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POTASSIUM EXCHANGE BETWEEN BATHING SOLUTION AND MIDGUT OF *HYALOPHORA CECROPIA* AND TIME DELAY FOR POTASSIUM FLUX THROUGH THE MIDGUT

BY KARL ZERAHN

Institute of Biological Chemistry A, University of Copenhagen Universitetsparken 13, D.K.-2100 Copenhagen Ø, Denmark

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SUMMARY

Exchange between potassium of the isolated midgut of the American silkworm *Hyalophora cecropia* and the bathing solutions has been determined by different authors, but with contradictory results. Therefore the experiments were repeated with another technique and the exchange determined for varying time intervals. It was found that the exchange was fast – half of the midgut K was exchanged in 2–3 minutes, which confirms the earlier findings by Harvey & Zerahn (1969).

INTRODUCTION

When the midgut of larvae of the large silkmoth *Hyalophora cecropia* is bathed in a solution containing potassium ions and sucrose it will actively transport potassium from the blood-side to the lumen (Harvey & Nedergaard, 1964). Harvey & Zerahn (1969) found that it takes about 12 min for the total midgut K to reach a relative specific activity of 70% with respect to the specific activity of the labelled K in the blood-side solution. This determination included both intra- and extracellular K in the tissue. Since the specific activity of the K in the extracellular spaces can be assumed to be low because the activity in the lumen solution was nearly zero, it can be concluded that the specific activity of the intracellular potassium in 12 min is even higher than the 70% which was reported.

Harvey & Wood (1972, 1973) have since reported experiments which they claim show that the increase in specific activity of cell potassium is a much slower process. They find that it takes about 1 h for the cell K to reach a level of 50% of the activity of the blood-side K.

Different techniques have been used for various series of measurements, and it was therefore reasonable to investigate the problem in more detail in the hope of solving the discrepancy. The results I report here confirm the earlier findings that it takes only minutes before a high level of specific activity is reached inside the cells.

METHODS

The midgut was put up in the apparatus described by Harvey, Haskell & Zerahn (1967). The syringe which was used for controlling the distension of the gut was also used to measure its volume when it was blown up as a sphere. The area of the sphere was calculated from the measured volume. The thickness of midgut samples varied from 10 to 20 mg/cm^2 (100–200 μm). The bathing solutions were the following: K-32: 30 KCl, 2 $KHCO_3$, 166 sucrose mmol/l; S-1: the same plus 5 $CaCl_2$, 5 $MgCl_2$ mmol/l.

The guts were keeping their natural potential during the experiments. No experiments were done with short-circuited guts because it would be very difficult to do the short time experiments (2–5 min) properly with short-circuited guts. Harvey *et al.* (1967) found that the flux of K from blood-side to lumen in the short-circuited midguts is on the average 2·1 times as large as the flux in the guts which have not been short-circuited. Thus the K exchange in the guts with natural potential cannot be expected to exceed that of the short-circuited midguts.

Equilibrium was assumed to have been established after the gut had been put up 10–15 minutes with the bathing solution on both sides. Radioactive potassium was then added simultaneously to both blood-side and lumen in such amounts that the specific activity was the same on both sides. At the appropriate time the total lumen solution was removed and an aliquot taken for measurement of the radioactivity. The gut itself was immediately removed, blotted slightly to remove adhering fluid, weighed and then mashed with 1 ml of perchloric acid (0·3 M). One ml of blood-side solution was finally taken.

The radioactivity of midgut and bathing solution were measured with a NaI crystal in a scintillation well counter from the Danish firm Selectronic and the potassium content was measured with a flame photometer (Unicam SP 90B). The sucrose content was determined by the glucose oxidase method after hydrolysing.

The bathing solutions have a high concentration of sucrose so the amount of sucrose present in the midgut was used as a measure for the extracellular space. In the first series of experiments it was found to be 48% (range 42–55%) and 45% in the second series (range 39–54%). The determinations are based on the assumption that sucrose does not enter the cells. To check the method, control experiments were made where the bathing solution was labelled with ^{35}S as sulphate in 8 mM cold sulphate as carrier. The concentration of ^{35}S was the same on both sides and the extracellular space was calculated both from the tissue content of ^{35}S measured on a Packard Tricarb Scintillation Spectrometer and from the sucrose content. Five determinations showed the sucrose space to be 82% of the ^{35}S space. Normally one would expect the sulphate content and sucrose content in the extracellular space to be identical. The difference however is not surprising because the sulphate molecule is smaller than the sucrose molecule, so it may penetrate further and also be bound to cations to some degree. Sucrose may also give too small a value as some of it may be metabolized by the midgut. However, under the experimental conditions used, the possibility was minimized because the time interval in which the gut was not in contact with the sucrose-containing solution was less than 1 min in all cases.

Even if the two methods produce somewhat different values, they are comparable.

In the earlier experiments only the solution on the blood-side was labelled, so the extracellular and adhering solution on both lumen and blood-side should have been determined separately in order to calculate cellular specific activity. In the present experiments both solutions were labelled equally. Therefore, in order to determine cellular specific activity, it is only necessary to know the total extracellular space given by the content of sucrose in the gut.

The radioactivity of the midgut was calculated as a percentage of the radioactivity of 1 ml of bathing solution, and the specific activity determined by dividing by the gut K. The relative specific activity is the ratio of specific activity of gut K to specific activity of K in solution. In curve (*a*) of Fig. 1 the relative specific activity of the K in the bathing solution is normalized to 100%. Values for cell K used in determining specific activity were corrected for extracellular K content and radioactivity. When a pronounced exchange has occurred, the correction for extracellular K has only a small influence on the specific activity of gut K, even with an extracellular space of around 50%. The K concentration in the midgut cells is constant during the experiments, and on the average was found in series 2 to be 137 ± 8 mM. This is in agreement with other experiments made in this institute and with the same technique, where no drop in the K concentration could be found with increasing length of the experiment, but is higher than the values found by Harvey & Zerahn (1969). The reason for the difference is because the guts in 1969 were washed in sucrose solution before the K was determined, and the concentration given was for the gut weight and not for the cells.

RESULTS

Fig. 1 shows the change in relative specific activity of total midgut K (curve (*a*)) and intracellular K (curve (*b*)). It can be seen that the relative specific activity approaches a value of 88% for the total K of the midgut and 84% for cell K. The initial slope of curve (*a*) reached the 50% level at 2·2 min whereas the corresponding time for curve (*b*) was 3 min.

Two series of experiments were done with similar results. One series used larvae grown on willow with a mean weight of 10 g and a potential difference of 110–30 mV during the experiment (mean value 40 mV). The other series used larvae grown on the diet given by Riddiford (1968), mean weight 8 g, 90–20 mV (on the average 61 mV). For the first series the level of 50% was reached in 2·2 min, for the other series in 2·6 min. The final level for labelling was 88% for the first series and 80% for the other series.

These experiments were done with a bathing solution free of Ca (K-32) but two experiments done with Ca and Mg containing solution (S-1) gave points which fell on curve (*a*) shown in Fig. 1. Thus there seems to be no difference in the rate of exchange whether or not Ca and Mg are present.

As seen from curve (*b*) there is a part of the cell K which does not become labelled. It was found that 10–20% of cell K remains unlabelled after 10 min. This K which is a small fraction of total gut K and has a very slow exchange, if any, will not be able to significantly influence a fast K flux through or into the midgut.

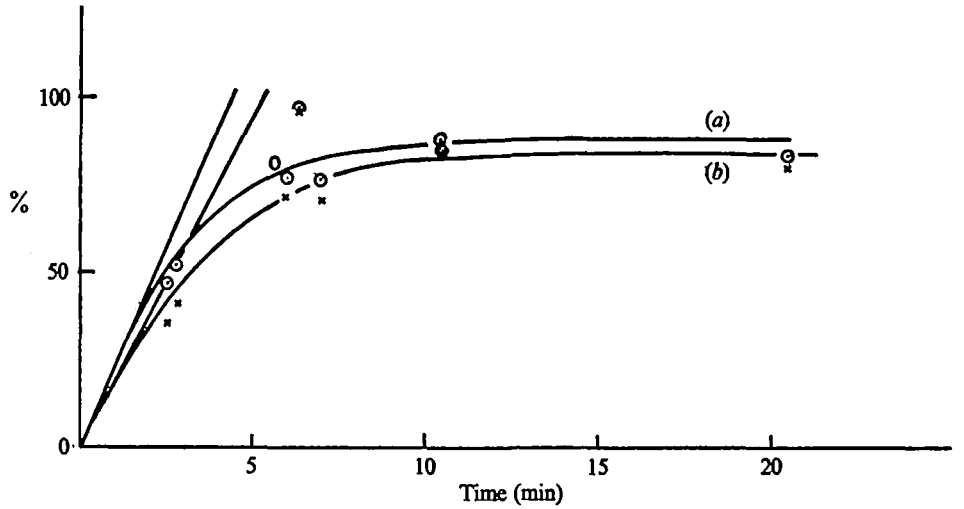

Fig. 1. Exchange between radiopotassium in the bathing solution and potassium in the midgut of *Hyalophora cecropia*. Time is plotted on the abscissa and the relative specific activity of K in the midgut and in the midgut cells on the ordinate. ⊙(*a*), Total K; ×(*b*), cell K;

$$\frac{\text{radioactivity of midgut}}{\mu\text{equiv K}} \text{ in \% of } \frac{\text{radioactivity of solution}}{\mu\text{equiv K}}.$$

The specific activity of the K in solution is taken as 100%. As seen from the curve a constant level is reached about 6 min after adding ^{42}K to the bathing solution on both sides of the midgut. The initial slope of curve (*a*) at time zero shows a specific activity of 50% in 2·2 min and of curve (*b*) in 3 min. The mean thickness of the guts was 15 mg/cm^2 or 150 μm.

DISCUSSION

The fast exchange between the gut K and labelled K in the bathing solution which was found earlier by Harvey & Zerahn (1969) has been confirmed in this investigation. Furthermore the value of 50% exchange of gut K in 2·5 min shown in Fig. 1 is not compatible with the value of about 50% in 1 hr found by Harvey & Wood (1972, 1973).

What can be the reason for this disagreement?

The first results by Harvey & Wood in 1972 were obtained from pieces of gut suspended as big lumps in the solution, so the diffusion to the middle of the tissue may have been rate-limiting and this could be the explanation for the long time for exchange. However, the results by Harvey & Wood in 1973 were obtained from experiments done under similar conditions to those used in the present investigation.

The thickness of the midgut has a definite influence on the delay of ^{42}K flux from blood-side to lumen. A twofold increase in the thickness of the midgut gave an increase of about two times in the lag time (Zerahn (1973); thickness of the guts varied from 170 to 440 μm in the mean of the two series).

No thickness of the midgut is given in the paper by Harvey & Wood (1973), but the weight was given to be about 100 mg, and with the guts distended the area should be in the order of 2–5 cm^2. This would give a thickness of about 30 mg/cm^2 = 300 μm compared to a mean gut thickness of 150 μm in the present investigation. It is hard to believe that this difference in thickness should be the only reason for the very different results in exchange rate reported by the different authors.

However, another difference between the investigations is that Harvey and Wood find a very low value for the extracellular space (5·6 μl on lumen and 1·2 μl on blood-side as mean values) and this will influence the calculation of the relative specific activity in the midgut cells.

Harvey and Wood made the determination by first labelling the solution with ^{35}S for 5 min, after which they washed away the labelled bathing solution. They do not indicate how rapidly the washing was carried out, so that it is not possible to estimate how much of the label in the extracellular space was washed away.

The difference in evaluating the extracellular space may give an explanation for the degree of labelling found by the different authors, but not for the rate.

In this study both sides of the bathing solution of the midgut are labelled in contrast to the earlier work where only the blood-side solution was labelled. The advantage is that we only need to make a correction for the total extracellular space and not for both sides separately.

Obviously no significant exchange should take place between the K in the lumen solution and the cells as confirmed earlier by Harvey and Zerahn (1969) for the solution S-1. Therefore neither the level of exchange nor the rate can be explained in this manner.

The fraction of cell K (about 10–20%) which does not exchange with the rest of the gut K may be present in either epithelial cells or in other cells, such as muscle cells or trachea cells. We know nothing of the exchange of K for the different cell types, but we know that the columnar cells are the larger part of the midgut tissue. These cells must exchange all or a large part of their cell K at a fast rate. In some experiments a midgut was labelled as described for about 20 min, after which the bathing solutions on both sides were replaced with unlabelled solutions. After 5 min in unlabelled solution the specific activity of the cells was only 20% and after 15 min only 2·5% of the original value. This shows that labelled K is not significantly taken up by the unexchangeable K pool.

The fast exchange of the K in the midgut cells is in agreement with the fast uptake of sodium which occurs when the K in K-32 solution is substituted by Na. In a previous study we found that Na reached the maximum concentration in the midgut cells in about 5 min (Harvey & Zerahn, 1972).

CONCLUSION

A careful reinvestigation of the exchange of radiopotassium between the midgut cells and the bathing solution has shown that it takes about 6 min for the intracellular K to become maximally labelled. In addition, only 10–20% of the total K of the midgut is left unlabelled. These results which confirm the earlier findings of Harvey & Zerahn (1969) are not compatible with those of Harvey & Wood (1972, 1973), who found both a slower rate of exchange and lower total labelling. Although it is possible to propose a basis for the difference in the total level reached it is not possible from the results obtained in the present study to explain the difference in rate of exchange.

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