

## COMPETITION BETWEEN POTASSIUM AND RUBIDIUM IONS FOR PENETRATION OF THE MIDGUT OF *HYALOPHORA* *CECROPIA* LARVAE

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The midgut of the American silkworm transports potassium actively from blood-side to lumen (Harvey and Nedergaard 1964). The use of radiopotassium to study this process is limited by the short half-life of  $^{42}\text{K}$  (12.4 h).  $^{86}\text{Rb}$  which has a half-life of 19 days has, therefore, been used in place of potassium on the assumption that Rb and K can substitute for each other. This assumption was tested by Harvey, Haskell & Nedergaard (1968) who showed that the short-circuit current does not change significantly when all K in the bathing solution is substituted by Rb. This experiment does not, in fact, prove that the two ions compete equally. However, Nedergaard and Harvey (1968) measured the net flux of Rb and K by means of absorption spectrophotometry and found that with a K/Rb concentration ratio of 1.1 the mean K/Rb flux ratio was 1.4 (5 determinations). The difference was disregarded and the conclusion made 'the midgut is unable to distinguish between potassium and rubidium'.

It is obviously an advantage to use Rb-86 when working with the midgut, so we have retested the competition between K and Rb fluxes. A 100% agreement between the fluxes could not be found, the values varied and on the average a 50-60% Rb flux was found for a 100% K flux. The variations between different guts were, however, much larger so the mean value cannot be used for a specific experiment. It made no difference if we considered the active K flux from blood-side to lumen or the flux from lumen to blood-side.

Fifth-instar larvae of *Hyalophora cecropia* (L) of 10-15 g were used for the experiments. They were grown on artificial diet (Riddiford, 1968) and on leaves of willow (*Salix babylonica*). The larvae were chilled on ice for at least 1 h before the gut was removed and placed in the apparatus described by Harvey, Haskell & Zerahn (1967) and Harvey & Zerahn (1972). Generally the midguts were short-circuited, but a few experiments were performed on unshorted guts.

Potassium flux was measured by labelling with  $^{42}\text{K}$  and the rubidium flux by labelling with  $^{86}\text{Rb}$ . The necessary amount of  $\text{K}^{42}$  and  $\text{Rb}^{86}$  were mixed and simultaneously added to the solution. In this way K and Rb were influenced identically, for example, by inaccurate short-circuiting.

The  $\text{K}^{42}$  was measured with a NaI crystal in a scintillation well counter and pulse height analyser (Selectronic) adjusted to minimize the effects of  $^{86}\text{Rb}$  gamma rays. After decay of the  $^{42}\text{K}$  the  $^{86}\text{Rb}$  was counted on a Packard beta spectrometer (Tricarb) with an accuracy of 2% or better. Radio tracers were added to the solution of one side

Table 1. *Competition between Rb and K flux from blood-side to lumen in the isolated, short-circuited, midgut of Hyalophora cecropia grown on artificial diet*

The fluxes are corrected for difference in concentration by multiplying the Rb flux with the concentration ratio of K/Rb. Ratio of rate constants given below. Fluxes obtained from double labelling experiments.)

Date	Concentration in bathing solution		Ratio	Rb flux/K flux					Mean value
	K	Rb		5 min	10 min	20 min	40 min	60 min	
	14. 11. 78	32		1	32	0.46	0.46	0.58	
15. 11. 78	32	3	10.6	0.41	0.45	0.48	0.53	0.59	0.49 ± 0.04
15. 11. 78	30.4	1.6	19	0.60	—	—	0.65	0.67	0.64 ± 0.03
15. 11. 78	30.4	1.6	19	0.40	0.37	0.40	0.48	0.49	0.43 ± 0.03
16. 11. 78	28.8	3.2	9	0.23	0.28	0.22	0.23	0.19	0.23 ± 0.02
16. 11. 78B	28.8	3.2	9	0.70	0.67	0.63	0.72	0.73	0.69 ± 0.02
16. 11. 78	25.6	6.4	4	0.55	0.59	0.59	0.59	—	0.58 ± 0.01
16. 11. 78B	25.6	6.4	4	0.54	0.51	0.50	0.52	—	0.52 ± 0.01
17. 11. 78	16.0	16.0	1	0.55	0.61	0.60	0.65	0.71	0.62 ± 0.03
17. 11. 78B	25.6	6.4	4	0.55	0.55	0.53	0.55	0.59	0.55 ± 0.01
17. 11. 78	25.6	6.4	4	0.80	0.91	0.91	0.86	0.98	0.89 ± 0.03
Mean value (not valid)				0.53	0.54	0.54	0.58	0.61	0.56

Table 2. *Competition between Rb and K flux from lumen to blood-side in the isolated, short-circuited, midgut of Hyalophora cecropia reared on artificial diet*

(The fluxes are corrected for difference in concentration by multiplying the Rb flux with the concentration ratio of K/Rb, ratio of rate constants given below. Fluxes obtained from double labelling experiments.)

Date	Concentration in bathing solution		Ratio	Rb flux/K flux					Mean value
	K	Rb		5 min	10 min	20 min	40 min	60 min	
	27. 11. 78	25.6		6.4	4	0.69	0.80	0.61	
27. 11. 78	28.8	3.2	9	0.54	0.48	0.60	0.55	0.58	0.55 ± 0.02
28. 11. 78	25.6	6.4	4	0.53	0.51	0.47	0.54	0.49	0.51 ± 0.01
28. 11. 78B	32.0	1.0	32	—	0.65	0.63	0.70	0.73	0.68 ± 0.03
28. 11. 78	28.8	3.2	9	0.39	0.41	0.46	0.46	0.49	0.44 ± 0.02
28. 11. 78B	30.4	1.6	19	0.89	0.79	0.79	0.69	0.79	0.79 ± 0.04
29. 11. 78	32.0	1.0	32	0.56	0.64	0.45	0.55	0.62	0.56 ± 0.04
29. 11. 78B	16.0	16.0	1	0.59	0.57	0.76	0.84	0.86	0.72 ± 0.07
29. 11. 78	30.4	1.6	19	0.32	0.51	0.50	0.58	—	0.48 ± 0.06
29. 11. 78B	16.0	16.0	1	0.59	0.59	0.54	0.54	0.55	0.56 ± 0.01
Mean value				0.57	0.60	0.58	0.61	0.66	

of the gut at the start and samples taken from the other side at times given in the tables.

The concentration of K was identical on both sides as was the concentration of Rb. The ratio of the concentration of the two ions was varied from experiment to experiment to test the influence of the concentration on the K/Rb flux ratio. The bathing solution used with  $^{42}\text{K}$  had the following composition: K + Rb = 32 mM; sucrose, 166 mM; Cl, 30 mM;  $\text{HCO}_3$ , 2 mM.

Table 1 shows the measured Rb/K flux ratios. To correct for the concentration

Table 3. Competition between Rb and K flux from blood-side to lumen, in the isolated midgut of *Hyalophora cecropia larva reared on willow*

(The fluxes are corrected for difference in concentrations by multiplying the Rb flux with the concentration ratio of K/Rb ratio of rate constants given below. Fluxes obtained from double labelling experiments.)

Date	Concentration in bathing solution		Ratio K/Rb	Rb flux/K flux					Mean value
	K	Rb		5 min	10 min	20 min	40 min	60 min	
18. 6. 79	16.0	16.0	1.00	0.74	0.74	0.70	0.70	0.75	0.73 ± 0.01
19. 6. 79A	16.0	16.0	1.00	0.86	0.81	0.80	0.81	—	0.82 ± 0.02
19. 6. 79B	16.0	16.0	1.00	0.60	0.49	0.54	0.60	—	0.56 ± 0.03
19. 6. 79C*	16.0	16.0	1.00	0.50	0.51	{ 0.48 0.65†	0.58‡	0.63	0.62 ± 0.03
20. 6. 79A*	16.0	16.0	1.00	0.34	0.40	{ 0.40 0.49†	0.45‡	0.47	0.47 ± 0.01
20. 6. 79B*	2.35	30.0	0.078	0.77	0.75	{ 0.77 0.74†	0.82‡	0.82	0.79 ± 0.03
20. 6. 79C*	2.35	30.0	0.078	1.13	0.98	{ 0.85 0.95†	0.94‡	0.90	0.92 ± 0.02
21. 6. 79A	2.70	30.0	0.090	0.71	0.72	0.74	0.71	0.84	0.74 ± 0.03
21. 6. 79B*	29.7	3.00	9.9	0.40	0.53	{ 0.36 0.44†	0.42‡	0.46	0.44 ± 0.01
21. 6. 79C*	29.7	3.00	9.9	0.59	0.58	{ 0.58 0.59†	0.56‡	0.58	0.58 ± 0.01
Mean value 0 mV not valid				0.73	0.69		0.66	0.58	
Mean value 100 mV						0.60			
Mean value 0 mV same guts						0.64			

\* First 20 min unshortened gut over 100 mV.

† 25-30 min period shorted after period of 20 min unshorted.

‡ 30-40 min period shorted after + period.

differences between K and Rb in the bathing solutions the Rb flux is multiplied by the K/Rb concentration ratio. The ratio is thus given for a 1 mM solution of both ions or for the rate constants. From Table 1 it can be seen that the mean values of the Rb/K ratio do not vary significantly with time. Rb carries only 56% of that expected for a perfect tracer for K. Because of individual variation, between 19 and 98%, an appropriate mean value cannot be obtained.

The inability of Rb to substitute for K might be true for the active K transport only. Accordingly flux experiments from lumen to blood-side were made. The results shown in Table 2 show that Rb does not behave differently when it traces for K from the blood- or from the lumen side.

Larvae are reared on leaves or on artificial diet. It is conceivable that differences in rearing might change the Rb/K ratio for transport. Table 3 summarizes results for animals reared on willow and show that under these conditions Rb is also not a reliable tracer for K. With the midgut at its normal potential the flux of K from blood-side to lumen can be diminished by a factor of 2-5. Nevertheless the Rb/K ratio is still far from 1 even with a potential over 100 mV across the midgut.

The above results indicate that Rb is not a perfect tracer for K. Only about half of the K flux is measured when Rb is used as a tracer for K, regardless of the Rb

concentrations. However, the wide biological variations between the guts implies that while Rb partially reflects the K flux, a constant factor between Rb flux and K flux cannot be found. The conclusion is that Rb cannot be used quantitatively as a tracer for K. This is true regardless of whether the active K flux is measured from blood-side to lumen or from lumen to blood-side.

The diffusion rates in water for potassium and rubidium are very close, so if the ions were passing the gut by diffusion they should compete almost equally. We know that the transport of K is active and accordingly the Rb/K ratio is often far from 1. But for the K flux from lumen to blood-side we do not know how the K crosses the membrane. It is interesting to note that under short-circuit conditions the Rb/K ratio is about the same as for the active K flux. We must conclude that in general K does not cross the gut by diffusion, but more probably uses the same route as the active K flux, possibly as a leak through the K pump.

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