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ACTIVE TRANSPORT OF CAESIUM BY THE ISOLATED AND SHORT-CIRCUITED MIDGUT OF *HYALOPHORA CECROPIA*

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INTRODUCTION

The isolated midgut from the American silkworm transports potassium, when it is bathed with a solution containing only potassium and sucrose (Nedergaard & Harvey, 1968).

The only other ion shown to be transported by the midgut so far is rubidium; this ion is considered by Harvey & Nedergaard to behave exactly as potassium. This is proved by them but only with a very rough method allowing 30% difference. Ammonium ion decreases the potential difference across the midgut as well as the short-circuit current and is considered not to be transported actively, and the same is true for sodium and lithium. Even in high concentrations like 30 mM on both sides sodium and lithium are not considered to be transported actively by the midgut (see also Harvey, Haskell & Nedergaard, 1968).

It was therefore thought worth while to determine whether the next member of the alkali metals could be transported actively by the isolated midgut.

However, the midgut proved to be very unpredictable when bathed with solutions containing Cs; some preparations would maintain a potential difference and short-circuit current for longer than 2 h, others would have no potential difference left after a few minutes (Fig. 1). It has not been possible to find the reason for these differences in behaviour towards Cs solutions. It was no remedy to add appreciable amounts of K to the solution; the K will interact with the Cs transport and even with 50% K in the solution several midguts lost their potential rapidly. It was possible to show that in pure K-free Cs solutions there was an appreciable active transport of Cs, when the midgut could maintain the potential difference for a sufficiently long time. The mean short-circuit current and the mean Cs flux from blood side to lumen were rather close, whereas the flux from lumen to blood side was much lower. When the competition between Cs and K was studied, however, it turned out to be a rather unusual one. With 50% K in the solution, the transport of Cs from the blood side to lumen was as low as the flux from lumen to blood side and the net Cs flux was only a minute fraction of the short-circuit current. With 90% or 80% Cs in the bathing solution and only 10 or 20% K, the short-circuit current was accounted for by the amount of Cs transported and no active transport of K was apparent.

This is different from the competition found between the active transport of K and Rb; the rates of active transport were shown by Harvey and Nedergaard to be almost equal when these ions were present in the same concentration in the bathing solutions.

From other tissues when two ions compete for a transport mechanism, as do Na and Li in the frog skin, it also was found that they will compete on an almost equal basis (Zerahn, 1955).

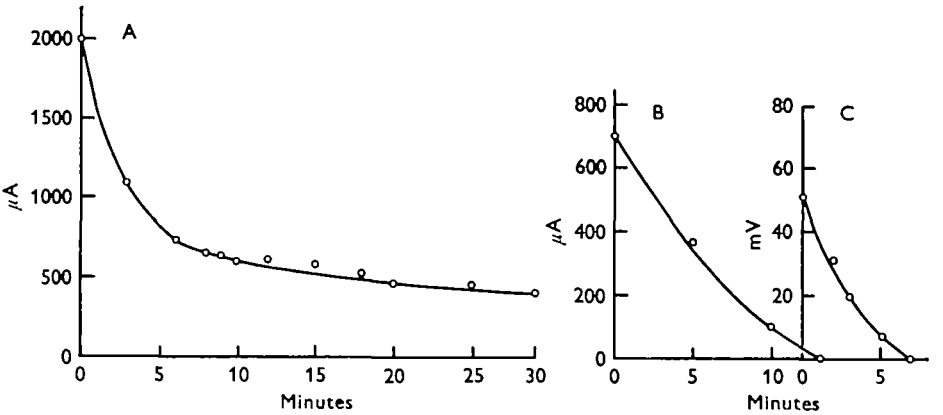

Fig. 1. A Time course of short-circuit current when a midgut is bathed in Cs solution and tolerates it fairly well. B, The same curve when Cs is not tolerated. C, The curve if the potential difference is measured.

METHODS

For the determination of the Cs flux the apparatus described by Harvey & Nedergaard (1964) was used. There has been shown some discrepancy between the short circuit current and the potassium transport measured with ^{42}K (Harvey & Nedergaard, 1964); however, it is uncertain whether this discrepancy is real, because the perfect short-circuiting of the midgut is difficult, because of the high K transport and the low conductivity of the solution. Furthermore, it has not been possible to demonstrate that any other ion is transported actively under the experimental conditions given by these authors. To improve the short-circuiting some changes were made in the placement of the Ag, Ag-Cl electrodes and this is shown in Fig. 2. The larvae, weighing about 12 g, were chilled for an hour in crushed ice, carefully dissected, and 100–200 mg of the gut was mounted as described by Nedergaard & Harvey (1968). The solutions were oxygenated by bubbling oxygen through and in addition the outside chamber was stirred magnetically. When the midgut seemed to tolerate the Cs solution, 25 μl of carrier-free ^{137}Cs solution was added, and the Cs flux was determined by removing samples from the opposite side of the gut at the different time intervals given in the tables. The samples were measured against diluted standards from the side on which the ^{137}Cs was added. The Cs solutions used were prepared from the following two solutions by mixing in the required ratio.

S–1. K solution: KCl 30 mM, $KHCO_3$ 2 mM, $MgCl_2$ 5 mM, $CaCl_2$ 5 mM, and sucrose 166 mM.

Cs–S–1. Cs solution: CsCl 30 mM, $NaHCO_3$ 2 mM, $MgCl_2$ 5 mM, $CaCl_2$ 5 mM, and sucrose 166 mM.

The radioactive measurements were performed on a well scintillation counter from

the Danish firm Selectronic. The midgut was removed at the end of the experiment, washed for 1 min in 260 mM sucrose solution, slightly blotted by resting on a soft tissue paper and weighed. The rinsing in sucrose solution will remove the solution adhering to the gut, and possibly a fraction of the solution in the extracellular spaces and in the folds of the gut, but not necessarily any of the Cs in the cells. After weighing the midgut was placed in a plastic counting vial and 1 ml of water was added. The gut was stirred with the water and assayed for ^{42}K. Later the vial was heated for 1 h and the K and Cs content was determined by flame photometry.

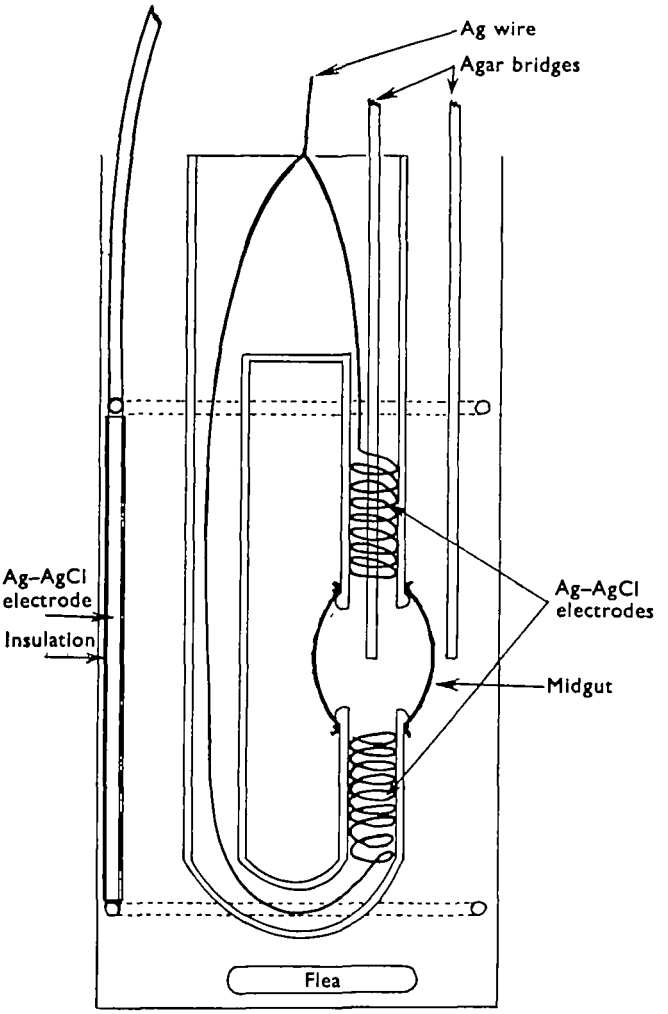

Fig. 2. The apparatus described by Harvey and Nedergaard is modified only for the placement of the silver silver-chloride electrodes. These are placed as follows: The ring-shaped electrodes in the outer chamber (usually the blood side) are placed at a distance away from the midgut but symmetrically with respect to the gut. The electrode in the inside chamber is made of two silver cylinders also placed symmetrically with respect to the gut. The end of one agar-agar bridge is placed in the centre of the gut. The end of the other is placed symmetrically on the blood side of the gut.

RESULTS

Table 1 shows the Cs flux from blood side to lumen, in different time periods; it is obvious that in the first 5 min period the flux is far less than the steady-state flux because equilibrium is not reached. The next three periods are treated as representative determinations even if it is quite clear that there is still not equilibrium in the first

Table 1. *Cs flux through the isolated short-circuited midgut of* Hyalophora cecropia *larvae from blood side to lumen*

(Bathing solution 30 mM-Cs and free of K. The respective short-circuit current is placed just below the Cs flux. The framed figures only are used for determining mean values. Larvae grown on lilac.)

Date	μmoles Cs transported per hour in intervals				
	0–5 min	5–10 min	10–15 min	15–30 min	30–60 min
14. vii.	3·4	10·6	11·2	11·2	
	14·9	11·2	9	5·4	
15. vii.	4·0	12·1	16·8	16·8	11·4
	24	19·4	16	12	6·7
16. vii.	6·0	14·3	16	15·5	3·2
	21	17	14	11	3·6
17. vii. A	2·4	6·0	7·7	7·6	—
	10	9·0	7·5	5·6	—
17. vii. B	5·8	12·5	13·4	12·7	—
	13	8·6	6·7	4·9	—
18. vii.	1·8	8·2	10·3	10·4	7·9
	18	16	13	8·6	3·9

Mean values

Cs flux blood side to lumen 2133/18 =	11·8 μ-equiv/h
Cs flux lumen to blood side	0·8 μ-equiv/h
Net Cs flux	11·0 μ-equiv/h
Short-circuit current 1949/18	10·8 μ-equiv/h

period, and the change in the labelling of the gut may influence the determined flux. When the flux from blood side to lumen is corrected for the flux from lumen to blood side the agreement with the short-circuit current is satisfactory, the net flux being about 20 times as high as the flux from lumen to blood. Furthermore, it can be seen that the lumen to blood side flux is much smaller then the same flux for K which is usually about 10 μ-equiv/h. These results are obtained from animals grown on lilac. The results from animals grown on artificial diet (Riddiford, 1968) gave similar results under these conditions. The Cs flux from lumen to blood side was determined on larvae grown on the artificial diet; on four animals it was determined to have a mean value of 0·80 μ-equiv/h varying from 0·36 to 1·2 μ-equiv/h. The results when the solutions contained 16 mM-K compared to 15 mM-Cs are given in Table 2. The Cs flux from blood side to lumen has now a mean value of 1·05 μ-equiv/h. The Cs flux from lumen to blood side determined under the same conditions was found to be 0·60 μ-equiv/h. The small net flux of 0·45 μ-equiv/h is hardly significant compared to the short-circuit current of 20 μ-equiv/h. This table (2) demonstrates that Cs is not

able to compete with K for the sites on the transport mechanism. It seemed possible that competition might become effective if the K concentration was brought down to a smaller fraction, such as one-tenth of the Cs concentration. However, Table 3 shows that with 10% K in the bathing solution the amount of Cs transported from blood side

Table 2. *Cs flux through the isolated short-circuited midgut of* Hyalophora cecropia *from blood side to lumen*

(Bathing solution 15 mM-Cs and 16 mM-K. For each midgut the short-circuit current is given just below the Cs flux. Only the framed figures are used for computing the mean values. Larvae grown on lilac.)

Date	μmoles of Cs transported per hour in intervals				
	0–5 min	5–10 min	10–15 min	15–30 min	30–60 min
5. viii.	0·23	0·43	0·44	0·38	0·33
	28	24	21	17	12
4. viii.	0·12	0·25	0·31	0·48	0·66
	40	40	20	20	8·0
30. vii.	0·41	1·8	1·2	0·78	0·58
			17	14	10
29. vii. C	0·55	1·04	1·18	1·27	1·24
			24	22	17
29. vii. C	1·7	2·2	2·7	2·2	1·6
			34	26	16

Mean values

Cs flux blood side to lumen 1·05 μ-equiv/h
Cs flux lumen to blood side 0·60 μ-equiv/h

Net flux 0·45 μ-equiv/h
Short-circuit current 20·00 μ-equiv/h

Table 3. *Cs flux through the isolated short-circuited midgut of* Hyalophora cecropia *from blood side to lumen*

(Bathing solution 27 mM-Cs, 3·1 mM-K. For each midgut the short-circuit current is given just below the Cs flux. Only the framed figures are used for computing the mean values. Larvae grown on lilac.)

Date	μmoles of Cs transported per hour in intervals				
	0–5 min	5–10 min	10–15 min	15–30 min	30–60 min
24. vi.	6	12	12	11·4	8·1
		13·4	12·3	10·8	5·6
22. vii.	3·5	9	11·2	11·1	10·8
	20·9	17·9	15·7	12·7	9
23. vii.	3·6	9·2	8·8	9	—
	14·9	8·8	5·6	3·4	—
25. vii.	4·7	14·9	16·9	15·7	13·6
	24·3	18·3	15·3	11·6	8·2

Mean values

Cs flux blood side to lumen 1412/12 = 11·8 μ-equiv/h
Cs flux lumen to blood side 0·8 μ-equiv/h

Net Cs flux 11·0 μ-equiv/h
Short-circuit current 1458/12 12·2 μ-equiv/h

to lumen is very close to the short-circuit current and the Cs flux is not really disturbed by the presence of K. Table 4 shows that in solutions containing 20% K and 80% Cs the midgut will still transport Cs. If the percentage of K is raised to 30% the mean values in Table 5 show that only one-third of the short-circuit current is accounted for by net Cs transport, but the variation between different experiments is pronounced.

Table 4. *Cs flux through the isolated short-circuited midgut of* Hyalophora cecropia *larvae from blood side to lumen*

(Bathing solution 24·0 mM-Cs and 6·2 mM-K. For each flux value the short-circuit current is given just below. Only the figures framed are used for computing the mean values. Larvae grown on artificial diet except* which was grown on lilac.)

Date	μmoles of Cs transported per hour in intervals				
	0–5 min	5–10 min	10–15 min	15–30 min	30–60 min
25. vi.	3·4	6·2	8	—	—
	5·6	3·5	2·8	—	—
5. viii.*	1·7	3·8	4·5	4·4	4·4
	4·7	1·7	0·4	0	0
25. ii.	2	3·7	4·3	—	—
	5·6	0	0	—	—
26. ii. A	3·4	5·0	4·6	3·9	3·8
	14	7·5	4·7	3·3	2·4
26. ii. C	4·2	6·1	6·0	—	—
	13·4	3·0	0		
27. ii. A	3·4	5·6	5·4	5·1	5·3
	4·3	4·1	2·2	2·4	3·1
27. ii. B	4·3	7·8	7·5	6·6	10·8
	17·1	12·7	9·5	7·8	5·4

Mean values

Cs flux blood side to lumen	5·5 μ-equiv/h
Cs flux lumen to blood side	0·8 μ-equiv/h
Net flux	4·7 μ-equiv/h
Short-circuit current	3·7 μ-equiv/h

Any explanation would involve the question: is metabolism affected by the presence of Cs in the bathing solution? The experiments for measuring the oxygen consumption were performed using the chamber described by Harvey, Haskell & Zerahn (1967). No change in oxygen consumption of the midgut could be found by replacing K in the bathing solution with Cs.

In Table 6 the concentrations of K and Cs are given, when the midgut has stayed in the bathing solution for more than 30 min. After this time the movements of Cs and K into and out of the midgut are slow. However, in the experiments with high concentrations of Cs in the bathing solution, the midgut constantly loses K. When the solution in the lumen is replaced with caesium solution, there is no effect on the p.d. or short-circuit current. After removing the gut, appreciable amounts of Cs were found in the gut, but this can very well be attributed to the extracellular space, even when the gut has been washed with sucrose solution.

Replacing the solution on the blood side with the Cs–S–1 reduces the p.d. somewhat, but the short-circuit current decreases to values around one-third to one-quarter of the value in S–1.

Table 5. *Cs flux through the isolated short-circuited midgut of* Hyalophora cecropia *larvae from blood side to lumen*

(Bathing solution 21 mM-Cs and 9·3 mM-K. For each flux value the short-circuit current is given just below. Only the framed figures are used for computing the mean values. Larvae grown on artificial diet except * which was grown on lilac.)

	μmoles of Cs transported per hour in intervals				
Date	0–5 min	5–10 min	10–15 min	15–30 min	30–60 min
25. vi.	0·7	1·3	1·2	1·1	—
	6·2	5·2	4·3	2·8	—
28. vii.*	2·8	4·3	9·6	7·3	5·6
	41	33	28	24	16·0
2. iii.	0·4	0·7	0·7	0·8	—
	10·3	4·1	2·1	1·7	—
3. iii. A	1·0	2·0	1·9	—	—
	4·9	3·6	3	—	—
3. iii. B	1·0	2·0	1·9	1·7	—
	18·3	9·2	4·1	1·5	—
4. iii.	2·6	3·4	3·4	3	—
	9·1	3	0·6	0	—
5. iii.	1·9	2·9	5·3	—	—
	6·3	1·5	0	—	—

Mean values without *

Cs flux blood side to lumen 2·1 μ-equiv/h
Cs flux lumen to blood side 0·8 μ-equiv/h
Net flux 1·3 μ-equiv/h
Short-circuit current 2·9 μ-equiv/h

Table 6. *Concentrations of Cs and K in isolated midgut (μM/g wet weight), no correction for adhering solution*

(The guts were washed 1 min in 260 mM sucrose solution before gentle blotting.)

	mM in bathing solution		mM in midgut		
No. of expts.	K	Cs	K	Cs	K+Cs
6	0	30	8·4	28·8	37
3	3·2	27	11·8	22·8	34·6
2	6·4	24	19·8	22·2	42
14	16	15	42·2	9·5	51·7

DISCUSSION

Because many guts do not tolerate Cs solutions all the figures in the tables relate to guts which were transporting for a sufficiently long time. This means that midguts which lose their potential after a few minutes are not used for flux measurements, and may have abilities not described in this paper. During short-circuiting, the solutions

on both sides of the midgut were identical, so any appreciable net flux is active. Furthermore, the flux from blood side to lumen was very close to the short-circuit current, and the flux ratio around 10–20 instead of 1. Even if the gut is not in perfect steady state the tables show values which can be used for evaluating the active transport because the flux ratio relationship will hold even when steady state is not obtained (Ussing, 1970).

Tables 1, 3 and 4 show that active transport of Cs takes place through the midguts which were tested. The Cs transport probably uses the same mechanism as the K transport and does not interfere with metabolism. How can this transport, however, compete with K transport in such an unusual way? The first suggestion is that the ions move in a single file as suggested by Hodgkin & Keynes (1955). If this took place, however, one would expect to get this phenomenon for all ions involved, and it does not seem to be apparent for the competition between potassium and rubidium (Harvey & Nedergaard, 1964; Nedergaard & Harvey, 1968).

If movement in single file is not the disturbing factor, one can only guess that the peculiar competition is due to the transport mechanism being changed by the substance it transports. If potassium is transported, the carrier or binding sites are able to bind only potassium in any appreciable amounts. If, however, K in the solution is substituted gradually by Cs, at a certain point the mechanism will stop having binding sites for K and only sites for Cs transport. With the amount of experimental material at hand it is not possible to tell how sharp the transition is and exactly how and where the changes occur; there may even be some differences between the way the animals have grown so this is the reason why the dietary regime has been reported. So far it can be said at 100, 90, and 80% Cs in the solution, the main ion transported is Cs. At 50% Cs and 50% K, Cs is not transported actively. At 30% K, 70% Cs, Cs accounts for only one-third of the short-circuit current and K for the rest.

These results force one to consider the possibility that ions other than K are actively transported by the midgut when K is present in low concentrations. With appreciable amounts of K in the bathing solutions an active transport of other ions may very well be hidden as is shown with Cs in Table 2.

Table 6 shows that there is not any close relationship between the amount of Cs in the midgut and its ability to transport Cs or K. There is a definite loss of K from the midgut when Cs is known to be transported. This is not certain when Cs is not transported significantly, as shown in the last line of the table when the solution contains 50% of K. The K concentration is here found to be 42·2 mM. When corrected for an extracellular space of 30% containing 16 mM-K, we find a value for cellular K of 53·5 mM. This should be compared to the values found by Harvey & Zerahn (1969) of 65 mM for 32 mM-K in the bathing solution and 47 of 16 mM-K; it is obvious that the loss of K is not significant. When the Cs content is corrected in the same way the concentration will be 5 mM in the gut cells. The Cs on the blood side often abolishes the potential difference of the midgut within 5–10 min. In these experiments the K of the gut will still be high and the Cs concentration low. The action of Cs as inhibitor of the K pump thus is not exerted through changes in the ionic concentration in the cells of the midgut, but must work on some more specific point of the transport system.

SUMMARY

1. The short-circuited midgut from the larvae of *Hyalophora cecropia* can actively transport potassium and rubidium from blood side to lumen. In this paper it is demonstrated that caesium also can be actively transported.

2. Transport of potassium does not interfere with the metabolism of the midgut; nor does transport of caesium.

3. Potassium and rubidium compete for the transport mechanism in a one-to-one ratio but competition between potassium and caesium is more complicated. At high caesium:potassium ratios the active transport taking place is transport of caesium; when the ratio is 2 there is more transport of potassium than of caseium; when the ratio is 1 there is no transport of caesium.

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