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ACTIVE ION TRANSPORT IN THE LARVAL HINDGUT OF *SARCOPHAGA BULLATA* (DIPTERA: SARCOPHAGIDAE)

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SUMMARY

1. The potential difference across the hindgut of *Sarcophaga* is 30 mV, *in vivo*, the lumen being negative with respect to the haemolymph. A potential difference of the same polarity exists in the isolated hindgut.

2. The potential difference is not a simple diffusion potential, since it is maintained in the absence of any ionic concentration difference across the gut, and is dependent on energy supplies.

3. The potential across the gut is the algebraic sum of two separate electrogenic pumping mechanisms; a cation system which moves K^+ or NH_4^+ and an anion system which moves Cl^- into the gut lumen.

4. Since a potential exists across the gut, the rate or amount of cation and anion movement into the gut cannot be equal; alternatively, various shunt pathways may exist for one or more of the ions involved.

INTRODUCTION

Hyperosmotic excreta in the majority of insects are produced by water reabsorption in the hindgut. The primary urine produced by the Malpighian tubules is isosmotic with the haemolymph, enters the hindgut at the junction of mid- and hindguts and then flows posteriorly. Water reabsorption, generally associated with active ion movements, occurs in the hindgut, especially in specialized rectal structures (Berridge & Gupta, 1967; Wall & Oschman, 1970). This reabsorption of water in the hindgut can produce excreta which are considerably more concentrated than the surrounding haemolymph.

In the blowfly larva, *Sarcophaga bullata*, hyperosmotic excreta are produced not by the reabsorption of water by the hindgut epithelium, but by solute secretion from the haemolymph into the hindgut lumen (Prusch, 1973). The secretion of solutes into the hindgut of the blowfly larva is associated with nitrogen excretion, essentially in the form of NH_4^+ (Prusch, 1972). As is shown in Table 1, the isolated hindgut under appropriate conditions is capable of concentrating Na^+, K^+, NH_4^+, and Cl^- in the hindgut lumen. In the absence of free external NH_4^+, but in the presence of exogenous metabolic energy sources and free amino acids, the isolated hindgut epithelium secretes mainly K^+ and Cl^-. When small amounts of NH_4^+ are added to the outside medium, Cl^- secretion is maintained, but K^+ secretion decreases, while NH_4^+ secretion greatly increases. These events were originally interpreted as providing evidence for a cation

Table 1. *Concentrations (mM) of various ions in the lumen of the isolated hindgut of* Sarcophaga bullata *larva equilibrated in control medium (Table 2) and control medium with 1 mM NH_4^+ for 90 min (Prusch, 1972)*

	Outside medium	Hindgut lumen	Outside medium	Hindgut lumen
Na^+	140	190	140	165
K^+	12	110	12	90
NH_4^+	0	28	1	120
Cl^-	151	200	151	220

pump in the hindgut epithelium capable of moving either K^+ or NH_4^+ against its concentration gradient. It was felt that the specificity of the pump site was higher for NH_4^+ than K^+, but would move K^+ in the absence of free NH_4^+. Chloride secretion was thought to be secondary to cation secretion, Cl^- following passively active cation movements.

It was decided to further investigate the secretion of ions in this system by measuring the electrical potential difference across the wall of the hindgut, it being previously established that the isolated hindgut is capable of maintaining a potential difference (unpublished results). Measurements of potentials have previously been used in a variety of systems, including insect midgut (Harvey & Nedergaard, 1964), to characterize ionic movements. In isolated Cecropia midgut, potential measurements have shown that K^+ is the principal ion transported into the gut. Potential measurements in the isolated hindgut of *Sarcophaga bullata* larvae have demonstrated the presence of two electrogenic pumping mechanisms, a cation pump (K^+ and/or NH_4^+) and an anion pump (Cl^-). The potential generated across the isolated gut under these conditions is therefore the algebraic sum of two different electrogenic systems.

MATERIALS AND METHODS

The hindgut of third instar larval *Sarcophaga bullata* was removed as described previously (Prusch, 1971) and mounted in a perfusion chamber, as is shown in Fig. 1. Because of the large overall length of the hindgut (approximately 3·5 cm), only the last 2 cm of the most posterior portion of the gut was used in this study in order to reduce any variability due to regional functional differences which may exist along the length of the gut. The isolated piece of hindgut was tied in place on the finely pulled-out pieces of polyethylene (p.e.) tubing in the perfusion chamber with silk thread. Initially, the mounted gut was bathed and perfused with a dissection medium given in Table 2. Perfusion of the hindgut was accomplished by connecting one end of the gut to a glass syringe. Rate of perfusion could be controlled by elevating the syringe system a given distance above the base of the perfusion chamber. This perfusion of the gut served the function of both flushing out the original gut contents and maintaining a constant internal ionic environment. Rate of perfusion of the medium through the gut was generally about 0·05 ml/min. The potential difference across the hindgut was unaffected by large changes in the rate of internal perfusion. The outside bathing medium in the perfusion chamber could be changed by aspirating the medium out of one end of the chamber, while adding another medium through the other end of the chamber.

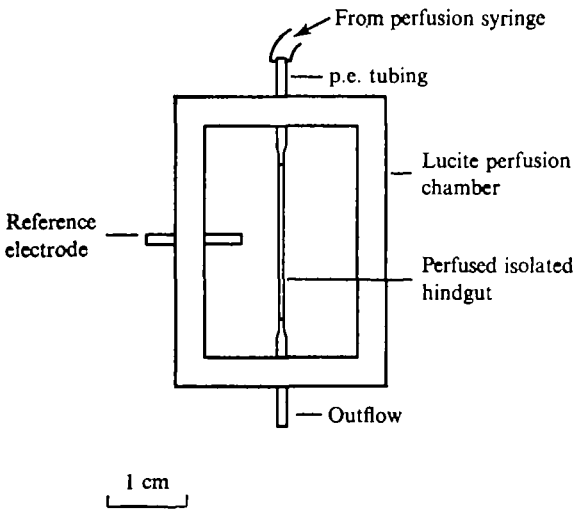


Fig. 1. Diagram of perfusion apparatus. The isolated hindgut was mounted in the chamber and tied on to finely pulled-out pieces of p.e. tubing with silk thread. The gut was then perfused from a syringe system. Potentials were monitored across the gut by connecting both sides of the gut to an electrometer through calomel half cells and agar bridges. The reference bridge was placed in the bath, while the other bridge was placed into the outflow tubing from the chamber.

Electrical potential differences across the wall of the isolated midgut were measured with a Keithley 602 electrometer. A fine piece of p.e. tubing, filled with 0·1 M-KCl in 2% agar, was inserted into the lumen of the perfused gut. The other end of the agar bridge was placed in a beaker of 0·1 M-KCl which also contained a calomel half cell. The outside bathing medium was also connected to a calomel half cell through an agar bridge: this arrangement is shown diagrammatically in Fig. 1. The two calomel half cells were in turn connected directly to the electrometer. Potentials across the gut were read from the electrometer after the system had first been zeroed. Potential measurements *in vivo* were made by placing one agar bridge in the haemolymph of a dissected larva, while another was placed into the open lumen of the hindgut which had been pulled up out of the haemolymph.

RESULTS

In vivo *potential measurements*

The mean potential difference across the intact blowfly hindgut was $31{\cdot}9 \pm 2{\cdot}47$ (S.E.M.) mV. The lumen of the hindgut was negative in respect to the haemolymph in all cases measured (27 determinations).

In vitro *potential measurements*

The potential difference across the isolated hindgut bathed with dissection medium on both sides as a function of time is shown in Fig. 2. Initially, the potential was approximately -25 mV (lumen negative) and then approached 0 mV with time. After 60 min the potential had fallen to $-0{\cdot}61 \pm 1{\cdot}05$ (9) mV (mean ± S.E.M. and the number determinations). If a metabolic energy source is added to the outside medium, in

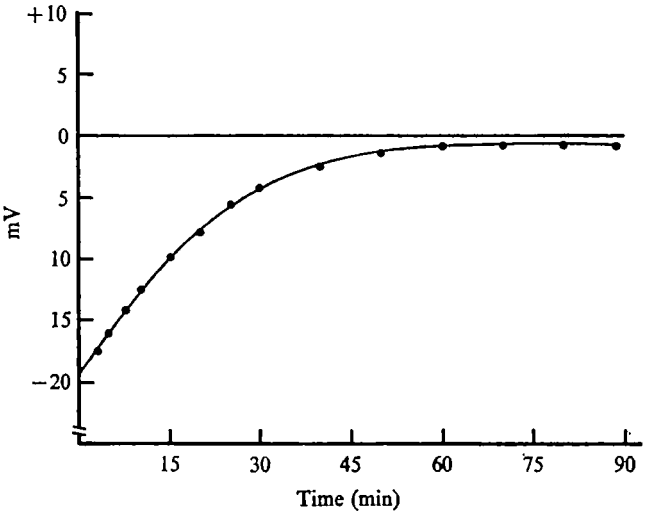


Fig. 2. Potential difference with time across the isolated hindgut equilibrated in dissection medium.

this case 5 mM each of trehalose and glucose, the rate with which the potential falls with time decreases. Furthermore, the level to which it falls is not as great as without the sugars, the potential across the gut under these conditions being $-12{\cdot}3 \pm 1{\cdot}59$ (8) mV.

The potential difference measured across the isolated hindgut resembles that measured *in vivo* in that in both cases the lumen of the hindgut is negative in respect to the outside medium. They differ though in that the potential across the isolated gut decreases slowly with time. Without a metabolic energy source, the potential difference decreases close to 0 mV, while in the presence of metabolic energy sources the potential decreases to only 12 mV. Under these conditions, no ionic gradient exists for any inorganic ions between the outside and inside media. Any potential difference that can be maintained under these conditions cannot simply be a diffusion potential across the gut wall. The fact that the potential difference can be maintained for a longer length of time and at a higher level in the presence of metabolic energy sources indicates that the potential maintained across the isolated hindgut may be due to an active ion transport system in the gut wall.

Effects of external nitrogen sources on the potential difference

It has previously been established that the isolated hindgut of *Sarcophaga bullata* can both move NH_4^+ against its concentration gradient and deaminate various amino acids (Table 1, Prusch, 1972). For these reasons, it was decided to investigate the effects of both free amino acids and NH_4^+ ions on the potential difference across the isolated hindgut. For these experiments the isolated gut was again perfused with the dissection medium, while control medium (Table 2) was placed outside. As in previous experiments, the initial potential was negative and fell with time (Fig. 3). The initial potential was generally around -25 mV, increasing in 30 min to approximately -35 mV and then falling to $-15 \pm 2{\cdot}31$ (11) mV in 60 min. The reason for the c

Table 2. *Experimental media**

Compound	Dissection medium (g/l)	Control† medium (g/l)
NaCl	7·62	7·62
KCl	0·894	0·894
$NaHCO_3$	0·886	0·886
$MgCl_2.6H_2O$	2·0	2·0
$CaCl_2$	1·8	1·8
Proline	—	0·6
Glutamine	—	0·8
α-alanine	—	0·4
Glycine	—	0·5
Trehalose	—	1·8
Glucose	—	1·8

* Adapted from Berridge (1966).
† pH adjusted to 7 with 0·01 N-HCl.

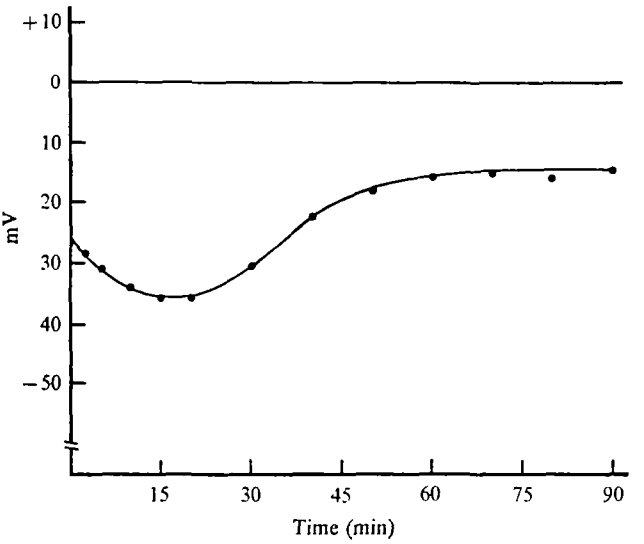


Fig. 3. Potential difference with time across the isolated hindgut equilibrated in control medium.

served initial changes in the potential difference across the hindgut are unknown, but may reflect intracellular compartmental shifts in various ion concentrations when the freshly excised gut is equilibrated in various media, or the absence (or depletion) of some metabolic energy source or control substance required for the maintenance of the potential. The level to which the potential falls in the presence of mixed amino acids does not differ significantly from the level to which the potential falls in the absence of amino acids (Fig. 1), although the initial hyperpolarization of the potential is much greater in the presence of amino acids. There is no difference in the potential across the gut if control medium is perfused through the gut instead of dissection medium, while the outside of the gut is bathed in control medium.

The effects of the addition of 1 mM NH_4^+ to the outside control medium are shown

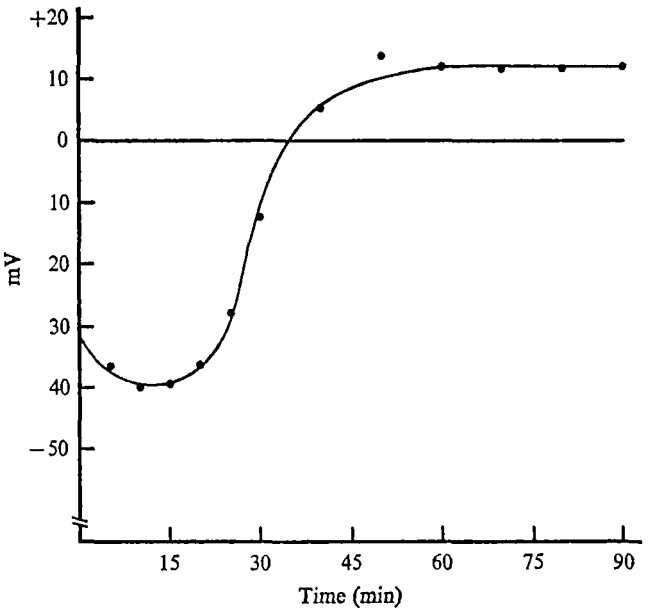


Fig. 4. Potential difference with time across the isolated hindgut equilibrated in control medium containing 1 mM-NH_4^+.

in Fig. 4. As seen in Table 1, the addition of 1 mM-NH_4^+ to the control medium results in a very large increase in NH_4^+ secretion into the hindgut lumen. The increased NH_4^+ secretion is accompanied by a decreased K^+ secretion, while Cl^- secretion remains high into the isolated hindgut. The potential across the hindgut under these conditions initially resembles that without amino acids or NH_4^+ (Figs. 2, 3), but differs significantly in that the potential difference across the gut reverses polarity and the gut lumen is now positive with respect to the outside medium, the potential across the gut after 60 min being $+12 \pm 1{\cdot}38$ (17) mV. Increasing the external NH_4^+ concentration in the outside medium to 10 mM increases the potential difference to $+26 \pm 2{\cdot}14$ (19) mV.

Cl^--free medium

Both *in vivo* and *in vitro*, with dissection or control medium outside the gut, the gut lumen is negative in respect to the outside medium. Since the gut is secreting large amounts of cations under these conditions, to what is this potential difference across the gut due, especially since it has been established that the potential is, in part, metabolically dependent and not due to ionic concentration gradients between the inside and outside media? In order to investigate this problem more directly, the effects of Cl^--free control medium on the potential difference were determined.

The Cl^--free control medium was made up by substituting Cl^- with SO_4^{2-}. The effect of Cl^--free control medium on the potential across the isolated hindgut is shown in Fig. 5; the lumen of the hindgut was perfused with normal control medium, while Cl^--free control medium was outside. As is the case with normal control medium (Fig. 3), the initial potential was negative. But in the case of the Cl^--free medium t

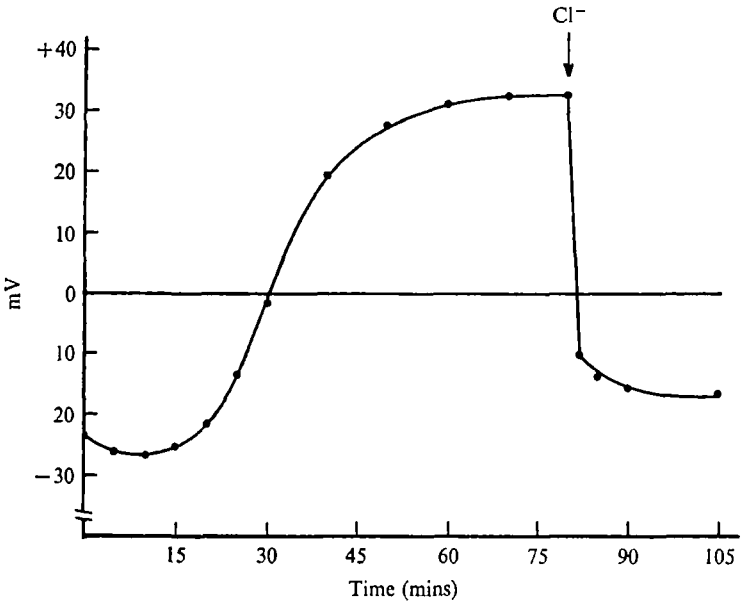


Fig. 5. Potential difference with time across the isolated hindgut equilibrated in Cl^--free control medium. Initially the gut was bathed in Cl^--free control medium and then normal control medium was added at 80 min (indicated by the arrow).

potential fell to 0 mV in about 30 min and then reversed polarity, so that the gut lumen now becomes positive. In the control medium, the potential difference across the hindgut was -15 mV after 60 min, while in the Cl^--free control medium the potential was $+32{\cdot}5 \pm 2{\cdot}3$ (14) mV after 60 min and remained at this level for at least another 60 min.

If the lumen of the gut is perfused with Cl^--free control medium, while the outside of the gut is bathed in Cl^--free control medium, no difference in potential is observed from when the gut was perfused with control medium (Fig. 5). On the other hand, there is no difference in the potential across the gut compared with the control situation (Fig. 3) when the gut is perfused with Cl^--free medium, while normal control medium is outside the gut. That is, the potential difference across the isolated hindgut is sensitive to changes in Cl^- concentration only in the outside medium and not to changes in Cl^- concentration at the luminal surface.

In order to determine whether or not this effect of Cl^--free medium on the potential is reversible, the Cl^--free medium bathing the outside of the hindgut was replaced with normal control medium. As was stated previously, the positive potential maintained in Cl^--free medium is stable for at least 90 min. If normal control medium replaces the external Cl^--free medium at 80 min (Fig. 5) the potential, instead of remaining at its previous positive level, decreases rapidly and again becomes negative, closely approaching the control level potential of -15 mV. This relatively fast change in potential due to the change in external Cl^- concentration demonstrates the reversibility of ion changes on the potential across the isolated hindgut, but it also suggests that the initial slow change in potential observed under most recording conditions is due to time required to exhaust the ion at the pump site, or changes in ion com-

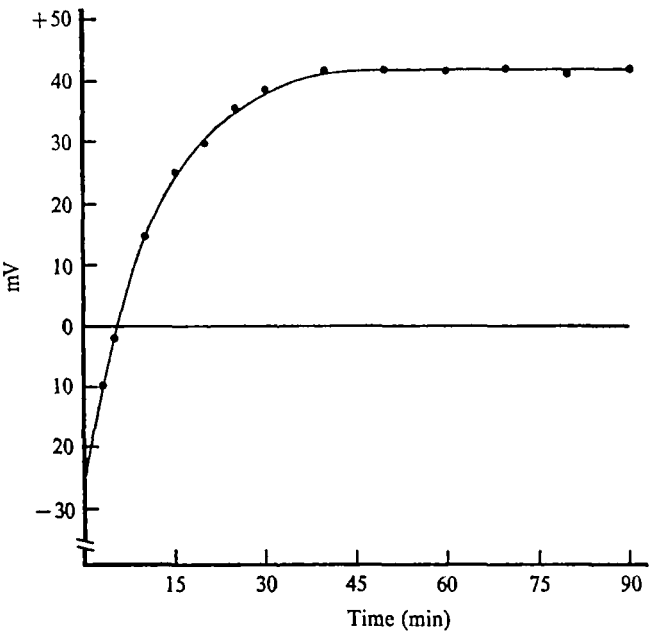


Fig. 6. Potential difference with time across the isolated hindgut equilibrated in Cl^--free control medium containing 1 mM NH_4^+.

partmentalization at the pump site, as was originally suggested. If this were the case, then it should have taken just as long to reach a new steady-state potential level when Cl^- was reintroduced into the outside medium as it did when the gut was initially exposed to Cl^--free medium (Fig. 5). This leaves the possibility that the initial changes in potential across the isolated gut may be due to the exhaustion of some exogenous metabolic energy source or to the elimination of some control substance.

The effect of adding 1 mM-NH_4^+ to the Cl^--free control medium is shown in Fig. 6. The potential difference under these conditions was similar to the potential change with time in the Cl^--free medium (Fig. 5), but in this case the potential difference switched polarity faster and became more positive after 60 min equilibration, $+41 \pm 2{\cdot}51$ (8) mV. The addition of NH_4^+ to the Cl^--free medium results in a much faster response in the recorded potential than when it is added to normal control medium (Fig. 4). The reason for this difference is not known, but it may reflect an increased NH_4^+ permeability in the absence of external Cl^-.

K^+-free medium

The positive potential elicited across the isolated hindgut in Cl^--free medium is interesting, but inconclusive as to what is the basis of the potential in the blowfly larva hindgut. Since the potential across the hindgut is negative in the control medium and *in vivo*, and because the potential becomes positive in the absence of Cl^-, the potential may arise in part from the electrogenic transport of chloride. The cations secreted into the gut lumen could then follow passively down an electrical gradient, although against their chemical gradient. But if the cations follow the transport of Cl^- passively, it is difficult to explain how the hindgut lumen becomes positive when

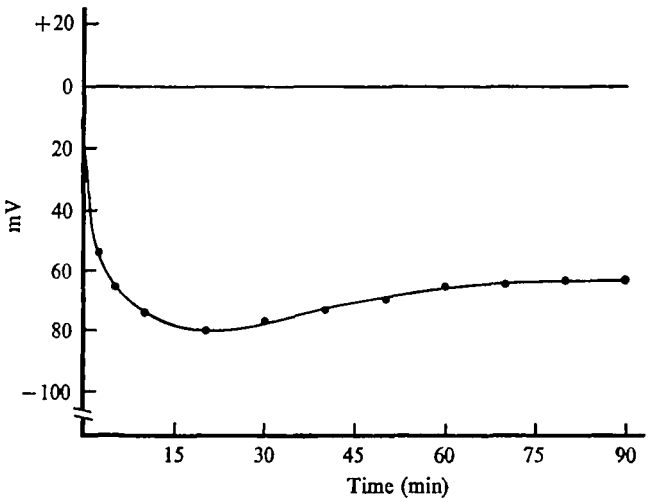
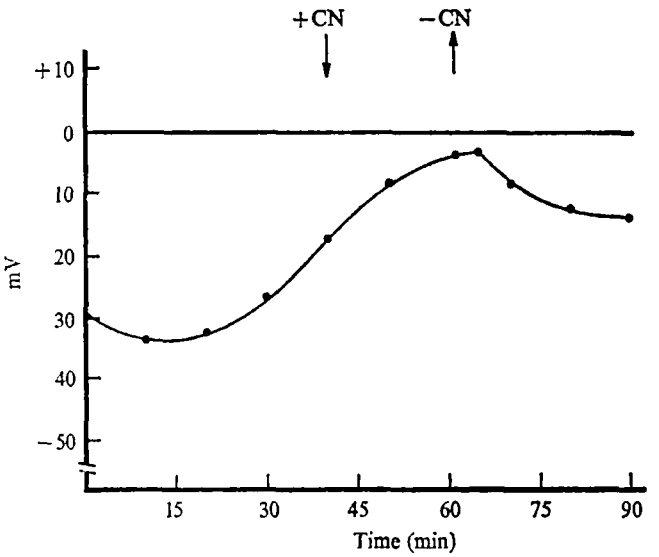


Fig. 7. Potential difference with time across the isolated hindgut equilibrated in K^+-free control medium.

is removed from the control medium and no cation gradient exists across the hindgut wall, unless an active cation transport system is involved as well. In order to investigate this possibility, the effects of K^+-free control medium on the hindgut potential difference were examined; K^+ being the major cation secreted by the isolated hindgut under these conditions (Table 1).

K^+-free control medium was made up by simply deleting the KCl normally added to the control medium (see Table 2). Since KCl makes up only a small fraction of the medium, it contributes approximately 6 per cent to the total osmolality; its deletion does not significantly change the total osmolality or ionic strength of the medium. Also, since only the KCl is deleted, the ratio of the remaining ions remains the same.

Fig. 7 shows the change of potential with time when the hindgut is exposed to K^+-free control medium. The potential begins at the same level as in the control medium, rapidly hyperpolarizes, and then slowly depolarizes, but not at the same rate or to the same level as in the control medium. In K^+-free control medium, the potential difference is $-62{\cdot}3 \pm 3{\cdot}93$ (15) mV after 60 min equilibration. Conceivably, the hyperpolarization of the hindgut transepithelial potential brought about under these conditions could come about, in part, by the effect of K^+ on the membrane potential of the hindgut cells. Decreasing the external K^+ concentration should increase or hyperpolarize the potential across the haemolymph side or basal membrane of the hindgut epithelial cells, which could contribute to the hyperpolarization of the transepithelial potential seen in K^+-free control medium. The effect of this hyperpolarization on the hindgut potential is presently unknown and can only be resolved by measuring intracellular potentials. Its actual effect may be small when it is considered that the contribution of K^+ to the membrane potential may be much less than that expected from the Nernst relationship. When external K^+ is decreased to very low levels, changes in membrane potential are much smaller than expected, presumably due to decreased K^+ permeability at low external K^+ levels or increased Na^+ permeability [illegible]rman, 1960). Perfusion of the hindgut lumen with $K\frac{1}{2}$-free control medium, with



ig. 8. The effects of CN (10^{-4} M) on the potential difference across the isolated hindgut. CN was added externally at 40 min and removed 60 min after the beginning of the experiment.

normal control medium outside, elicits no observable difference in potential from the control level.

Metabolic inhibitors

In order to determine further whether or not the potential difference across the isolated hindgut is metabolically dependent, the effect of several metabolic inhibitors, including anoxia, dinitrophenol (DNP), cyanide (CN) and iodoacetic acid (IAA) was determined. Anoxia was induced by bubbling N_2 gas into the perfusion chamber, while monitoring the potential difference across the isolated hindgut bathed and perfused with control medium, resulting in an almost immediate and reversible decrease in potential. Both CN and DNP (10^{-4} M) also brought about reversible decreases in the transgut potential. Fig. 8 shows the effect of 10^{-4} M-CN applied externally on the potential recorded across the hindgut in control medium (compare with Fig. 3). The potential measured across the hindgut in control medium initially declines and levels off at -15 mV. If Cn is added to the control medium, the potential does not level off at this level, but continues to fall toward zero. This reduction in potential by CN is generally reversible, as is shown in Fig. 8. Alternatively, if CN is present for a longer period of time, so that the potential actually becomes zero, the effect is rarely reversible. IAA, up to 5×10^{-4} M, had no observable effect on the potential difference.

DISCUSSION

The potential difference which exists across the hindgut of the blowfly larva is the result of a metabolically dependent, electrogenic ion-transport system. The dependence of the potential on exogenous energy sources, such as trehalose and glucose, as well as the reversible decrease in the potential in the presence of metabolic inhibitors, supports the conclusion that the potential is indeed metabolically dependent. That

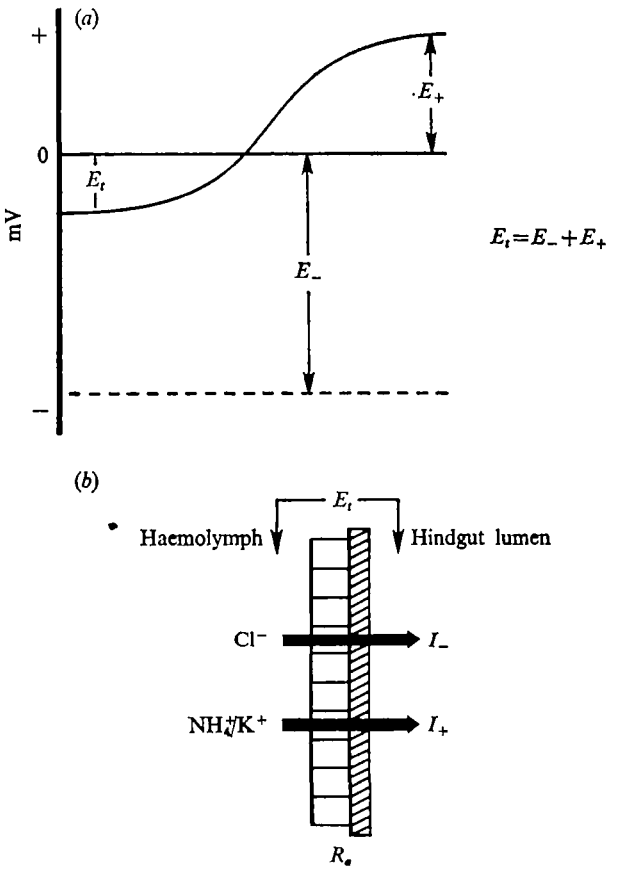


Fig. 9. Diagrammatic representation of the electrical events occurring across the hindgut. (*A*) Graphical summary of potential measurements across the isolated hindgut; E_t, potential across gut in control medium; E_+, potential measured in Cl^--free control medium and representing the potential generated by cation movement, and E_-, the potential measured in K^+-free control medium representing the potential generated by anion movement. (*b*) Schematic drawing of the hindgut (the hatched area representing the hindgut cuticle): I_- and I_+ representing anion- and cation-generated currents moving across the gut resistance, R_g.

potential difference is simply an ionic diffusion potential is ruled out by perfusing both sides of the gut with identical ionic solutions and still observing a transgut potential, and by the sensitivity of the transgut potential to changes only in external ion concentration. Changes in the polarity and level of the potential reflect changes in the rate of at least two different pumping mechanisms which exist across the wall of the hindgut. One of these pumping systems is anion-specific, and most probably moves Cl^- from the outside medium into the hindgut lumen, while the other pumping mechanism is cation-specific and will transport either K^+ or NH_4^+ into the hindgut lumen. The specificity of the cation pump is such that it will move K^+ against its concentration gradient under normal conditions, but in the presence of NH_4^+ it switches to a predominantly NH_4^+ transport system.

A diagrammatic representation of the hindgut is shown in Fig. 9. The overall transgut potential, E_t, is the algebraic sum of two separate potentials, E_-, the potential difference generated by the anion pump and E_+, the potential difference generated

by the cation pump. That is, $E_t = E_- + E_+$.* If this is the case, the final level of t[illegible] potential difference shown in Fig. 5 in Cl^--free control medium (+32·5 mV) should represent E_+. Removal of chloride decreases E_- to zero and now $E_t = E_+$. Alternatively, removal of K^+ from the control medium, K^+ being the major cation secreted into the hindgut under these conditions, should set E_+ to zero, so that now $E_t = E_-$. From Fig. 7 it is seen that $E_- = -62{\cdot}3$ mV. Since $E_t = E_- + E_+$, then $E_t = -62{\cdot}3 + (+32{\cdot}5) = -29{\cdot}8$ mV. The measured potential difference in the normal control medium, or E_t, is -15 mV. Although the experimentally measured value of E_t in the control medium qualitatively resembles that of the calculated value of E_t in both polarity and decreased level from E_-, quantitatively it is different. This discrepancy between the measured and calculated values of E_t indicates that the simplistic model representing transport processes across the hindgut epithelium needs modification. For example, it is known that Na^+ is also secreted into the hindgut lumen when the gut is equilibrated in the control medium (Table 1). Although K^+ is the major cation secreted into the hindgut from the control medium and probably carried most of the positive charge across the gut wall, Na^+ may also contribute in part to the potential difference. If this is true, then in K^+-free medium, E_+ may not be zero, resulting in erroneously calculated E_t values. The error in calculating E_t if E_+ is not zero under K^+-free conditions could be compounded if the charge carried by Na^+ increased under these conditions. This model may have to be revised after unidirectional flux measurements and membrane potentials have been determined under these same conditions.

Although the electrical potentials in the hindgut of the blowfly larva cannot yet be fully quantified, their measurement has added considerably to the elucidation of the ionic events occurring in the hindgut of this animal. First of all, chloride is transported against an electrochemical potential gradient from the haemolymph into the gut lumen. From Table 1, it is seen that the isolated hindgut accumulates Cl^- and it is now known that the hindgut lumen is electrically negative in respect to the outside medium. Secondly, it appears that the hindgut is capable of transporting K^+ and/or NH_4^+. Active K^+ transport is indicated by the large K^+ concentration gradient that can be maintained across the isolated hindgut and the shift to a positive potential when chloride is removed. If only Cl^- were actively moved across the gut wall, then its removal (provided no cation concentration gradient existed) would eliminate the potential difference across the gut. Evidence for NH_4^+ transport comes from the following observations: (1) the large concentration gradient can be maintained for NH_4^+, (2) its competition with K^+ for secretion into the hindgut and (3) the increase in the positive level of the transgut potential when a small amount of NH_4^+ is added to the outside medium.

Since it appears that both K^+ and Cl^- are actively transported into the isolated hindgut at the same time and a potential difference exists, the rate at which K^+ and Cl^- are moved into the hindgut cannot be the same. If both K^+ and Cl^- were being moved at the same rate into the hindgut lumen when the gut is equilibrated in the control medium, assuming that these are the major substances being actively moved, then the potential difference across the gut should be zero instead of the observed

* More correctly, $I_t = I_- + I_+$ where I_- is the current generated by anion transport and I_+ is the current generated by cation transport, but the potentials can be added, given that R_g, the gut resistance, is relatively constant.

gative value. Conceivably, Cl^- is being moved into the hindgut lumen faster than K^+, which should result in a greater change in Cl^- concentration across the gut wall than in K^+ concentration. Since this is not the case (Table 2), i.e. the K^+ concentration gradient is greater than that for Cl^-, the rate of Cl^- transport cannot exceed that of the K^+ transport rate, unless a relatively large 'leak' or passive backflux exists for Cl^- across the gut. If this were the case, Cl^- would be transported faster than K^+; the gut lumen would be negative; but the Cl^- concentration gradient established would be smaller than the K^+ gradient due to a greater Cl^- backflux from the lumen to the outside medium. Alternatively, various 'shunt' or extracellular pathways may exist for part of one or both of the active transport systems (Frizzell & Schultz, 1972), which would not contribute to the observed total transgut potential difference.

The cation pumping mechanism in the hindgut epithelium will move either K^+ or NH_4^+ against its concentration gradient into the hindgut lumen. The specificity of this pumping mechanism is much greater for NH_4^+ than for K^+, as demonstrated by the concentrations that can be maintained for K^+ and NH_4^+ (Table 1) and from the present electrical measurements. In the absence of free NH_4^+ in the external control medium, the hindgut secretes and maintains an approximate tenfold concentration gradient for K^+. Addition of 1 mM-NH_4^+ to the control medium results in decreased K^+ secretion and establishment of a 100-fold NH_4^+ concentration gradient.

The potential difference maintained across the hindgut equilibrated in the control medium is -15 mV, but addition of 1 mM NH_4^+ outside brings about a polarity reversal, so that the potential across the gut is now $+12$ mV (Fig. 4). If the total potential difference across the gut E_t is the sum of two separate electrogenic potentials E_+ and R_-, then E_+, due to electrogenic cation movement, must be greater when NH_4^+ is being transported than when the pump site is occupied by K^+. Alternatively, E_-, the potential generated by Cl^- secretion, could decrease during NH_4^+ secretion. But increased Cl^- concentration in the hindgut (Table 1) under these conditions argues against a decrease in E_-. Both the concentration gradient maintained for NH_4^+, as compared to the gradient maintained for K^+ in the hindgut, and the increase in E_+ in the presence of NH_4^+, indicates that the cation pumping mechanism is much more specific for NH_4^+ than for K^+ even though both ions probably compete for the same transport mechanism.

Active secretion of K^+ into the hindgut lumen is similar to the situation in the midgut of *H. cecropia* larva in which K^+ is also actively transported into the midgut lumen (Harvey & Nedergaard, 1964). Active K^+ secretion in Cecropia midgut is also similar to K^+ secretion in *Sarcophaga* hindgut in that the K^+ secretion in Cecropia is electrogenic (Harvey, Haskell & Nedergaard, 1968) and the potential difference in Cecropia midgut is sensitive to various metabolic inhibitors (Haskell, Clemons & Harvey, 1965). Structurally, the two systems are quite different. Cecropia midgut consists of a single layer of two cell types (Anderson & Harvey, 1966), while the hindgut of the blowfly larva has a single cell layer which may consist of three different cell types arranged in longitudinal rows along the hindgut cuticle as observed in *Lucilia* (Waterhouse, 1955).

Functionally, the two systems are even more different. The Cecropia midgut is basically a system for transporting large amounts of K^+ from the haemolymph into e gut lumen. When the K^+ concentration of the haemolymph side bathing medium

is reduced, the K^+ transport system can move caesium (Zerahn, 1970), Na^+ or [redacted] (Harvey & Zerahn, 1971) into the midgut lumen. Changes in external Cl^- concentration have no effect on the transgut potential difference in Cecropia midgut (Harvey *et al.* 1968); and K^+ in Cecropia cannot be replaced by NH_4^+ (Nedergaard & Harvey, 1968). The electrogenic transport system in *Sarcophaga* larva moves both K^+ and Cl^- into the gut lumen; and NH_4^+ competes at relatively low concentrations with K^+ secretion. The hindgut of the blowfly larva serves primarily in the active excretion of NH_4^+/K^+ and Cl^-.

Potential measurements in the midgut of the cockroach have demonstrated the presence of a linked Na^+–K^+ pump mechanism (O'Riordan, 1969). The lumen of the midgut is approximately 12 mV negative in respect to the outside medium and is again decreased by the application of various metabolic inhibitors. The cockroach midgut resembles that of Cecropia in that there is no apparent anion transport. Substitution of SO_4^{2-} for Cl^- on both sides of the isolated cockroach midgut resulted in an increased potential across the gut. This indicates that Cl^- movements follow passively the active movements of cations in this system; and SO_4^{2-} ions being presumably much less permeable than Cl^- ions result in greater charge separation and consequently a higher potential.

The isolated hindgut of *Sarcophaga* larva is capable of moving both anions and cations against their electrochemical potential gradient into the hindgut lumen. Energetically, it would cost less if either a cation or an anion, but not both, was transported across an epithelial layer with the co-ion following passively. The most obvious advantage, obtained at the expense of increased metabolic energy in transporting both ionic species across the epithelial layer, is the resulting fine degree of control of ion movements into the gut lumen. Transport of only one ion species across an epithelial barrier would result in the control of the transport system being regulated almost entirely by concentrations of the transported species at the transport site, while changes in co-ion concentration, especially increases in co-ion concentration at the transport site, probably would not change the rate of primary ion transport.

In systems where both the cation and anion are both transported, e.g. *Calliphora* salivary glands (Berridge & Prince, 1971), they may be under separate control. Cation movement in the salivary gland appears to be influenced by cyclic AMP, while anion movement is increased by the presence of 5-hydroxytryptamine. The mechanism of control of ion movements in *Sarcophaga* larval hindgut is presently unknown, but it is conceivable that the cation and anion transport systems are also under separate control. Further studies of this system, including measurements of unidirectional ion fluxes and short-circuit current, may lead to much more information concerning ionic movements in the blowfly hindgut and the control of these ion movements.

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