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ACTIVE TRANSPORT OF POTASSIUM AND OXYGEN CONSUMPTION IN THE ISOLATED MIDGUT OF *HYALOPHORA CECROPIA*

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INTRODUCTION

Oxygen uptake is essential for active ion transport across the isolated silkworm midgut as well as across other isolated epithelial membranes such as the frog skin and toad bladder. The study of the chemical relationship between these two processes in the skin and bladder has been rewarding. The short-circuit current across the isolated frog skin and toad bladder is accounted for entirely by the active transport of sodium and a substantial fraction of the oxygen consumption is used for Na-transport. When sodium is being transported, extra energy is utilized and the rate of respiration is increased. The increase in respiration rate is proportional to the amount of sodium transported, and therefore the ratio between extra oxygen consumed and the amount of sodium transported remains constant (Zerahn, 1956; Leaf and Renshaw, 1957; Leaf, Anderson & Page, 1959).

After Harvey & Nedergaard (1964) demonstrated that potassium is actively transported from blood side to lumen of the isolated midgut of *Hyalophora cecropia* (L), it seemed feasible to measure the relationship between K-transport and oxygen uptake. Following the approach of Zerahn (1956), it seemed sufficient merely to measure the oxygen uptake of the midgut when it was transporting potassium and to subtract the oxygen taken up when the transport was stopped. This value for the net oxygen uptake could be compared to the net K-transport as calculated from concurrent measurements of the short-circuit current. Harvey & Nedergaard showed that more than 80 % of the short-circuit current is carried by potassium. Because of the importance of this figure for calculating the net K-flux, and because of the high respiration of the midgut tissue when experiments are performed in oxygen-saturated solutions, the method of short-circuiting the midgut was modified and the relationship between the K-flux toward the lumen and the short-circuit current was verified.

The omission of potassium from the blood-side solution stopped the K-transport at once so that oxygen uptake measured during such a period could serve as a blank. Periods of low transport were also produced by the following method. When the lumen exhibits a large positive potential relative to the blood side, the transport should be retarded and the influx of potassium should approach zero. However, measurements of the ^{42}K influx (toward the lumen) showed that the natural potential

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(in excess of 100 mV.) reduced the influx to only about 50% of the short-circuited value. Therefore, an additional potential was added from a battery to reduce the active transport to about 10–20% of its short-circuit value.

Both methods of obtaining a blank value with low or zero K-transport gave the same results. Oxygen consumption does not increase when the midgut transports potassium and the negligible changes in oxygen uptake that do occur fall within the limits of experimental error. It is concluded that although the transport of potassium by the midgut is strongly dependent on the oxygen consumption, the oxygen consumption is not dependent on the K-transport. This result is contrary to the findings for the active transport of sodium in the isolated frog skin and indicates a marked difference at some point in the transporting mechanism.

METHODS

Apparatus

The technique developed by Ussing & Zerahn (1951) for measuring ion fluxes and short-circuit currents in the isolated frog skin was modified by Harvey & Nedergaard (1964) in their study of the isolated midgut. Using air to stir the solution and aerate the midgut tissue Harvey & Nedergaard measured currents not exceeding 1 mA. In the present study it was necessary to provide a sealed chamber to measure oxygen uptake and it was practicable to bathe the midgut in oxygenated solutions. The resulting large currents in solutions with low conductivity led to high potential gradients in the solutions. The technique for short-circuiting the midgut was therefore modified (see p. 238).

Fig. 1 is a diagram of the new apparatus. An inner chamber was constructed by ring-sealing two sections of glass tubing (5 mm., O.D.) into the inner member of a 24/25 standard-taper ground-glass joint. One section of tubing emerging from the narrow end of the joint was shaped to form an inverted question mark, the opening of which was separated by a 10 mm. gap from the opening of the other section of tubing. When a short cylindrical piece of midgut was tied in place in this gap, a continuous channel for bathing the lumen of the midgut was formed. An outer chamber was constructed by sealing two 5 mm. (O.D.) T-tubes to the outer member of the standard-taper joint, the end of which was sealed to form a vessel. The entire outer joint assembly was ring-sealed into a glass water-jacket to enable the temperature of the bathing solutions to be regulated within 0·1° C. All measurements were carried out at 25°C. except those reported in Table 5 which were carried out at 15° C.

Dissection and cannulation of the midgut

Mature feeding fifth instar larvae of *Hyalophora cecropia* weighing from 5 to 16 g. were used in the experiments. A cylindrical section of midgut (100–200 mg., wet wt.) was carefully dissected out and tied in place in the gap between the two glass channels as described by Harvey, Haskell & Nedergaard. The inner chamber with the tissue in place was inserted into the outer chamber which had been filled with previously oxygenated standard solution (S-1) containing 32 mM-K^+, 5 mM-Mg^{2+} 5 mM-Ca^{2+}, 2 mM-HCO_3^-, 50 mM-Cl^- and 166 mM sucrose. To prevent damage to the luminal surface while this operation was carried out, the inside chamber was closed with a section of rubber tubing. The ground-glass joint was greased with

petroleum jelly and all of the air bubbles in the outer compartment were forced out as the inner joint was sealed in place. The solution in the blood-side compartment was stirred with a magnetic flea which rotated 600 rev./min.

After the outer compartment was closed, the lumen compartment was assembled by attaching T-tubes and rubber tubing to the upper ends of the two glass channels.

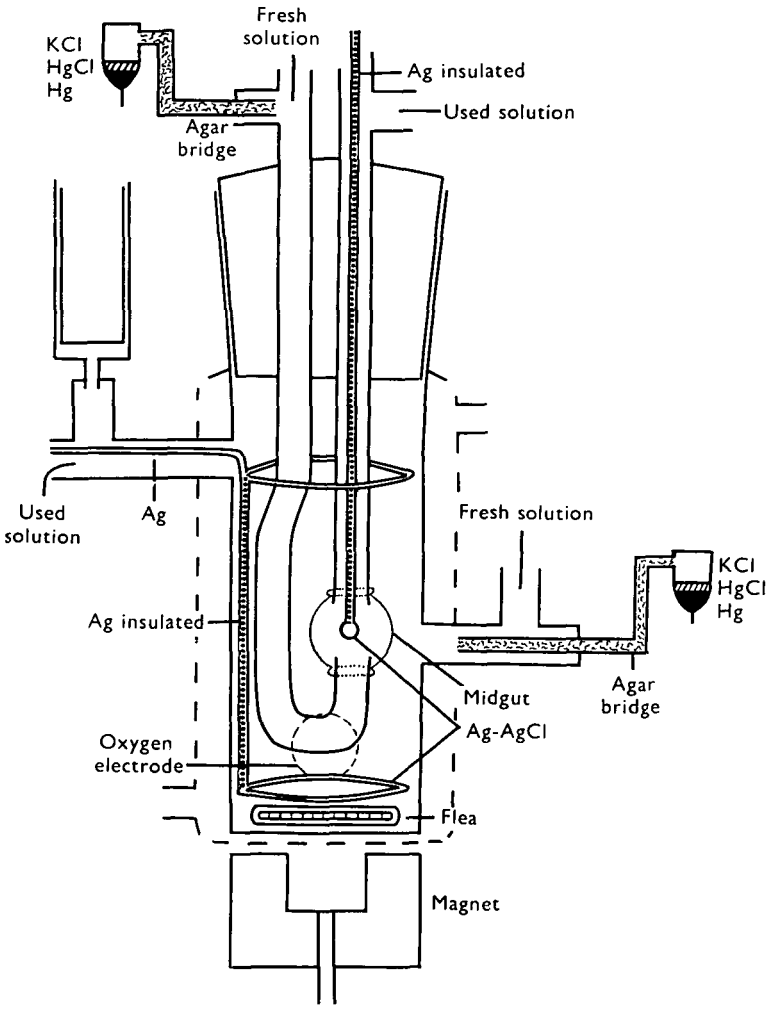

Fig. 1. Apparatus for short-circuiting the isolated midgut of *Hyalophora cecropia*. The potential is measured by calomel electrodes which connect via agar bridges to the solutions bathing blood-side and lumen of the isolated midgut. To short-circuit the midgut, an external potential is applied across the Ag-AgCl electrodes so that the small Ag-AgCl sphere in the centre of the midgut sphere is negative. The placement of the midgut as a large sphere surrounding the central electrode ensures that the entire surface of the midgut is equipotential with respect to the lumen solution. The potential gradient in the blood-side solution is kept as uniform as possible by forming the Ag-AgCl electrode as two large rings placed symmetrically as far as possible from the gut. The midgut is made a sphere by adjusting the volume of fluid in the outer compartment by means of the syringe. The dotted circle shows the location of the oxygen electrode near the stirrer in the wall of the outer compartment. A short section of iron rod enclosed in glass tubing serves as a stirrer. The entire outer compartment is enclosed in a glass jacket (dotted line) through which water is circulated from a constant temperature circulator.

The inner compartment was then filled with standard solution and closed. The gut was distended to form a sphere by withdrawing a little fluid from the outer compartment with the syringe while the lumen side was opened temporarily. If the gut was leaky the sphere soon collapsed. A gut which remained as a sphere for 5 min. usually remained so for hours so that a 5 min. test for leakiness proved sufficient. A tight gut was allowed to equilibrate for about ½ hr. before experiments were begun. Immediately prior to each measuring period the outer compartment (blood-side of the gut) was flushed with fresh oxygenated insect-saline. In this way the tissue was exposed to the same oxygen tension at the start of each measurement.

Preliminary experiments revealed that stirring of the lumen solution was not necessary to maintain the potential and the K-transport. This means that very little oxygen could have been given off or taken up by the gut from the unstirred lumen solution. When the flux of ^{42}K through the gut wall was measured the lumen solution was circulated by a stream of air. Because under these circumstances the gut may consume oxygen from the lumen solution no oxygen determinations were made.

Oxygen uptake was measured polarographically. The electrode (Beckman, Model 777) was standardized by determining the oxygen content of the solution using Krogh's modification (1935) of the Winkler method. Readings were taken over a 20 min. period during which they were linear with respect to time and accurate to about 1 %. At 25° C. the oxygen pressure dropped about 10–15 % during the 20 min. period. The volume of the blood-side solution was about 26 ml. and contained 140 μ-equiv. of oxygen at the outset. Therefore, about 14 μ-equiv. of oxygen were used in 20 min. The amount of potassium transported was approximately 50 μ-equiv./hr. If the oxygen uptake of the midgut were stimulated by potassium to the same extent that the respiration of the frog skin is stimulated by sodium, the oxygen uptake would increase by about 3·7 μ-equiv. in 20 min. This amount would be a sizeable fraction of the 14 μ-equiv. of oxygen used by the midgut during this time and would be easy to measure with the method just described.

Measurement of potential and short-circuit current

If the midgut is maintained in its normal cylindrical shape, the potential across its wall can be measured with reasonable accuracy by placing the potential-measuring electrode in the middle of the cylinder. However, for measuring the short-circuit current by balancing the natural potential with an externally applied potential, geometrical conditions become more critical. If the external E.M.F. for bringing the potential to zero is applied more strongly from one end of the cylinder, that end will have too much potential applied and the other end too little.

To obtain a more uniform application of the applied potential on its surface, the midgut was distended so that its shape approximated that of a sphere. Although the distension enhanced the current, it did not shorten the survival time of the midgut *in vitro*. The potential-measuring electrode in the lumen was the opening of the lower glass channel which was connected *via* the bathing solution to an agar bridge at the top of the channel. An Ag-AgCl sphere was placed in the centre of the midgut sphere, thereby making the equipotential surfaces spherical. Because the central Ag-AgCl electrode was small (2 mm. diameter), it was difficult to cover its surface with sufficient AgCl to carry a large current for more than ½ hr. when it was negatively

charged. However, when the AgCl was spent, current continued to pass undisturbed through the pure silver electrode surface that remained, but now produced molecular hydrogen. Bubbles of hydrogen gas rose to the top of the lumen compartment and caused the volume of the midgut lumen to increase slightly. This gas was allowed to escape by leaving one of the glass channels open as a vent. The pH change is not important in the short experimental period.

The potential gradients in the 1 cm. diameter sphere were very steep when currents of the order of 1–4 mA. were passed through it. It was therefore necessary to keep the Ag-AgCl electrode accurately centred and the midgut as nearly spherical as possible. Although the opening of the lower tube was a part of the surface of the outer (tissue) sphere, it drew no current. It was so small, however, that the electrical field should not be disturbed significantly. A study of the longitudinal properties of the midgut becomes feasible using this system because only a rather short segment of midgut tissue is required. The electrical accuracy of the system was tested by comparing the current measured as just described with the flux of potassium measured with ^{42}K.

The conductivity of the standard midgut solution (S-1) was lower than that of frog Ringer by a factor of approximately 3. Moreover, the active transport of potassium through the gut was very large and the gut was placed in a rather small volume of fluid so that the potential gradients in the solution were very steep. It was possible to correct for the potential difference which arises between the two agar bridges when current is passed through the solution with no gut present in the gap. However, these corrections in S-1 were as much as 10–20 mV. for 1 mA. The error is due to the electrical field from the Ag-AgCl sphere plus the effect from the field produced by the upper and lower Ag-AgCl ring. These current electrodes have opposite signs, and by aligning the gap which holds the gut slightly assymetrically with respect to the opening for the blood-side potential electrode this correction can be minimized. This can be done by having the gap either higher or lower than the opening or by turning the inner chamber to alter the lateral alignment slightly. The short-circuit currents measured were often so large that one could expect that the field was changed when the gut was in place and that the corrections might be wrong. For these reasons the results in Table 1 are given both with and without corrections.

To measure the short-circuit current more accurately it was found feasible to increase the conductivity of the solution. When the potassium concentration was increased to 73·5 mM the active transport of potassium was usually higher than in 32 mM-K (S-1). Furthermore the conductivity was increased by a factor of 2 or more and the corrections discussed above were made almost nil by placing the electrodes favourably. Under these conditions the short-circuit current and K-flux through the gut were determined without the need for corrections and, as shown in Table 6, the difference between the short-circuit current and the K-transport became insignificant ($2 \pm 8\%$). This result indicates that the difference between the short-circuit current and the K-transport in S-1 may be mainly an artifact arising from the difficulty of controlling the steep potential gradients. However, it does not give any explanation for the potential and transport which occurs when no potassium is available in the blood-side solution.

RESULTS

Relationship between current and potassium flux

The magnitude of the short-circuit current is compared with that of the influx (lumen-directed flux) of ^{42}K in Table 1. The mean value for the influx was 89 % of the current. This value lies between the 112 % for influx and 83 % for net flux toward the lumen reported by Harvey & Nedergaard. The discrepancy between current and K-flux may be due to experimental error although the active transport of some other ion cannot be ruled out. When potassium is omitted from the blood-side solution a net transport of this ion toward the lumen becomes impossible. However, the potential and current do not drop to zero. The remaining current amounts to about 15 % of the total current. This result suggests that some ion other than potassium may be transported actively under these conditions.

Table 1. *The short-circuit current compared to the unidirectional flux of ^{42}K toward the lumen of the isolated midgut measured at 25° C.*

Date	Corrected current (μ-equiv./hr.)	Potassium flux toward lumen (μ-equiv./hr.)	Calculated* uncorrected current (μ-equiv./hr.)
9 July (1)	64	59	49
9 July (2)	78	69	60
8 July (1)	78	65	60
	75	63	58
8 July (2)	78	74	64
	70	67	57
Mean	74	66	58
K-influx in % of I	100	89	
		114	100

* Uncorrected current calculated by multiplying the corrected current by the ratio of the potentia just prior to short-circuiting to that potential plus the correcting potential.

Relationship between potassium transport and oxygen uptake in potassium-free solution compared to potassium-containing solution

To eliminate the possibility that damage to the gut might occur in the absence of potassium on the blood side, a period of measurement with no potassium present was sandwiched between periods of measurement with potassium present. The oxygen uptake during the no-potassium period was compared with the mean of the uptake during periods with potassium present. There is very little difference in the oxygen consumption during the transport and non-transport periods as shown in Table 2 and Fig. 2. This result might be criticized on the grounds that the current remaining in the absence of potassium may be carried by ions which use more oxygen per equivalent of ion transported than does potassium. A series of measurements was therefore made in which the potassium concentration in the solutions was not changed but the K-transport was blocked by imposing a high positive potential on the lumen.

Table 2. *Net oxygen consumption and short-circuit current in the isolated midgut*

(The net oxygen consumption is obtained by subtracting the oxygen consumption with heightened potential or with no potassium on the blood side from the oxygen consumption determined during short-circuiting the midgut at 25° C. The K-flux toward the lumen was taken as 90% of the short-circuit current. The units are μ-equiv./hr.)

Date	Oxygen consumption: Shorted	Oxygen consumption: High P.D.	K-flux	Net oxygen consumption	μ-equiv. K / μ-equiv. O
14 July	66	62	70	4	—
15 July (1)	60	64	81	−4	—
15 July (2)	62	57	73	5	—
16 July (1)	61	60	47	1	—
16 July (2)	41	39	73	2	—
Mean	—	—	70	1·6	44
	Shorted	Minus K			
19 July (1)	56	54	61	2	—
	49	50	43	−1	—
19 July (2)	43	40	68	3	
Mean	—	—	57	1·3	44

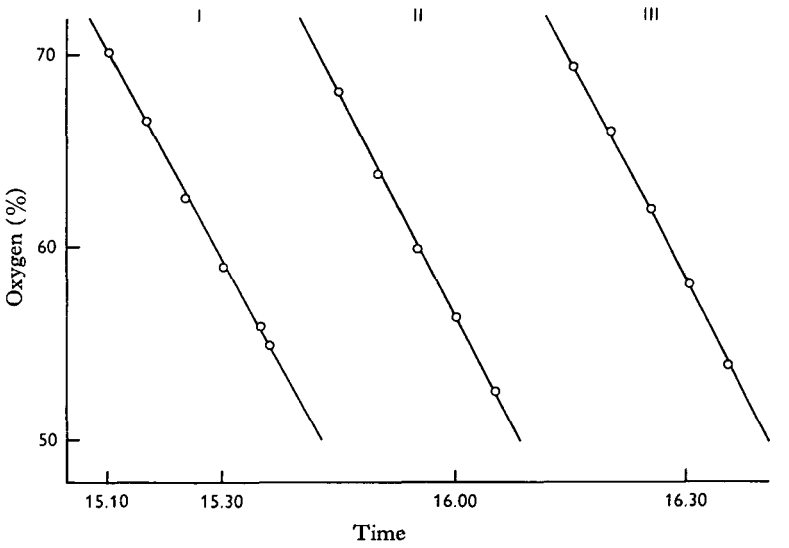

Fig. 2. A representative experiment in which the time-course of oxygen uptake is plotted when the midgut is transporting potassium (period II) and when the potassium transport has been stopped (periods I and III). In period II, the midgut is producing a short-circuit current of 1400 μA. (equivalent to 47 μ-equiv. potassium/h. if 90 % of the current is carried by potassium). In periods I and III an external potential of 60 mV. is added to the natural potential, making the lumen about 150 mV. positive to the blood side. The rate of oxygen uptake as indicated by the slope of the lines is virtually identical under the two conditions.

Relationship between potassium transport and oxygen uptake with a high applied potential, the natural potential, and zero potential

The ^{42}K flux against the natural potential (about 120 mV.) remains about half as large as the flux measured in the short-circuited condition. A further positive potential of 60 mV. applied on the lumen side reduced the transport to about one-fifth of that

in the short-circuited gut (Table 3). In other words, the K-flux could be brought tc about 15–20 % of its short-circuited value by applying a large positive potential to the lumen-side. However, this procedure had little or no effect on the oxygen uptake as seen in Table 2.

Table 3. *Effects of natural potential and externally applied high potential on lumen-directed flux of ^{42}K in the isolated midgut*

(The flux in the short-circuited gut is given in the second column, the ratio of this flux to that against the natural potential is given in the fourth column and the ratio against the natural potential plus 60 additional millivolts is given in the last column.)

Date	Short circuit (μ-equiv./hr.)	Natural potential (mV.)	s.c.-flux / Nat Pd-flux	s.c.-flux / High Pd-flux
8 July (1)	65	116	2·1	8·7
8 July (2)	74	—	2·3	6·7
9 July (1)	64	114	2·1	4·5
9 July (2)	78	140	2·1	4·9
Mean	—	—	2·1	6·2

Table 4. *Total oxygen consumption and short-circuit current in the isolated midgut at* 25° C.

(The K-flux is calculated as 0·90 times the current. The last column is the ratio of K-flux in μ-equiv. to the total oxygen consumption in μ-equiv.)

Date	Total oxygen consumption (μ-equiv./hr.)	Current (μ-equiv./hr.)	K-flux (μ-equiv./hr.)	μ-equiv. K / μ-equiv. O
14 July	66	78	70	1·06
15 July (1)	60	90	81	1·35
15 July (2)	62	82	74	1·19
15 July (3)	51	64	58	1·13
16 July (1)	61	52	47	0·77
16 July (2)	51	81	73	1·43
19 July (1)	73	104	94	1·29
	62	86	77	1·24
	49	48	43	0·88
19 July (2)	52	86	77	1·48
	34	65	58	1·71
Mean	56	76	68	1·23

Potassium transport and oxygen uptake in S-1.

Both ways of measuring the relationship between K-transport and oxygen uptake gave the same result—that within the limits of experimental error the oxygen uptake is not influenced by K-transport. The ratios of the mean value of potassium transported in μ-equiv. to the negligible net oxygen consumption in μ-equiv. are listed in the last column of Table 2. In experiments in which K-transport was partially quenched by the natural potential, or more fully quenched by a high potential, 44 μ-equiv. of potassium were transported for each μ-equiv. of extra oxygen taken up; in experiments in which the transport was stopped by omitting potassium from the blood-side solution the value was also 44.

The ratios of net K-flux to *total* oxygen consumption are shown in Table 4. The net flux (toward the lumen) was obtained by multiplying the short-circuit current by 0·9 (from Table 1). The efflux of potassium from the lumen to the blood side is very small in guts with high initial potentials especially when the potential is short-circuited. The influx and net flux are therefore almost the same. It can be seen that the ratio of μ-equiv. potassium transported to μ-equiv. oxygen taken up is 1·2. This value is too small because the oxygen uptake of the non-transporting edges of the gut was not deducted.

When the measurements were repeated at a lower temperature (15° C.) both oxygen consumption and short-circuit current decreased by about 36% so that the mean values for K/O ratios remained the same as they were at 25° C., namely about 1·2 (Table 5).

Table 5. *Total oxygen consumption and short-circuit current in the isolated midgut at* 15° C.

(The K-flux is calculated as 0·90 times the current. The last column is the ratio of K-flux to the total oxygen consumption).

Date	Total oxygen consumption (μ-equiv./hr.)	Current (μ-equiv./hr.)	K-flux (μ-equiv./hr.)	$\frac{\mu\text{-equiv. K}}{\mu\text{-equiv. O}}$
21 July (1)	35	37	33	0·94
21 July (2)	45	61	54	1·20
	36	49	44	1·22
22 July	54	75	67	1·24
	40	56	50	1·25
	28	31	28	1·00
23 July (1)	24	34	31	1·29
23 July (2)	35	68	61	1·74
	25	28	25	1·00
Mean	36	49	44	1·22

Potassium transport and oxygen uptake in 73·5 mM *potassium*

As discussed in the section on Methods, the midgut potential can be short-circuited more accurately when the potassium concentration (and the conductivity) of the bathing solutions is increased to 73·5 mM-K. A comparison of Tables 1 and 6 reveals that the current tends to be higher in 73·5 mM-K than in 32 mM-K. In the high-potassium solution the K-flux toward the lumen is equal to the short-circuit current within ±8%. The K-efflux (from lumen to blood side) was measured in the high-potassium solution and found to be less than 10% of the current in most cases (Table 7). We therefore conclude that in solutions containing 73·5 mM-K the current is accounted for entirely by the net flux of potassium within the accuracy of about 10% achieved in these determinations.

The oxygen consumption in the solution with high potassium concentration was not greatly changed from its value in S-1 (mean of 56 in both Tables 4 and 8) whereas the K-transport in 73 mM-K was double that in S-1. In almost all experiments the ratio of potassium transported to oxygen consumed in 73·5 mM-K was about 2 (mean of 2·0). At the end of each of these measurements the K-transporting segment

of the gut was cut from the inner chamber and the oxygen uptake of the remaining edges was determined. The oxygen uptake of the gut was corrected by subtracting half of this amount (10% of the total oxygen uptake in most cases).

Table 6. *Relationship between short-circuit current and potassium-flux from blood-side to lumen of the isolated midgut bathed in high potassium concentration* (73·5 *m*M-K^+, 5 mM-Ca^{2+}, 5 *m*M-Mg^{2+}, 89 *m*M-Cl^-, 2 *m*M-HCO_3^-, *and* 83 *m*M-*sucrose*)

Date	Short-circuit current (*μ*-equiv./hr.)	K-flux (*μ*-equiv./hr.)	K-flux as % of currents
3 June	108	93	86
	108	106	98
	101	101	100
9 June (1)	78	82	105
	66	76	115
9 June (2)	78	75	96
	77	80	104
	73	75	103
9 June (3)	58	65	112
10 June	89	88	99
10 June	86	87	101
Mean	—	—	102
S.E.	—	—	±8

Table 7. *Efflux of potassium from lumen to blood-side with* 73·5 mM *potassium on both sides of the isolated short-circuited midgut*

Date	Efflux (*μ*-equiv./hr.)	Current (*μ*-equiv./hr.)	% M_{out}/I (α/α)
1 July (1)	5·6	112	5·0
	10·3	—	9·2
1 July (2)	11·0	134	8·2
	15·7	112	14·0
1 July (3)	7·8	50	15·6*
	14·2	45	32*
6 July (1)	6·0	112	5·4
	7·2	—	6·4
6 July (2)	11·8	120	10·0
	10·2	—	8·5
6 July (3)	7·8	145	5·4
	6·6	138	4·8
6 July (4)	10·4	120	8·6
	11·0	116	9·5
6 July (5)	8·6	99	8·7
	12·2	96	12·8

Gut was pulled both up and down on chamber and retied; rough treatment.

Table 8. *Relationship between short-circuit current and oxygen consumption in 73·5 mM potassium solution under steady-state conditions*

(At this potassium concentration the current is equal to the net potassium transport within the error of measurement.)

Date	K-transport (μ-equiv./hr.)	Total oxygen consumed (μ-equiv./hr.)	$\frac{\mu\text{-equiv. K}}{\mu\text{-equiv. O}}$
12 July (1)	120	61	2·0
	112	55	2·0
12 July (2)	155	72	2·1
	88	62	1·4*
13 July (1)	75	34	2·2
	56	29	1·9
13 July (2)	135	70	1·9
	120	55	2·2
13 July (3)	149	67	2·2
	127	55	2·3
Mean	114	56	2·0

* Current not steady

DISCUSSION

The active K-transport in the midgut requires oxygen and stops rapidly in its absence (Haskell, Clemons & Harvey, 1965). We have shown that there is no significant difference between the oxygen consumption when potassium is being transported and when it is not. If it were the case that extra oxygen were consumed during the transport period and used to transport potassium, then an average of 40 μ-equiv. of potassium would have to be transported for every μ-equiv. of extra oxygen consumed. We shall show that this relationship is not thermodynamically possible and that energy must be used from the basal oxygen consumption. When bathed in the standard solution containing 32 mM-K the isolated gut has an initial potential of about 120 mV. In most cases the flux ratio exceeds ten (Harvey & Nedergaard, 1964). The work required to move one equivalent of potassium can be calculated from these data using the equation derived by Ussing (Zerahn, 1956):

$$W = 0{\cdot}239 \times 96{,}500 \left(0{\cdot}058 \log \frac{C_{in}}{C_{out}} + E + 0{\cdot}058 \log \frac{M_{in}}{M_{out}}\right).$$

The first term will disappear because the concentration of potassium is the same on the lumen-side and blood-side solutions. The value of E can be taken as 120 mV and the last term as about 60 mV. for a total of 180 mV. Therefore $W = 4200$ cal. But the energy derived from metabolizing substrate with 1/40 equiv. of oxygen amounts to but 25,000/40 = 600 cal. or only one-seventh of the energy necessary to transport the potassium when the efficiency is 100%. So the extra oxygen consumption cannot account for the transported potassium.

The total consumption is 40 times larger than the net oxygen consumption. Therefore only a fraction of the oxygen would be needed for transport if the efficiency of the midgut for potassium is as high as that of the frog skin for sodium. Because the

oxygen consumption in the midgut does not change when active K-transport ensues, the mechanism for K-transport must be idling; the energy must be lost as heat or geared to some undetermined endergonic process. The ion transport has no triggering effect on the oxygen consumption as it has in the frog skin.

The 1·2 ratio of active K-transport and total oxygen consumption in Tables 4 and 5 depends on the potassium concentration of the blood side. Because the complete absence of potassium on the blood side does not change the oxygen consumption, it is certain that at potassium concentrations lower than 32 mM the lower K-transport will lead to K/O ratios much smaller than 1. Nedergaard & Harvey (unpublished results) found that the amount of active K-transport often increases when high concentrations of potassium are used in the solutions bathing the blood side of the midgut. The increase is not directly proportional to concentration through all values but at concentrations of potassium higher than the 32 mM solution used in this study the current increases. At the higher potassium concentration of 73·5 mM the ratio between potassium transported and oxygen consumed was found to have a mean value of 2·0 (Table 8). This value may not be the maximum figure because we do not know whether or not the transport might increase further with still higher potassium concentrations. However, when the values found for 32 mM-K are corrected for the oxygen consumed by the non-transporting edges of the gut the value for K/O of 1·2 will be increased by about 10 % to 1·3. The increase of the K/O value from about 1·3 at 32 mM-K to 2·0 at 73·5 mM-K is not proportional to the 2·3-fold increase in potassium concentration which may indicate that the value of 2·0 at 73·5 mM-K is close to the maximum value. In summary, the K/O ratio varies from zero with no potassium on the blood side to 2·0 at the highest potassium concentration tested.

It is quite clear that in the midgut there is no stoichiometric relationship between oxygen consumption and ion transport as there is in frog skin and toad bladder. However, there are cells in the gut which presumably consume oxygen but take no part in K-transport, e.g. the muscle cells. Therefore the K/O ratio of the cells involved in transport must be larger than 2·0. More serious is the prospect that only a single type of epithelial cell takes part in the transport, e.g. the goblet cells which account for but $\frac{1}{3}$–$\frac{1}{4}$ of the cell population. If both goblet and columnar cells respire at the same rate and the goblet cells were alone responsible for K-transport, the K/O value would be perhaps 6–8. Considering that the goblet cells may use energy for other purposes than K-transport the ratio would have to be even higher, perhaps in the vicinity of 10. Such a high value would make almost impossibly high demands on the exergonic capabilities of the midgut cells. These considerations make it desirable to consider a mechanism in which both types of cells are engaged in the transport process or in which the most prevalent type, the columnar cells, are involved. Another possibility is that the two cell types have greatly different rates of respiration.

Although the oxygen consumption is independent of the K-transport the opposite is not the case. The inhibition of K-transport by anoxia is complete but highly reversible. Under constant potassium concentrations the degree of transport depends almost directly on the rate of oxygen consumption. The oxygen consumption of the isolated midgut depends in turn on the oxygen pressure in the bathing solution but the dependency is complex and a delay is usually encountered between changing the oxygen pressure and its effect on the oxygen uptake. With the methods used in this

study the drop in oxygen pressure is used to measure the oxygen uptake. For this reason the current cannot be maintained steadily at lower oxygen pressure and an assessment of K/O ratios at, say, 20% oxygen or lower was not possible.

CONCLUSION

The two mechanisms for active transcellular ion transport, the sodium system in frog skin and the potassium system in the midgut of *H. cecropia*, might have been different only with respect to the specificity of ion transported. Because the response of the oxidative metabolism to the active transport is not the same in the two tissues, there must also be a difference in the coupling of transport to energy supply. During Na-transport in the frog skin metabolism is stimulated, and the increase in metabolism is proportional to the amount of active transport. Although the K-transport in the midgut is 5–10 times larger than the Na-transport in the skin for a similar mass of tissue, the K-transport has no effect on the oxygen consumption. The ratio of potassium transported to total oxygen consumed was found to be approximately 1·3 with 32 mM-K^+ and 2·0 with 73·5 mM-K. Presumably only a fraction of the total oxygen consumed is available for K-transport so that, as in other epithelial membranes, several equivalents of ion are transported for each equivalent of oxygen used by the transport process. This result indicates that the ion-transport mechanism of the midgut is complex, with undefined links between oxygen and K-transport. Furthermore there is a link between Na-transport and respiration in the frog skin which is missing for K-transport in the midgut.

SUMMARY

1. Flux measurements with ^{42}K reveal that in the isolated midgut of *Hyalophora cecropia* 90 to 100% of the short-circuit current is carried by the active transport of potassium from the blood-side to the lumen.

2. When K-transport is strongly depressed, either by withholding potassium from the blood side or by imposing a large positive potential on the lumen, the oxygen uptake of the isolated gut remains virtually unchanged. If the K-transport were to be energized by the negligible increase in oxygen uptake about 40 μ-equiv. of potassium would have to be transported for every μ-equiv. of extra oxygen taken up. This ratio of K-transport to oxygen uptake is thermodynamically impossible.

3. The ratio of potassium transported to total oxygen consumed when the midgut is bathed with 32 mM potassium on both sides is about 1·3 at temperatures of 25° and 15° C. The ratio must be smaller at lower potassium concentrations and is 2·0 at 73·5 mM-K, which may be approaching the maximum value.

4. Although the oxygen uptake is independent of the K-transport, the reverse is not true. There is a close dependency of K-transport on oxygen consumption.

5. K-transport by the midgut contrasts with Na-transport by the frog skin because Na-transport stimulates oxidative metabolism whereas K-transport does not. Evidently the coupling of transport to energy supply is different in the two systems.

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