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ACTIVE TRANSPORT BY THE CECROPIA MIDGUT

III. MIDGUT POTENTIAL GENERATED DIRECTLY BY ACTIVE K-TRANSPORT

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INTRODUCTION

The isolated midgut of the fifth-instar larva of *Hyalophora cecropia* maintains a potential difference in excess of 100 mV., the lumen positive to the blood-side, for several hours with identical solutions bathing both sides. During this time, the tissue actively transports approximately 20 μ-equiv. of potassium per cm.^{2} of gut tissue per hour from the blood-side to the lumen. The present paper deals with the role of the active K-transport in establishing the electrical potential difference.

To date, potential differences arising in systems transporting sodium have been investigated more thoroughly than those in systems transporting potassium. The main subjects have been plasma membranes of cells such as neurons and red blood cells and epithelial membranes such as those in the frog skin and toad bladder. It has been customary to assume that active ion transport across epithelial membranes is accomplished by active transport across some area of the plasma membrane of at least some of the cells making up the epithelium. The simplest and most widely accepted hypothesis is that both cellular and epithelial potentials are due to the passive diffusion of ions between the interior and exterior of cells. This explanation requires that there be a mechanism capable of maintaining an uneven distribution of ions between the cells and their surroundings. The best documented mechanism, the active transport of sodium, is alleged to help maintain a low sodium and a high potassium concentration inside cells, and requires not only sodium ions but also small amounts of potassium ions in the bathing solutions. Potassium is thought to be the counter-ion for sodium in a non-electrogenic active process, i.e. a process in which sodium is actively transported out of the cell and an equal amount of potassium passes into the cell. A small potential at most is generated by this active exchange but large passive diffusion potentials arise secondarily from the higher potassium concentration inside cells and the higher sodium concentration outside (e.g. Hodgkin & Huxley, 1952).

To extend this explanation of active cellular ion transport to transepithelial ion transport seemed straightforward in the early paper of Koefoed-Johnsen & Ussing (1958). As the years have passed the model has been made more complex to encompass newer experimental findings, but recent models of the origin of the frog-skin potential (e.g., Ussing, 1966) still retain the concept that active transepithelial Na-transport is based on cellular Na-transport and (less strongly) that the Na-transport is non-electro-

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genic with potassium serving as the counter-ion for the actively transported sodium (Ussing, 1965).

From the outset it has not been easy to account for the Cecropia midgut potential on the basis of diffusion potentials arising secondarily from a cellular mechanism of non-electrogenic active K-transport. The only process resembling a diffusion potential in the isolated midgut is a decrease in the midgut potential when the blood-side potassium concentration is lowered. If the effects of the change in blood-side potassium concentration on the potential were to arise from changes in the rate of diffusion of potassium between cell interior and blood-side compartment, they would detract from the trans-epithelial potential (Harvey & Nedergaard, 1964).

To investigate whether the central idea of the origin of potentials in sodium-transporting cells or epithelia applies to potassium systems, i.e. to examine the hypothesis that potassium being actively transported across the midgut exchanges across some cell membrane with some other ion in a non-electrogenic process and that the midgut potential arises secondarily from diffusion potentials of resultant unequally distributed ions, the midgut system has been studied in further detail. The effects on the midgut potential of cations (potassium, sodium, magnesium, calcium, and rubidium) and of anions (chloride, sulphate, and isethionate) were studied.

The results show that the midgut potential cannot be explained in terms of a diffusion potential of any ion present or a combination of diffusion potentials of several ions, because the potential is very little dependent on sodium, calcium, and magnesium and is maintained without these ions. The only cation required is potassium. It is necessary to postulate that the active transport of potassium across the midgut is electrogenic.

MATERIALS AND METHODS

In the following paper of this series Nedergaard & Harvey (1968) describe in detail the rearing, narcotization and dissection of *Hyalophora cecropia* larvae. They describe the method for isolating and cannulating the midgut, the measurement of the midgut potential and the short-circuit current, and the composition of the solution (S 1) normally used to bathe the isolated midgut (Table 1). To express the effects of changes in external ion concentration on the midgut potential, the slow decay of the potential (see Results) must be taken into account. Moreover, several minutes may elapse after the concentration of an ion is changed before a new steady-state potential is attained. For these reasons the value of the potential in the experimental solution was subtracted from the average value of the potential in the standard solution just prior to the concentration change and just after the standard solution was restored. In this way increases in midgut potential are given a plus (+) sign which indicates that the lumen has become more positive to the blood-side, whereas decreases are given a minus (−) sign which indicates that the lumen has become more negative. In some cases the slope of the potential/log (C^+) curve has been calculated and expressed as follows:

$$\frac{E_{st}-E_{ex}}{\log [C^+_{st}]-\log [C^+_{ex}]} = \frac{\Delta E}{\Delta \log [C^+]},$$

where C is any univalent cation and the suffixes st and ex refer to standard and experimental conditions respectively.

RESULTS

(1) *The midgut potential*

When thoroughly aerated and bathed in the standard sodium-free physiological saline (S_1) described in Table 1 the midgut isolated from a mature fifth-instar larva produced a potential as high as 152 mV., the lumen-side always being positive to the

Table 1. *Composition of solutions* (mM/l.) *used to bathe the isolated midgut*

	32 mM./l. (S_1)	Choline	Added Na	Sulphate	Isethionate
KCl	30	30	30	—	—
$KHCO_3$	2	2	2	2	2
$CaCl_2$	5	5	5	—	—
$MgCl_2$	5	5	5	—	—
Sucrose	166	102	102	192	196
Choline chloride	—	32	—	—	—
NaCl	—	—	32	—	—
K_2SO_4	—	—	—	15	—
$CaSO_4$	—	—	—	4·5	—
K isethionate	—	—	—	—	30
$MgSO_4.7H_2O$	—	—	—	5	—

blood-side. The average time-course of the maintenance of the potential in eight preparations is presented as Fig. 1. An initial increase in magnitude of the potential almost invariably occurred while the midgut was equilibrating in the physiological solution. The time of maximum potential was arbitrarily designated zero time and the

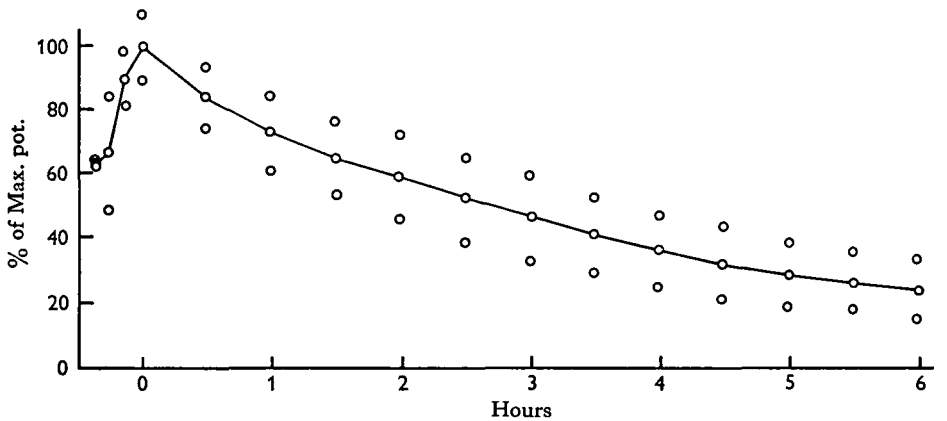

Fig. 1. Time-course of midgut potential. The points on the line represent mean values for eight preparations and the points above and below the line represent ± 1 standard deviation. For this series the average maximum potential (100%) was 93 mV.

magnitude (average of 93 mV.) was assigned a value of 100% in Fig. 1. The other points falling at half hour intervals from time zero are averages of the percentage of the maximum potential remaining at the indicated time. The time that elapsed between the start of cannulation of the midgut and the first potential reading was usually about 3 min. The bathing solutions were replaced every half hour during the measurements for Fig. 1. The experiments involving changes in ion concentrations in the bathing

solutions to be described in the following sections were begun only after the potential had reached its maximum and begun its characteristic slow decline.

(2) *Potassium concentration and the midgut potential*

When the potassium concentration was decreased in the solution bathing the blood-side of the midgut from 32 to 10 mM/l., by replacing part of the potassium by sodium or choline, the midgut potential decreased by an average of 22 mV. in fourteen experiments (Tables 2 and 3). A decrease from 32 to 2 mM/l. K^+ on the blood-side led to a

Table 2. *Effects on the midgut potential of changing the potassium concentration in the solution bathing the midgut from* 32 *to* 10 mM/l.

Prior E in 32 mM/l. K^+ (mV.)	E in 10 mM/l. K^+ (mV.)	Subsequent E in 32 mM/l. K^+ (mV.)	ΔE (mV.)	$\frac{\Delta E}{\Delta \log [K^+]}$ (mV.)
		Lumen		
80	74	66	+ 1·0	− 2·0
78	73	59	+ 4·5	− 8·9
100	98	85	+ 5·0	− 9·9
58	64	52	+10	−19
63	66	60	+ 3·5	− 5·5
42	52	44	+10	−19
100	100	91	+ 3·0	− 5·9
109	103	89	+ 4·0	− 7·9
52	47	30	+ 6·0	−12
103	99	82	+ 6·0	−12
Mean			+ 5·2	−10
		Blood side		
91	65	50	−28	+55
46	22	38	−20	+40
85	58	76	−22	+44
106	89	100	−14	+28
80	58	70	−18	+35
70	36	54	−26	+52
46	24	43	−21	+42
59	25	42	−25	+50
78	61	70	−13	+26
94	56	72	−26	+52
95	71	91	−22	+44
62	30	48	−25	+48
43	15	38	−26	+51
51	30	43	−17	+34
Mean			−22	+43
		Both sides		
64	44	45	−10	+20
79	60	67	−13	+26
78	64	67	− 8	+16
105	78	83	−16	+32
Mean			−12	+24

larger drop in potential (41 mV.). Increasing the potassium concentration on the blood-side from 32 to 64 mM/l. increased the midgut potential 5 mV. and from 32 to 128 mM/l. decreased it 9 mV. on the average (Table 3). The size of these potential changes depends on the size of the potential prior to the concentration change, the

slope of the potential/log [K^+] curve increasing with prior potential (Haskell, Harvey & Clark, 1968). Although the value of the slope appears to approach 59 mV. with prior potentials in the vicinity of 130 mV., there is no indication that 59 mV. is a limiting value and the agreement may be coincidental.

Table 3. *Effects on the midgut potential of changing the potassium concentration in the solution bathing the isolated midgut from* 32 mM/l. [K^+_{st}] *to the values listed in column* 2 [K^+_{ex}]

No. of experiments	mM/l. [K^+_{ex}]	Prior E in 32 mM/l. K^+ (mV.)	$\Delta E \pm$ S.E. (mV.)	$\frac{\Delta E}{\Delta \log [K^+]} \pm$ S.E. (mV.)
		Lumen		
9	0·2	46	+12±0·5	−5·3±0·9
18	2	54	+8·7±1·1	−7·2±1·2
10	10	79	+5·2±0·9	−10±1·7
2	64	54	−12	−41
2	128	40	−9·8	−17
		Blood side		
6	0·2	43	−42±7·8	+19±3·5
18	2	59	−41±3·3	+34±2·1
2	3	52	−37	+36
14	10	72	−22±1·3	+43±2·5
14	64	74	+5·2±1·9	+17±6·6
8	128	42	−8·9±4·3	−16±8·7
		Both sides		
2	0·2	31	−20	+9±4·0
11	2	40	−26±3·2	+22±2·5
4	10	82	−12±1·7	+24±3·5

The potassium concentration was similarly changed on the lumen side from 32 mM/l. to the same concentrations as just reported for the blood-side. Decreases in potassium concentration on the lumen-side led to small potential increases and increases in potassium concentration led to small potential decreases (Table 3).

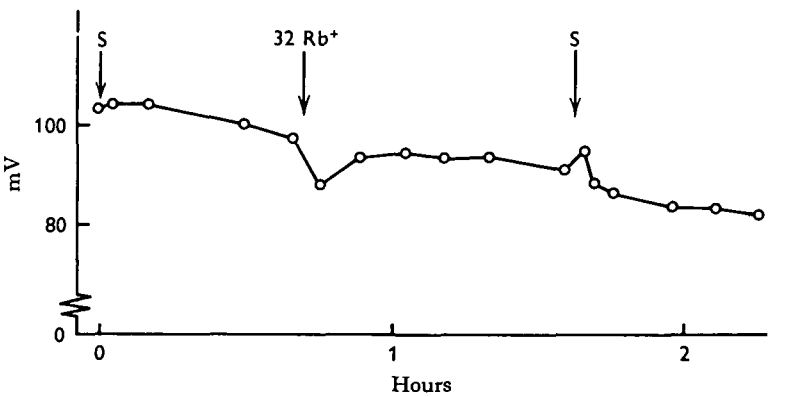

Fig. 2. Effects of replacing all of the potassium in both bathing solutions by rubidium. At the time marked 32 Rb^+ all of the potassium was replaced by rubidium and at the time marked S the standard bathing solution was restored.

When the potassium concentration was decreased from 32 mM/l. to 2, and 10 mM/l. on both sides of the midgut simultaneously, the potential decreased by 26, and 12 mV., respectively (Table 3).

(3) *Rubidium*

The ion which is chemically most similar to potassium is rubidium. The midgut potential is unaffected when all the potassium in the bathing solution is substituted by rubidium (Fig. 2). The potential can be maintained as well by solutions containing rubidium as by solutions containing potassium. Moreover, the effects of changing rubidium concentrations are similar to those of changing potassium concentrations, e.g. a decrease in rubidium concentration on the blood side from 32 to 2 mM/l. resulted in a potential drop of about 30 mV. (Fig. 3). A drop in rubidium concentration from 32 to 2 mM/l. yielded a Nernst slope of $+ 30 \pm 3{\cdot}3$ mV. (average of six experiments) compared to the $+ 34 \pm 2{\cdot}1$ mV. slope already reported for potassium in Table 3. We do not consider the difference to be significant.

(4) *Sodium*

The midgut potential is independent of the presence or absence of sodium ions (Harvey & Nedergaard, 1964). In order to add as much as 32 mM/l. sodium chloride without changing the osmolarity of the bathing solution, the amount of sucrose was reduced but the total ionic concentration was increased to as much as 270 m-equiv/l.

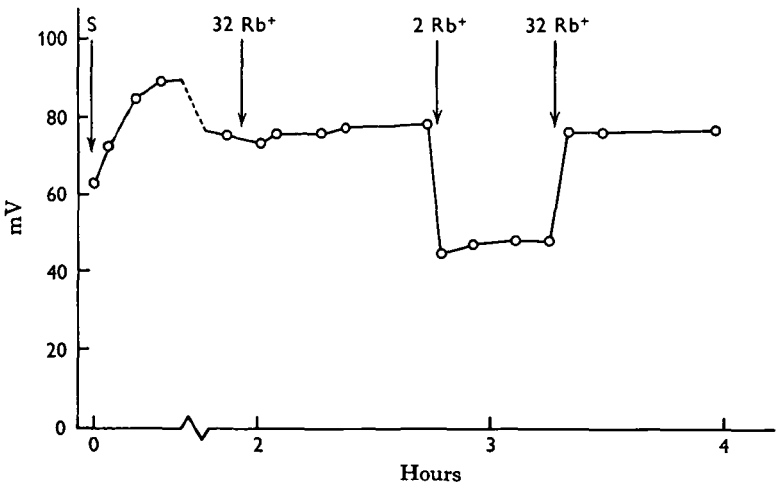

Fig. 3. Effects of changing rubidium concentration on the blood side of the isolated midgut. At the time marked 2 Rb^+ the rubidium concentration was diminished from 32 to 2 mM./l. and at the time marked by the second 32 Rb^+ the original rubidium concentration was restored.

Solutions with added choline chloride were tested and found to have small unpredictable effects on the potential. When finally the bathing solutions were changed from the sodium-free, choline-containing variation of the standard solution to solutions in which as much as 32 m-moles/l. of choline chloride were replaced by sodium chloride, no significant effect on the potential was detected (Table 4).

Table 4. *Effects on the midgut potential of adding sodium to the solutions bathing the isolated midgut*

No. of experiments	mM/l. $[Na^+_{ex}]$	Prior E in 0·01 mM/l. Na^+ (mV.)	ΔE (mV.)
		Lumen	
1	2	108	+7·0
1	10	79	+0·5
1	30	69	+2·0
7	32	54	−2·5
		Blood side	
3	2	51	−0·8
3	10	49	+1·0
3	30	36	−0·3
7	32	57	−1·2
		Both sides	
7	32	50	−0·9

(5) *Magnesium*

The magnesium concentration in the standard bathing solution, S-1, is 5 mM/l. The effect of magnesium on the midgut potential was studied by changing the magnesium concentration to zero, using sodium as a replacement ion, and by increasing the magnesium concentration to as much as 20 mM/l. by replacing part of the sucrose by magnesium chloride. It is clear both from the typical experiment shown in Fig. 4, and the results of seventy-six similar experiments summarized in Table 5, that magnesium has no effect on the midgut potential.

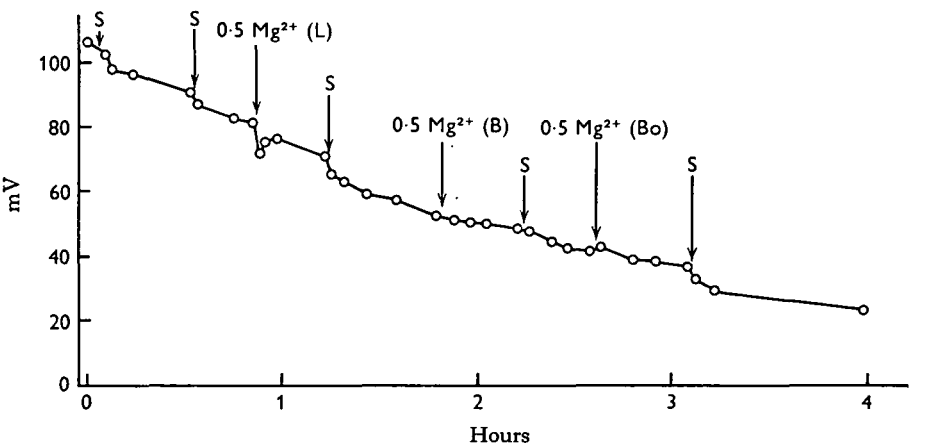

Fig. 4. Trivial effects of changing the magnesium concentration in bathing solutions from its standard value of 5 mM./l. to 0·5 mM./l. In this figure and subsequent ones, the symbol *S* stands for the standard solution, (*L*) for changes on the lumen-side, (*B*) for changes on the blood-side, and (*Bo*) for simultaneous changes on both sides.

Table 5. *Effects on the midgut potential of changing the magnesium concentration in the solution bathing the isolated midgut from* 5 mM/l. [Mg^{2+}_{st}] *to the values listed in column* 2 [Mg^{2+}_{ex}]

No. of experiments	mM/l. [Mg^{2+}_{ex}]	Prior E in 5 mM/l. Mg^{2+} (mV.)	ΔE (mV.)
		Lumen	
13	0	56	+1·5
6	0·5	52	−3·2
4	2	50	−1·2
4	10	55	−0·2
4	20	60	−0·2
		Blood side	
11	0	52	+1·4
4	0·5	62	+3·8
4	2	53	+0·5
4	10	31	−0·7
7	20	54	−1·3
		Both sides	
16	0	44	+1·3

(6) *Calcium*

The effects of calcium on the midgut potential were investigated in the same concentrations as magnesium. The results are shown in Table 6 and Fig. 5. In general, the effects are small and inconsistent; however, in one series of experiments (marked

Table 6. *Effects on the midgut potential of changing the calcium concentration in the solutions bathing the isolated midgut from* 4·5 mM/l. [Ca^{2+}_{st}] *to the values listed in column* 2 [Ca^{2+}_{ex}]

No. of experiments	mM/l. [Ca^{2+}_{ex}]	Prior E in 4·5 mM/l. C^{2+} (mV.)	$\Delta E \pm$ S.E. (mV.)	$\frac{\Delta E}{\Delta \log [Ca^{2+}]} \pm$ S.E. (mV.)
		Lumen		
10*	0·5	62	−14 ± 1·0	+14 ± 1·0
4	0·5	61	−1·8 ± 1·1	+1·8 ± 1·1
4	2	65	−1·5 ± 1·3	+4·2 ± 3·7
4	10	63	0·0 ± 0·9	0·0 ± 2·6
3	20	62	+4·7 ± 3·0	+7·3 ± 4·6
		Blood side		
10*	0·5	52	4·5 ± 3·0	−4·5 ± 3·0
3	0·5	47	0·0 ± 0·6	0·0 ± 0·6
3	2	51	+0·3 ± 2·7	−0·9 ± 7·6
4	10	59	+3·0 ± 2·2	+8·5 ± 6·3
4	20	75	+1·5 ± 1·6	+2·3 ± 2·4
		Both sides		
10*	0·5	38	−4·0 ± 1·8	+4·0 ± 1·8
4	0·0	86	+15	—
3	20	63	−0·8 ± 0·9	−2·0 ± 1·3

* Original concentration was 5 mM/l. Ca^{2+}

by an asterisk in Table 6) the midgut was isolated early in the morning and the ion changes were effected immediately after the midgut potential had reached its maximum. In this case a tenfold drop in calcium concentration on the lumen-side decreased

the potential by an average of 17 mV. The average is based on ten determinations and the standard error is but ± 1 mV. This value is too large, compared to the 29 mV. expected for a divalent cation, to be ignored. However, these results are in the opposite direction from those predicted for a change in diffusion potential of a cation between tissue and lumen and more probably reflect an influence of calcium on the permeability of the midgut tissue to some other ion or ions such as chloride.

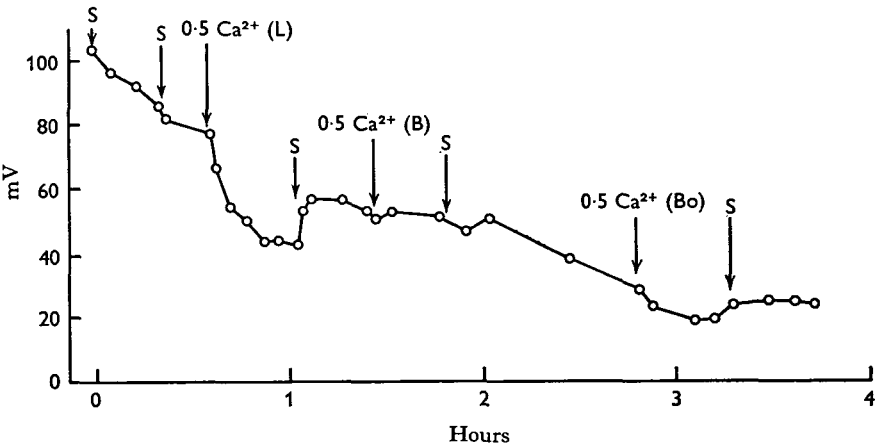

Fig. 5. Effects of changing calcium concentrations in the solutions bathing a fresh, sparingly washed midgut. The potential was diminished when the calcium concentration was reduced on the lumen-side contrary to predictions from the Nernst equation. When the midgut was washed repeatedly and soaked in calcium-free solutions, no effects of calcium changes were detected.

(7) *Anions*

Table 7. *Effects on the midgut potential of replacing the standard chloride bathing solutions with either isethionate or sulphate solutions*

No. of experiments	Experimental solution	Prior E in S_1 (mV.)	$\Delta E \pm$ S.E. (mV.)
	Lumen		
4	Isethionate	74	−14±7·4
4	Sulphate	98	+1·5±2·5
	Blood-side		
4	Isethionate	68	+12±2·4
4	Sulphate	76	+14±2·5
	Both sides		
4	Isethionate	50	−1·8±1·3
5	Sulphate	62	+4·5±2·6

In order to determine whether chloride, the principal anion in the bathing solutions, to some extent shunts the potential or affects it in some other way, such as by setting up chloride diffusion potentials, chloride was substituted by the larger divalent anion sulphate or the large organic anion isethionate. The effects were small in both cases (Fig. 6). The substitution of chloride by sulphate on the lumen-side alone or, more significantly, on both sides had negligible effects and the potential was increased by only about 14 mV. when chloride was removed in this way from the blood-side (Table 7).

Similarly, isethionate had little effect when substituted for chloride on both sides although its substitution for chloride on the lumen-side decreased the potential 14 mV. with a very large standard error (Table 7). The persistence of large potential differences across the isolated midgut in bathing solutions devoid of chloride argue against a direct role of this ion in generating the midgut potential.

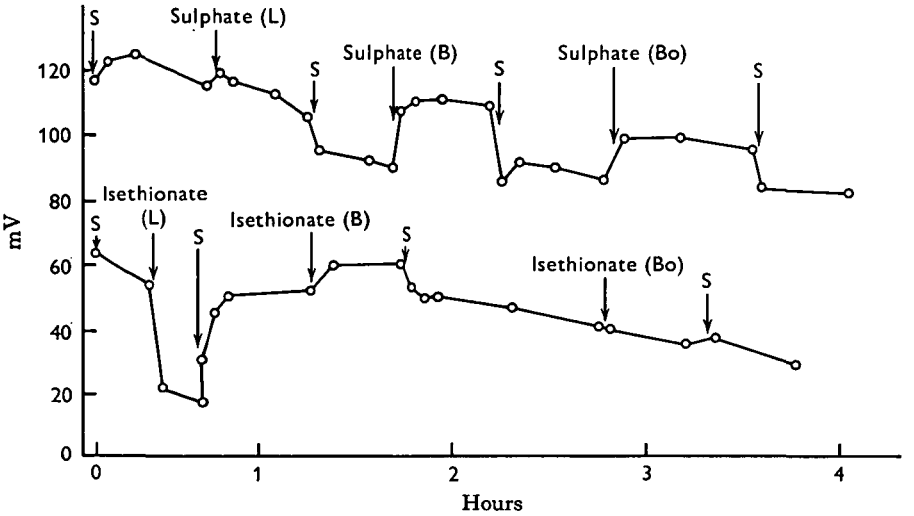

Fig. 6. The upper curve depicts the effects on the potential of replacing chloride in the bathing solution with sulphate, and the lower curve replacing chloride with isethionate. Attention is directed to the last part of each curve which depicts the substitution of sulphate or isethionate on both sides of the isolated midgut simultaneously. Clearly the potential does not require chloride ions to be present although the effects with sulphate are consistent with the view that the potential is partially shunted in chloride solutions.

DISCUSSION

How does the midgut potential in excess of 100 mV. arise across the isolated organ bathed on both sides with the same solution? In addition to sucrose the standard bathing solution contains K^+, Mg^{2+}, Ca^{2+}, Cl^-, HCO_3^-, and traces of H^+ (Table 1.) The midgut tissue contains these ions and in addition protein and other charged and uncharged molecules. Transient diffusion potentials or Donnan equilibrium potentials between the epithelial cells and the bathing solutions could arise from purely passive processes. However, transient diffusion potentials could not account for a midgut potential which is sustained for several hours and the Donnan potentials would not be large enough. Clearly some endergonic process must be involved.

Koefoed-Johnsen & Ussing (1958) proposed that an active exchange of cellular sodium for 'inside' potassium might secondarily sustain a sodium diffusion potential across the 'outside' surface and a potassium diffusion potential across the 'inside' surface which could sum to account for the frog skin potential. Could a similar non-electrogenic mechanism exchanging potassium for some other ion act to produce an asymmetric distribution of some ionic species between midgut tissue and bathing solutions which could secondarily sustain diffusion potentials large enough to account for the midgut potential? We have shown that neither sodium, magnesium, calcium,

nor any other cation except potassium is needed to generate the midgut potential and so could not be required as a counter ion for an electroneutral active process (Tables 3–6; Harvey & Nedergaard, 1964; Nedergaard & Harvey, 1968). Nor are diffusion potentials of Mg^{2+}, Ca^{2+}, or Na^{+} demonstrable on either side of the midgut (Tables 4–6). Hydrogen ions are present at too low a concentration (10^{-8} M) in the bathing solutions to serve as counter ions and the potential is independent of pH changes over at least two orders of magnitude (Harvey, unpublished data). Chloride ions can be replaced completely by sulphate or isethionate without loss of the potential (Table 7). Rubidium can substitute for potassium (Fig. 2), but is not normally present either in the midgut tissue or in the bathing solutions. Potassium is the only ion which must specifically be present and even potassium is required only on the blood-side of the midgut. (Table 3, and unpublished data). In the absence of a requirement for a counter ion for potassium and with no diffusion potentials demonstrable (with the possible exception of a potassium diffusion potential which is wrongly oriented as discussed in the next paragraph) we must reject the model of an electroneutral active mechanism giving rise secondarily to diffusion potentials as an explanation of the midgut potential.

The dependence of the midgut potential on the potassium concentration on the blood-side (Harvey & Nedergaard, 1964; Tables 2 and 3), may indicate, among other things, a potassium diffusion potential across the blood-side surface or the stimulation of an electrogenic mechanism by potassium. A potassium diffusion potential on the blood-side surface could only detract from the midgut potential unless very low potassium concentrations are present within some restricted space within the tissue. However, Quatrale (1966) found the potassium concentration of the midgut tissue to be approximately 90 mM/l. of tissue water.

A venerable suggestion is that if a metabolic anion such as bicarbonate were to diffuse preferentially from tissue to lumen carrying a stoichiometric amount of potassium in an electroneutral process, the secondary diffusion of potassium from blood-side to tissue might account for the potential. However, the amount of bicarbonate produced by the metabolism of the entire tissue is much too small. As many as 2 μequiv. of potassium are transported for every μequiv. of oxygen that is consumed (Harvey, Haskell, & Zerahn, 1967). This means that there are at least eight potassium ions transported for every bicarbonate ion produced metabolically even if all the oxygen taken up by the entire tissue were used for potassium transport.

The simple conclusion is that potassium and only potassium is required for the midgut potential. Indeed a solution of potassium chloride or potassium bicarbonate and sucrose on the blood-side and any other non-toxic ionic solution on the lumen is all that is required to sustain a potential for several hours. It follows that the midgut potential must be generated by the active transport of potassium ion itself.

SUMMARY

1. From two lines of evidence, we conclude that the potassium transport gives rise directly to the midgut potential, i.e. that the active potassium transport mechanism is electrogenic.

2. First, diffusion potentials of neither potassium, sodium, magnesium, calcium,

nor chloride could give rise to the large midgut potential if values for tissue concentrations are accepted for their respective activities in the epithelium.

3. Secondly, no externally added cation other than potassium is required to sustain either the potential or short circuit current, no specific anion is required, and no metabolic ion is known to be produced in sufficient amount to act as a counter ion for potassium in a non-electrogenic process.

4. Changes in the concentration of potassium on the blood-side of the midgut always lead to changes in potential in the direction predicted by the Nernst equation. Moreover, a tenfold change in potassium concentration leads to the expected 59 mV. potential change provided that the prior midgut potential is at least 130 mV. This effect could be attributed either to the stimulation of an electrogenic potassium pump or to a potassium diffusion potential across the blood-side barrier.

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