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Active transport of the alkali metals by the isolated mid-gut of *Hyalophora cecropia*

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The isolated midgut of the larvae of the American silkworm transports potassium from blood-side to lumen, when the bathing solution contains potassium, magnesium, calcium and sucrose.

When potassium is substituted by caesium the midgut transports caesium. The competition between potassium and caesium for the transport mechanism is unusual—with more than 30% potassium no caesium will be transported, with less than 20% no potassium will be transported.

Sodium, lithium and ammonium are not transported under these conditions.

When magnesium and calcium are removed from the solutions also lithium, sodium and ammonium are transported.

Potassium competes equally with rubidium for the transport mechanism, but in competition with the rest of the alkali metals potassium is the preferred ion. With 50% potassium in the solution only potassium will be actively transported.

The larva of the american silkworm is well known and large so it is rather easy to work on the isolated mid-gut of this animal. A piece of the mid-gut of the shape of a short tube is placed as a membrane between two identical solutions. The solutions contain potassium, magnesium, calcium and sucrose and the composition of the solutions used is shown in table 1. The solutions are oxygenated and the mid-gut will keep up a potential in the K solution for many hours of about 100 to 150 mV at the start.

Table 1. Solutions

Concentrations are mmol/l

S–1	30 KCl, 2 $KHCO_3$, 5 $CaCl_2$, 5 $MgCl_2$, 166 sucrose
Cs–S–1	30 CsCl, 2 $NaHCO_3$, 5 $MgCl_2$, 5 $CaCl_2$, 166 sucrose
K–32	30 KCl, 2 $KHCO_3$, 166 sucrose
Na–32	30 NaCl, 2 $NaHCO_3$, 166 sucrose

The potential originates from the active transport of potassium across the mid-gut; this was shown by Harvey & Nedergaard in 1964. They found that the potential is dependent on metabolism and decays when the supply of oxygen is cut off. The potential can be shorted with silver electrodes and the short-circuit current is independent of the presence of Na and Li ions These ions are not actively transported and neither is ammonium. The only other ion transported actively under these conditions is rubidium which will be transported just like K and compete equally for the transport mechanism.

The only other member of the alkali metals left to try was caesium. However, the mid-gut proved to be very unpredictable when bathed with solutions containing Cs. (Zerahn 1970). Some mid-guts would keep up a potential difference and short-circuit current for longer than 2 h, others would have no potential left after a few minutes.

Figure 1*a* shows a drop in current when the bathing solution is changed from S–1 to the solution where K is substituted by Cs. The current does not decay very fast and after some time it is steadily but slowly dropping. The other curves show that the potential difference decreases to zero in about 5 min and this also happens for the short-circuit current.

The stability of the potential or the short-circuit current could not be improved by adding potassium to the solution, because the potassium interferes with the Cs transport. Furthermore, even with 50 % potassium in the solution the mid-gut often lost the potential in 5 to 10 min. The first experiments were therefore done with Cs–S–1 (all K substituted with Cs). The Cs flux was measured by labelling the Cs with ^{137}Cs and the apparatus used was the one used by Harvey & Nedergaard.

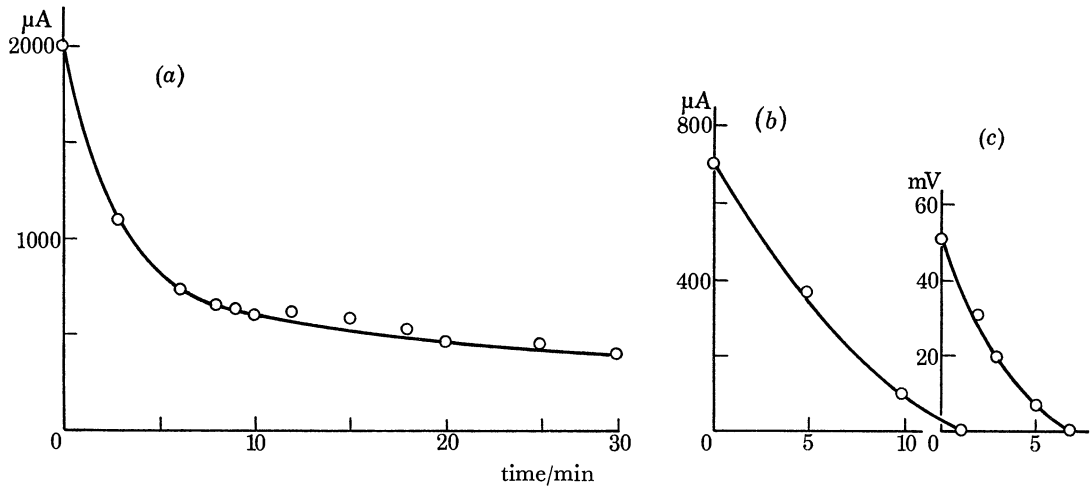

FIGURE 1

TABLE 2. CAESIUM FLUX THROUGH THE ISOLATED SHORT-CIRCUITED MID-GUT OF *HYALOPHORA CECROPIA* LARVAE FROM BLOOD-SIDE TO LUMEN

[Cs] = 30 mmol/l
μmol Cs transported per hour in intervals

	5–10 min	10–15 min	15–30 min
7 July	12.1	16.8	16.8 flux
	19.4	16.0	12.0 current
18 July	8.2	10.3	10.4 flux
	16.0	13.0	8.6 current

mean values Cs flux blood-side to lumen	11.8 μmol/h
flux lumen to blood-side	0.8
net Cs flux	11.0
short-circuit current	10.8

Table 2 shows the Cs flux from blood-side to lumen, in different time periods; the first 5 min period was discarded because the steady state was not obtained, but the next periods were treated as representative determinations and the mean values were calculated. We had to deduct the flux from lumen to blood-side; this flux was determined on other mid-guts by adding ^{137}Cs to the lumen and measuring the appearance of it on the blood-side. Furthermore, the flux ratio was determined to be about 20. As shown lately by Ussing (1971) the flux equations are applicable also when the steady state is not reached, if the two fluxes are measured over the same periods. As they were, there is no doubt Cs is actively transported.

Now we will naturally be interested in how the competition of Cs for K in the K pump. The short-circuit current of the mid-gut is in the beginning only about one-third with Cs–S–1 as the bathing solution compared to the solution K–S–1. One would be tempted to expect a competition like 1/3, when the two ions were present in the same concentration in the bathing solution.

The Cs flux from blood-side to lumen is diminished to only 1.0 μmol/h in the mean, and this is about the same size as the flux from lumen to blood-side, which was found to be 0.6 μmol/h. The small difference of 0.4 is hardly significant and a very small fraction of the short-circuit current of 20 μmol/h. We may conclude that only K is transported actively by the K pump when both ions are equally available. A much higher concentration of Cs than of K is the bathing solution seems to be needed. Other concentration ratios of Cs/K were chosen and the flux of Cs compared to the short-circuit current.

Surprisingly with 10 and 20 % K in the solution only Cs is actively transported; with 30 % K both ions are actively transported and as already mentioned with equal concentrations of the two ions only K is actively transported.

TABLE 3. CAESIUM FLUX THROUGH THE ISOLATED SHORT-CIRCUITED MID-GUT OF *HYALOPHORA CECROPIA* FROM BLOOD-SIDE TO LUMEN

Bathing solution 15 mmol/l Cs and 16 mmol/K

μmol of Cs transported per hour in intervals

5–10 min	10–15 min	15–30 min	
0.43	0.44	0.38	flux
24	21	17	current

mean values of 12 determinations on 5 mid-guts

Cs flux blood-side to lumen	1.05 μmol/h
Cs flux lumen to blood-side	0.60
net flux	0.45
short-circuit current	20

TABLE 4. CAESIUM FLUX THROUGH THE ISOLATED SHORT-CIRCUITED MID-GUT OF *HYALOPHORA CECROPIA*

Mean values of about 15 determinations per concentration (expressed as mmol/l)

% Cs	[Cs]	[K]	Cs to lumen	to blood	net	current
90	27	3.1	11.8	0.8	11.0	12.2
80	24	6.2	5.5	0.8	4.7	3.7
70	21	9.3	2.1	0.8	1.3	2.9
50	15	16	1.0	0.6	0.5	20

How can these observations be explained? The two ions must use the same transport mechanism. Cs is a rare ion in nature, and is always diluted with other alkali metals, so the chance for development of a special pump for Cs is small. The first suggestion which comes to mind is that the ions move in a single file as suggested by Hodgkin & Keynes (1955).

However, if this took place one would expect to have this phenomenon for all ions studied, and it is not apparent for the competition between potassium and rubidium, as found by Harvey & Nedergaard (1964).

If movement in single file is not the disturbing factor one can only guess that the peculiar competition is due to a change in the pump caused by the substance which is transported.

Another quite natural question will be: How does the K content in the mid-gut behave with Cs in the bathing solution? This can be seen in the table 5. When Cs is the ion being transported the K content in the mid-gut decreases, and to a rather low value so all the cells in the mid-gut are loosing K and taking up Cs. When Cs is not transported like in those guts which do not transport Cs due to the 50 % K in the bathing solution, the K content in the mid-gut is kept at a high value. The increase seen in the table is partly because the extracellular space is filled with 50 % Cs from the bathing solution.

The Cs in solution on the blood-side often abolishes the potential difference of the mid-gut within 5 to 10 min. In these experiments the K of the mid-gut will still be high and the Cs low. The action of Cs as an inhibitor of the pump thus is not exerted through changes in the ionic concentration in the cells of the mid-gut, but must work on some more specific point of the transport system.

TABLE 5. CAESIUM AND POTASSIUM CONCENTRATION IN ISOLATED MID-GUT
(μmol/g WET WEIGHT)

No correction for adhering solution. The guts were washed 1 minute in 260 mmol/l sucrose solution before gentle blotting

	solution		mid-gut		
expt.	[K]	[Cs]	[K]	[Cs]	[K+Cs]
6	0	30	8.4	28.8	37.0
3	3.2	27	11.8	22.8	34.6
2	6.4	24	19.8	22.2	42.0
14	16.0	15	42.2	9.5	51.7

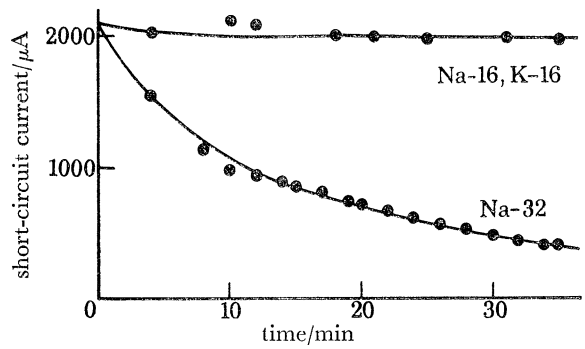

FIGURE 2

The unusual competition between Cs and K leads to the question: Do other cations really not get actively transported? Is this not hidden by a high concentration of K in the bathing solution? When I was working together with William Harvey this year, we discussed this problem. We wanted to study it but were rather reluctant. We knew from earlier work on the mid-gut, that when K is substituted by Na the potential will disappear fast and often be reversed. However, in our studies of the influence of the K concentration on the potential difference, we wanted to avoid other ions, because they might be actively transported. We left every unnecessary substance out of the bathing solution and had the following solution left, K-32, containing potassium, chloride, bicarbonate as buffer and sucrose. We know sucrose is a necessity for keeping an active K transport, we do not know why. This solution will transport K just as the earlier used solution S–1. We found no difference in the transport of K by omitting

Mg and Ca, but when we substituted the K with Na we found a great difference in the behaviour of the mid-gut. In this solution Na-32, the active transport of Na is going on well, and in the next table the time curve for the short-circuit current is shown.

The current decreases when the bathing solution is changed from K-32 to Na-32 but slowly so the Na transport is easily studied. When the bathing solution contains 50 % K and 50 % Na the current is stable and behaves as a K transport. This is in good agreement with the earlier findings by Harvey & Nedergaard that Na has very little influence on the short-circuit current when added in the same amounts as K to the bathing solution.

To make sure that we do have an active Na transport we first measured the Na flux from blood-side to lumen with ^{22}Na (see table 6). Na flux equals the current and the table contains data from all the experiments: none were discarded because the mid-gut lost the potential difference after a few minutes. However, it is useful to know the flux from lumen to blood-side even if table 6 shows it must be small, and the next table shows this.

TABLE 6. SODIUM FLUX FROM BLOOD-SIDE TO LUMEN OF THE SHORT-CIRCUITED, ISOLATED MID-GUT OF *HYALOPHORIA CECROPIA*

Bathing solution 30 mmol/l NaCl, 2 mmol/l $NaHCO_3$ and 166 mmol/l sucrose

date	period min	Na-flux μmol/h	current μmol/h
22 June	10–15	18.7	21.2
	15–30	18.7	16.5
23 June	10–20	26.8	21.5
	20–30	21.1	18.3
mean of 10 determinations and 5 mid-guts			
	—	22.5	22.9

TABLE 7. SODIUM FLUX FROM LUMEN TO BLOOD-SIDE OF THE SHORT-CIRCUITED, ISOLATED, MID-GUT OF *HYALOPHORIA CECROPIA*

The bathing solutions on both sides of the mid-gut contained 30 mmol/l NaCl, 2 mmol/l $NaHCO_3$ and 166 mmol/l sucrose and were oxyygenated

date	period min	sodium-flux μmol/h	current μmol/h
24 Jun (A)	10–20	0.67	22.0
	20–30	0.79	19.0
24 June (B)	10–20	1.40	28.0
	20–30	3.40	17.0
24 June (C)	10–20	1.00	10.1
	20–30	1.06	9.2
24 June (D)	10–21	0.50	22.4
	21–30	0.74	17.5
mean	—	1.2	18.2

The mean value of the flux is about 1 μmol/h and the flux ratio is about 15, demonstrating quite clearly an active transport after the flux equations of Ussing. How this Na-flux competes with K-flux is shown in table 8.

The Na flux from blood-side to lumen is about 2 μmol/h for the current. The net Na flux will be even smaller so the Na competes very unfavourably with K. However the active Na transport across the mid-gut is in another way behaving as the K transport, it is not reacting on the addition of ouabain.

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TABLE 8. SODIUM FLUX FROM BLOOD-SIDE TO LUMEN OF THE SHORT-CIRCUITED, ISOLATED MID-GUT OF *HYALOPHORA CECROPIA*

The bathing solutions on both sides of the mid-gut contained 16 mmol/l Na, 16 mmol/K, 30 mmol/l Cl, 2 mmol/l HCO_3 and 166 mmol/l sucrose and were oxygenated

date	period min	sodium-flux μmol/h	current μmol/h
26 June (A)	20–30	0.61	21
26 June (B)	10–20	0.20	32
	20–30	0.25	32
26 June (C)	10–20	1.95	69
	20–30	3.56	69
27 June (A)	10–20	1.43	37
	20–30	2.50	35
27 June (B)	11–20	1.73	74
	20–30	2.70	74
mean	—	1.7	49

TABLE 9. COMPETITION BETWEEN DIFFERENT CATIONS EXPRESSED AS FLUX OF COMPETING ION/FLUX OF K OR Na

Concentration ratio of competing ions 1/1

solution cation	+5 mmol/l Ca and Mg		free of Ca and Mg	
	16 mmol/l K	50 mmol/l K	16 mmol/l K	16 mmol/l Na
K	1.00	1.00	1.00	—
Rb	1.00	—	—	—
NH_4	0	—	0.25	7.00
Cs	0.05	0.15	—	1.6
Na	0	—	0.05	1.00
Li	0	—	—	1.00

Lithium, like sodium, does not interfere with potassium transport when they are present in the same concentrations. Because of the lack of a suitable radioisotope for lithium we have not treated this cation as we could study Na. However, we were able to demonstrate a short-circuit current for Li just as we were doing for Na, and this is a fair proof of the active transport of Li. It has also been possible later to show that under the same conditions with an NH_4-32 solution, there will be an active transport of ammonium, just as for Na and Li. Also the transport of Cs will, when Cs-32 is used as bathing solution be much easier to produce and will not be as unpredictable as in the solutions containing Mg and Ca ions.

So it is obvious that the mid-gut transports all the alkali metals actively, including ammonia, the question of how they compete is not answered completely. From the experiments with Cs it was clear that both the concentration and the concentration ratio between the ions is involved. But the question can be answered when both the concentration is kept constant and the ratio is 1/1 for the competing ions.

The left side of the table shows that the absolute concentration has some influence when Mg and Ca are present. The right side that both ammonium and Na are competing unfavourably with K, and that Li is competing equally with Na, and Na unfavourably with Cs and NH_4. The sequence at the outmost left gives the order for competition of the studied alkali metals. If the order of competition is compared with the physical data of the ions there is no agreement

with the ionic diameter taken from crystal data, and no agreement either with the mobility of the ions in aqueous solutions. A more specific property of the ions seem to play the important role in the process of active transport. However the fact that so many cations all have the ability to be actively transported may be a useful tool which, in time, may contribute new information and understanding of the ion pump in the midgut.

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