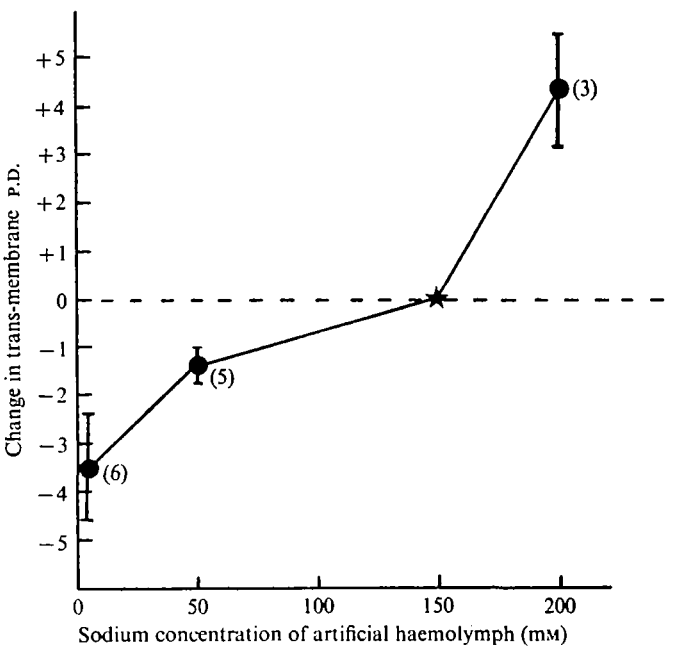

Fig. 5. The effect of varying the Na^+ concentration of the artificial haemolymph on the trans-rectal P.D. of the posterior segment. All values are expressed as the mean change in P.D. ± S.E. relative to that observed for recta bathed in normal artificial haemolymph (★; +10·5 ± 4·6 mV, n = 9). The numbers in brackets indicate the sample size for each point.

lymph. The effect of varying artificial haemolymph concentrations of Na^+ is shown in Fig. 5. The P.D. increases (lumen more positive) with increasing levels of Na^+ in the haemolymph. The P.D.s observed in the presence of 5 mM-Na^+ and 200 mM-Na^+ are significantly different ($P < 0.01$). The lowering of the P.D. in the presence of 5 mM-Na^+ is reversible upon return to normal haemolymph.

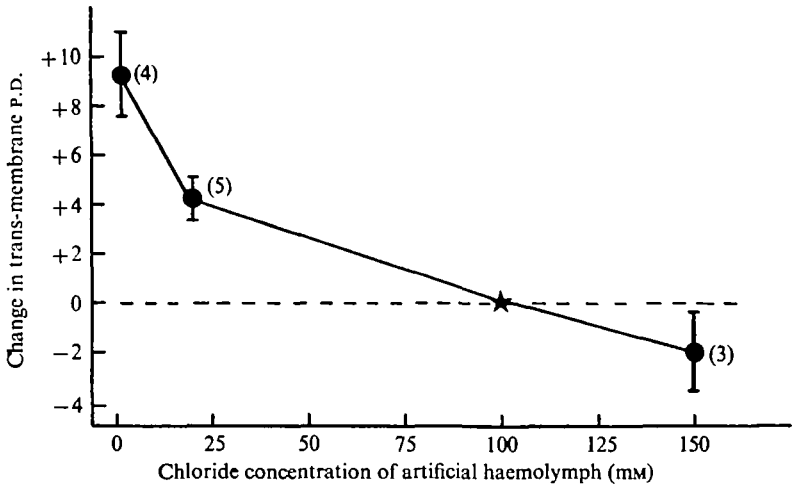

Fig. 6. The effect of varying the Cl^- concentration of the artificial haemolymph on the trans-rectal P.D. of the posterior segment. All values are expressed as the mean change in P.D. ± S.E. relative to that observed for recta bathed in normal artificial haemolymph (★; +10·5 ± 4·6 mV, $n = 9$). The numbers in brackets indicate the sample size for each point.

The trans-rectal P.D. decreased significantly with increasing Cl^- concentration ($P < 0{\cdot}01$). Decreasing the Cl^- level of normal haemolymph to 1·3 mM caused a twofold increase in the mean trans-rectal potential (Fig. 6). This P.D. increase was maintained for 20 to 30 min, after which time a partial or even total loss in potential occurred. Upon return to normal haemolymph, the trans-rectal potential was more negative by 3 to 4 mV than previously observed in normal haemolymph.

We have shown (Bradley & Phillips, 1977*b*) that high levels of either Na^+ or Cl^- in artificial haemolymph stimulated the rate of secretion of these ions. The rate of fluid secretion and, in the case of sodium, the osmotic concentration of the rectal fluid also increased as compared to values for normal artificial haemolymph. Therefore, it was of particular interest to observe the effect of these artificial haemolymphs on the trans-rectal potential. High levels of Na^+ (200 mM) in the artificial haemolymph were found to increase the trans-rectal potential in a manner which was not reversible within a 1 h period (Fig. 7). High levels of Cl^- (150 mM) in the artificial haemolymph, on the other hand, did not change the trans-rectal P.D. appreciably (Fig. 6). Although the effect of chloride shown in Fig. 7 is not completely typical, in that a slow increase in potential can be observed after a small initial dip, the increase in potential upon return to normal haemolymph, as compared to previous values in normal haemolymph, is clearly shown. Generally, the application of artificial haemolymph high in Cl^- led to a slight decline in trans-rectal potential ($-2{\cdot}0 \pm 1{\cdot}6$ mV, $n = 4$), but upon return to normal haemolymph, the potential rose to a level higher than that previously observed in normal haemolymph by a mean value of $3{\cdot}0 \pm 1{\cdot}0$ mV ($n = 4$).

The addition of ouabain (10^{-3} M) to the normal haemolymph did not produce a significant change in P.D. ($+0{\cdot}6 \pm 0{\cdot}6$ mV, $n = 3$). Serotonin (10^{-4} M) also did not significantly affect the trans-rectal P.D. over a period of 15 min ($+0{\cdot}8 \pm 0{\cdot}5$ mV, $n = 4$). The addition of cyclic AMP (10^{-3} M) and theophylline (10^{-2} M) to normal haemolymph induced no significant change in P.D. ($1{\cdot}0 \pm 0{\cdot}6$ mV, $n = 3$). These

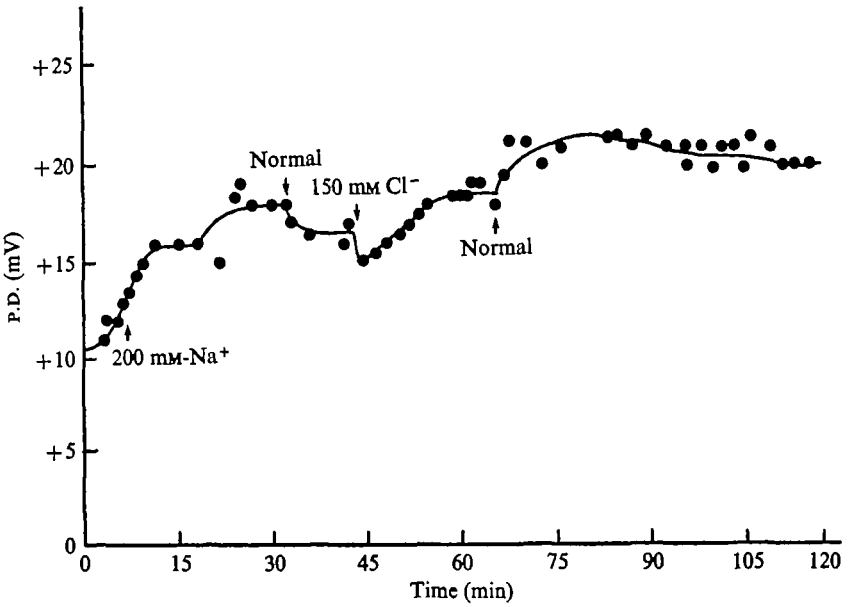

Fig. 7. The trans-rectal P.D. (lumen relative to haemolymph) observed in a posterior rectal segment bathed in normal artificial haemolymph or artificial haemolymphs differing in the concentration of the ion indicated. Haemolymphs were changed at the time indicated by arrows.

findings confirmed observations using the *in vitro* rectal preparation (Table 2), which showed that cyclic AMP + theophylline did not stimulate secretion, as indicated by an increased osmotic concentration of rectal fluid. Lack of an effect on the *in vitro* preparation could possibly be attributed to anoxia, but this can be excluded in the present experiments. With electrical preparations (1), the siphon and the tracheae were intact, yet electrical potentials were unaffected by these pharmacological agents. The results from these two separate experiments suggest that rectal secretion in saline-water mosquito larvae cannot be stimulated on a short-term basis by these hormone analogues applied on the haemocoel side of the rectum.

The P.D. observed across the posterior rectal segment was substantially and irreversibly reduced upon addition of KCN (10^{-3} M; Fig. 3). This rapid decline in P.D indicates a metabolic dependence of the potential generating mechanisms.

DISCUSSION

In vitro preparations of the larval rectum used in this study secreted a much less concentrated fluid than did the *in vivo* preparations of Bradley & Phillips (1975). Since the same artificial haemolymph bathed both preparations, the poorer performance of *in vitro* recta was probably due to disruption of tracheal connexions and possibly the absence of neural and hormonal stimulation. However, sufficient transport activity remained *in vitro* in the isolated posterior rectum to produce a secretion 366 mOsm more concentrated than the haemolymph (Table 1). This portion of the rectum is therefore clearly one, if not the only, area where hyperosmotic secretion occurs.

No firm conclusions can be drawn from the present data alone concerning the function of the anterior rectum. *In vitro*, this rectal segment appears to reduce the concentration of the fluid in the lumen of both whole recta and isolated anterior recta. The ultrastructural observations of Meredith & Phillips (1973) indicated that the anterior rectum of saline-water mosquito larvae resembles the whole rectum of strictly freshwater larvae, where salt resorption results in the formation of hyposmotic excreta. The evidence to date suggests that the two rectal portions serve separate functions. They show morphological differentiation, electrical potentials differing both in magnitude and polarity, and cause opposite changes in rectal fluid concentration *in vitro*. This supports the hypothesis of Phillips & Meredith (1969) and Meredith & Phillips (1973) that the anterior rectum is the site of salt and nutrient resorption in both fresh and saline waters and that the posterior rectum is the location of the hyperosmotic secretion of salts required for osmoregulation in saline waters. Another saline-water insect, *Ephydrella*, possesses two cell types in the hindgut which contains hyperosmotic fluid (Marshall & Wright, 1974). Marshall & Wright propose that the two cell types perform separate functions, either salt resorption or salt secretion by the small cells, and water resorption by the large cells.

Based on our present understanding of rectal function in two species of saline-water mosquito larvae (Bradley & Phillips, 1975, 1977*a*, *b*) and the blowfly larva *Sarcophaga bullata* (Prusch, 1974), we suggest that most aquatic larvae capable of hyposmotic regulation produce a concentrated urine by the secretion of a hyperosmotic fluid in some part of the hindgut. In accordance with this function, the portion of the gut in which final modication of urine concentration occurs will show two cell types, one engaged in resorption of essential metabolites and some ions and the other in secretion of hyperosmotic fluid when the animal is in a hyperosmotic medium.

The electropotential differences measured in this study can be compared to the ionic ratios previously measured under identical conditions (Bradley & Phillips, 1975) to determine which ions are actively transported across the rectal wall of *A. taeniorhynchus* living in 100% sea water. The P.D. required to support an ionic concentration difference across a diffusion barrier, in this case the rectal epithelium, is described by the Nernst equation which, at the experimental temperature used and for monovalent ions, simplifies to

$$E = 58 \log (c_1/c_2),$$

where E equals the electrical potential difference and c_1/c_2 the concentration ratio which can be maintained by the electrical P.D.

The ratio of the rectal fluid to haemolymph concentration observed for each ion *in vivo* using ligated recta, as well as the P.D. required to maintain that concentration ratio (lumen relative to haemolymph; shown in brackets) is as follows: Na^+ 1·9 (−16 mV), K^+ 9·9 (−57·7 mV), Mg^{2+} 5·0 (−58 mV), Cl^- 4·3 (+37 mV). These concentration ratios are for 2 h after ligation and must therefore be compared to average values for P.D. observed across the rectal wall at the same time (−1·8 and +6·4 mV respectively for anterior and posterior segments). Therefore, under these experimental conditions, it is necessary to postulate that Na^+, K^+, Mg^{2+} and Cl^- are all actively transported across the rectal wall from the haemolymph to the rectal lumen. (No distinction between primary and secondary transport is possible at this

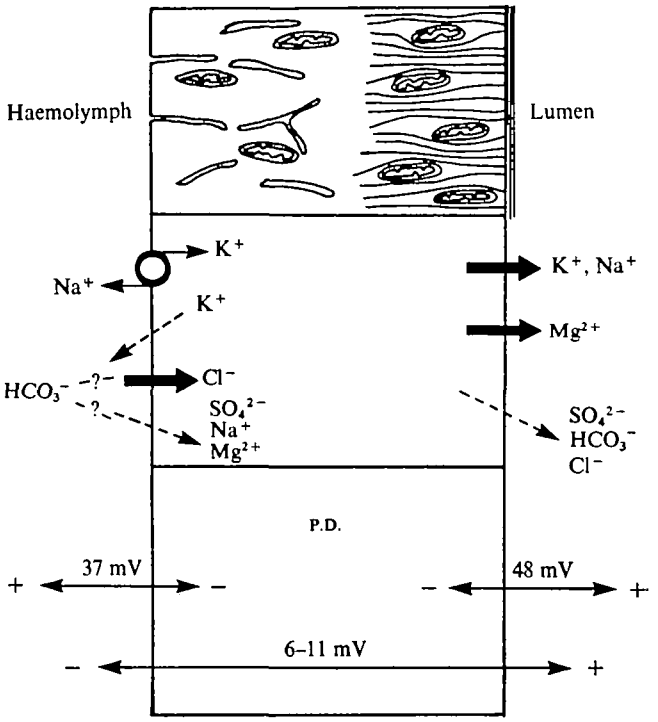

Fig. 8. A model for the organization of transport processes occurring in cells of the posterior rectal segment during the secretion of hyperosmotic fluid. The upper region is a diagrammatic representation of the ultrastructure of the one cell-type, after Meredith & Phillips (1973). The central block depicts the proposed sites of ion transport during fluid secretion. Solid arrows represent active transport while broken arrows depict passive movements. The bottom diagram shows the average P.D. measured across each plasma membrane and across the entire posterior rectal epithelium, when recta are bathed in normal artificial haemolymph.

time.) In particular, if one considers the evidence that hyperosmotic secretion occurs in the posterior rectal segment where the lumen is positive, then clearly all three cations are transported not only against concentration but also electrical potential differences.

We examined the P.D. across the posterior rectal wall in more detail because the morphological and physiological evidence indicated that hyperosmotic secretion occurred in this region. We have made three assumptions in constructing a model for ion transport across the posterior rectal epithelium (Fig. 8). (1) As indicated by electron micrographs, only two important barriers to ion diffusion are present in the posterior rectal wall, namely the basal (haemocoel side) and apical (lumen side) plasma membranes. (2) The fluid secreted across the posterior rectal wall is similar to or more concentrated than that collected from the lumen of the whole, ligated rectum. (3) The intracellular concentrations of ions are typical of those in most other cells; i.e. high K^+, low Na^+ and Cl^-. Like most cells, those of the posterior rectal segment have an interior which is negative to the haemolymph. This is partially attributed to a small K^+ diffusion potential, with the K^+ concentration gradient maintained by an active Na^+–K^+ exchange mechanism (reviewed by Schwarz, Lindenmayer & Allen, 1972). In support of this, when an artificial haemolymph abnormally

high in K^+ was placed on the posterior rectum, the interior of the cell became less negative. A reduction of the P.D. across the basal membrane should increase the trans-rectal P.D., since the electrical potentials across the basal and apical membranes oppose each other. This was experimentally observed when artificial haemolymphs high in K^+ were placed on the posterior rectum (Figs. 3, 4). On the basis of these observations, an outward diffusion of K^+ to the haemocoel, which is opposed by active transport involving a typical Na^+–K^+ exchange 'pump', is depicted in Fig. 8. This is envisaged as a low capacity system which maintains typical ion gradients in non-secreting recta and which is overshadowed by high capacity electrogenic pumps during secretion. This would explain the failure to demonstrate inhibition by ouabain; alternatively the basement membrane might be impermeable to this inhibitor.

The P.D. across the basal membrane was found to decrease upon reduction of the Cl^- concentration of the artificial haemolymph. Since this anion appears to be transported across the whole rectal wall we suggest that an electrogenic Cl^- 'pump' exists on the basal membrane which transports chloride into the cell during hyperosmotic fluid transport. Active transport of Cl^- at this location would not only explain the effect of low Cl^- haemolymph on the electrical potential difference across the basal membrane, but also would ensure the entry of Cl^- into the cell against the steep potential gradient across the basal membrane (Table 3). Indeed this pump may be the largest source of the P.D. across this border. This same P.D. would facilitate the movement of Na^+ and Mg^{2+} into the cell. These ions are generally present in cells at lower activities than in the blood (Palaty & Friedman, 1975). These cations could therefore enter the cell passively down both electrical and chemical potential gradients.

Schmidt-Nielsen (1975) has reviewed evidence that, in some epithelia secreting hyperosmotic fluid, the barrier to the diffusion of water is apparently at the basal membrane, since the intracellular osmotic concentrations are very high (1000–2000 mOsm). In other such epithelia, the cells are isosmotic to the blood. We have no data for the larval rectum and information of the location of the barrier to water diffusion must await further experimentation. The apical border seems the more likely site of osmotic work on ultrastructural grounds.

The P.D. across the apical border of the posterior rectal cells (34–61 mV) is approximately equal to that across the basal membrane (37–50 mV), the cell being negative to the exterior at both sites. It is at the apical membrane that the passive transport of the cations is opposed. It is therefore necessary to propose one or more cation pumps at this location to account for the ion concentrations observed in the hyperosmotic secretion. This membrane also has the greatest surface area in the cell and is associated with most of the mitochondria. Fig. 8 shows separate cation pumps for the monovalent and divalent cations. In some vertebrate tissues, Mg^{2+} is thought to be transported by a separate mechanism from that for other cations, with this transport being dependent on a Na^+ gradient to drive $Na^+ - Mg^{2+}$ exchange (Baker & Crawford, 1973; Palaty, 1974). Whether this applies to the larval rectum as well is at present unknown.

When the Na^+ concentration of the artificial haemolymph bathing the posterior rectum was raised from 5 to 200 mM, the trans-rectal potential increased significantly. This is consistent with stimulation of the electrogenic Na^+ pump proposed in our model. However, the failure to observe a concomitant change in potential across the

basal plasma membrane is puzzling, since more Na^+ is clearly secreted across the epithelium as haemolymph Na^+ levels are raised (Bradley & Phillips, 1977*b*). Possibly Na^+ permeability across the basal border of secreting recta is so high that intracellular levels of this cation equilibrate very rapidly with those in the blood, and no diffusion potential was detected across this membrane in our experiments because rapid mixing of the external medium was not feasible. Alternatively, increased Na^+ entry into the cell may stimulate chloride transport into the cell so that the basal P.D. is unchanged. For the sake of simplicity, K^+ and Na^+ are shown sharing the same monovalent transport mechanism (Fig. 8). Further studies may indicate that these ions actually use separate transport mechanisms.

The P.D. across the apical membrane is sufficient to support a tenfold concentration ratio for Cl^-. The normal intracellular Cl^- concentration for epithelia secreting hyperosmotic fluid is 80–120 mM (Schmidt-Nielsen, 1975). A concentration of only half of this value would be sufficient in the rectal cell to allow Cl^- to pass across the apical membrane and accumulate in the lumen by passive means. The intracellular Cl^- concentration could be maintained at this level by the active Cl^- transport proposed across the basal membrane in Fig. 8. We have followed the convention of not proposing an active transport mechanism where no thermodynamic requirement exists, and therefore suggest that Cl^- and other anions cross the apical membrane by passive means (Fig. 8).

The transport of other anions by the posterior rectal segment must be considered for larvae living in several natural waters. In all likelihood, HCO_3^- largely substitutes for Cl^- in the rectal secretion of animals reared in hyperosmotic media low in Cl^- (Bradley & Phillips, 1977*a*), resulting in hyperosmotic secretion with Cl^- concentrations as low as 40 mM. In waters high in $NaHCO_3$, HCO_3^- probably accompanies the active transport of cations at the apical membrane when cellular Cl^- concentrations are depressed.

It should be emphasized that the data for experiments in which the effects of different salines on P.D. were studied should be considered semi-quantitative. This is because the saline was changed by replacement of the bathing drop every 1–2 min rather than by perfusion. The slow change in P.D. in most experiments may reflect the time for diffusion through the resulting unstirred layers. Over this period substantial changes in ion concentrations within the rectal cells and also in the lumen contents may have occurred. Clearly more accurate measurements await the application of methods which eliminate these technical problems.

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