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KINETICS AND ROUTE OF ACTIVE K-TRANSPORT IN THE ISOLATED MIDGUT OF *HYALOPHORA CECROPIA*

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INTRODUCTION

The isolated midgut of the Cecropia silkworm actively transports large quantities of potassium from its blood-side to its lumen (Harvey & Nedergaard, 1964). The epithelium consists of but two types of differentiated cells, columnar cells and goblet cells in a ratio of approximately 2 to 1, arranged in a one-cell-thick mosaic and resting on a thick basement lamina (Anderson & Harvey, 1966). The surrounding muscle cells and tracheae, which are a minor fraction of the gut, do not form a diffusion barrier; the trans-membrane transport mechanism is therefore restricted to the epithelium.

The kinetics of ^{42}K flux from blood-side to lumen were studied in an effort to locate the massive stream of K being transported through the epithelium. The time lag between exposing the blood-side to labelled potassium and its reaching a steady state of appearance in the lumen was determined from the time-course of ^{42}K flux (Fig. 1). If the labelled K were to pass directly through the gut without being used to label the gut itself, such a plot of the K-flux, with time, would yield a straight line through the origin. However, a lag time of 2–4 min. invariably was observed. The existence of such a lag time means that some isotope must accumulate within the gut. The labelled K might be located either before or after the transport mechanism and, depending on the location of the latter, it might be localized within cells or in extracellular spaces.

The flux of ^{42}K through the gut was changed: (1) by short-circuiting the gut, (2) by changing the blood-side K concentration and (3) by decreasing the oxygen pressure in the bathing solutions. The lag time is independent of the ^{42}K flux, and we conclude that the potassium must be transported through the gut without mixing to any great extent with gut K. With 2 mM potassium in the blood-side solution the specific activity of the K appearing in the lumen under steady-state conditions must approach 100% of the blood-side value. However, the specific activity of gut K was found to be less than 10%, furnishing strong support for the conclusion just reached.

METHODS

Mature 5th-instar larvae of *Hyalophora cecropia* were reared and the midguts were isolated by the methods of Nedergaard & Harvey (1968). The midgut potential, short-circuit current, K-influx, and oxygen consumption were measured as described by Harvey, Haskell & Zerahn (1967) (also see Fig. 2). The standard perfusion

medium (S 1) contained 32 mM-K^+, 5 mM-Mg^{2+}, 5 mM-Ca^{2+}, 2 mM-HCO_3^-, 50 mM-Cl^- and 166 mM sucrose per litre.

In order to determine its lag time, the K-influx must be held as constant as possible. The midgut was therefore equilibrated in the appropriate solution for from 30 to 45 min. prior to the measurement of lag time, and the period of measurement was restricted to but 12 min. The K-influx depends on the oxygen consumption which in turn depends on the oxygen pressure. The latter was kept constant by injecting 1–2 c.c. of

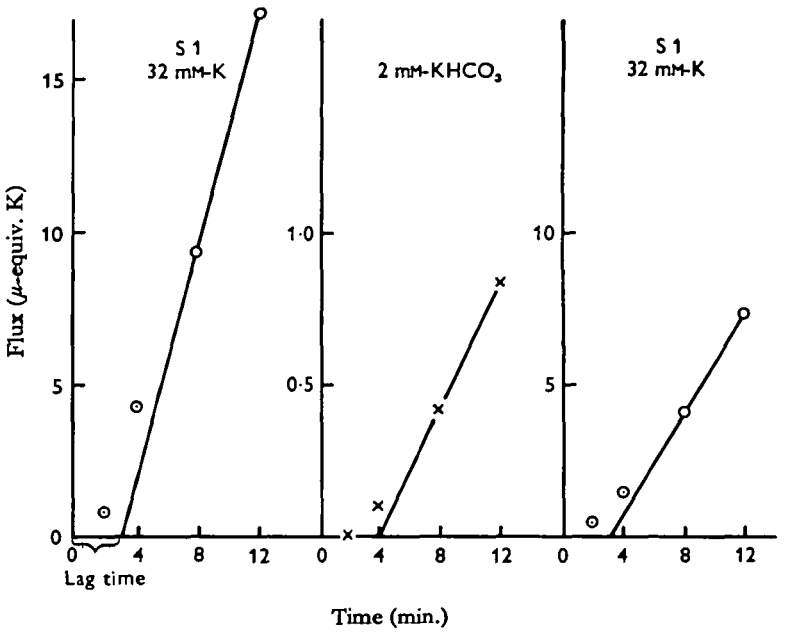

Fig. 1. The time-course of ^{42}K-movement from blood-side to lumen of an isolated midgut is plotted for a representative experiment in which the gut is equilibrated with S 1 (32 mM-K), with 2 mM-$KHCO_3$ (ordinate expanded 10 ×), and again with S 1. The lag time is estimated by extrapolating the steady-state line to the abscissa. Although the flux varied from 11·9 to 6·2 and back to 4·9 μ-equiv. K/hr. the lag time was virtually unchanged.

air or oxygen into the blood-side chamber, except when low oxygen pressures were desired. In this case the blood-side solution was equilibrated with nitrogen and the lumen solution was stirred with air, supplying sufficient oxygen to the gut to maintain a low and steady current. The oxygen pressure in the blood-side solution was monitored by an oxygen electrode.

The open circulation in the lumen disturbed the adjustment of the short-circuiting slightly, so the lumen was closed for about 10 sec. while this adjustment was made and then re-opened. Hydrogen bubbles produced by the central Ag electrode were removed by the lumen circulation.

For the determination of the K-influx and its lag time, ^{42}K was injected from a syringe through a thin cannula which penetrated the rubber tubing and ended in the solution in the blood-side chamber (Fig. 2). The injection of ^{42}K took about 1 sec., the amount of labelled solution being usually about 50 μl. of approximately 0·2 M-KCl. At appropriate intervals samples were taken from the lumen solution with a Krogh

syringe and the volume was restored with inactive solution. The samples in small test tubes were counted in a well-scintillation counter, and the fluxes were computed by comparing the radioactivity in the lumen samples with that in a 50–100 μl. sample of the blood-side solution made up to volume with inactive solution.

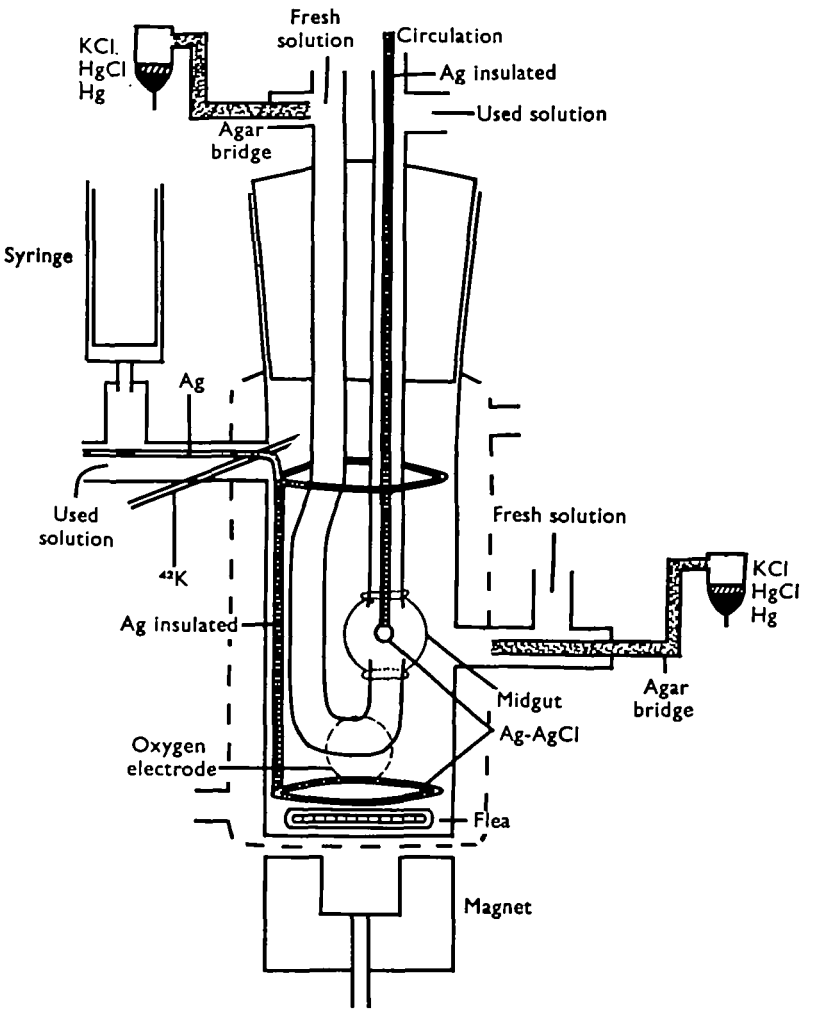

Fig. 2. Chamber used to perfuse the isolated midgut and measure the midgut potential, short-circuit current, K-influx and oxygen uptake (after Harvey *et al.* (1967)). ^{42}K was injected through the cannula. Aeration and stirring of the lumen solution was accomplished through the upper ends of the lumen chamber. Broken lines indicate position of water jacket to maintain constant temperature.

Inhibition of active K-flux by low oxygen pressure seemed to be completely reversible, so it was reasonable to assume that no significant changes in cellular K concentration occurred during inhibition. Inhibition of the K-flux by reducing part of the potassium available for transport was less straightforward. Substitution of blood-side K with Mg was unsuccessful because the potential dropped below zero and the process was far from being reversible, presumably indicating damage to the gut. When the

blood-side K was reduced to 2 mM by substitution with sodium or sucrose, the inhibition was much more reversible as judged from the partial reversibility of the drop in short-circuit current or K-flux. Moreover, the inhibition had no direct or subsequent effect on the lag time which was the same before, during, and after inhibition (Fig. 1). The maintenance of a high potential in the low-K solution indicated that the gut was still functioning. Chemical determinations by flame photometry showed that there was no significant change in gut K even at this low K concentration on the blood-side (Table 4).

The unchanged lag time and unchanged gut K during inhibition of K-influx indicate that the amount of K in front of the transport mechanism must be very small. However, the lack of change in lag time might have been due to a fast equilibrium of cell K with the blood-side ^{42}K. To determine the rate of exchange between blood-side ^{42}K and gut K, the midgut was isolated and perfused in the usual way, and the lag time in 2 mM-$KHCO_3$ + sucrose was determined in 10 min. Then the gut was removed from the blood-side solution, rinsed with 260 mM sucrose solution, and the specific activity was determined after 12 min. in all. Because the labelling of the gut was low, only 18% of blood-side K, it is a fair approximation to divide this value by 12 to get the exchange per minute, which amounts to around 2%/min in 2 mM-K (Table 5). The exchange varied with K concentration and from gut to gut.

Table 1. *Influence of inhibition of K-flux on lag time of the isolated short-circuited midgut*

The inhibition was obtained by reducing the oxygen pressure in the blood-side solution with nitrogen.

	K transport in oxygen		Inhibited K transport in nitrogen	
Date	K-influx (μ-equiv./hr.)	Lag time (min.)	K-influx (μ-equiv./hr.)	Lag time (min.)
26 May	60	3·1	24	3·3
27 May	50	3·8	18	3·7
3 June	70	2·3	15	1·6
Mean	60	3·1	18	2·9

RESULTS

Although the K-transport in nitrogen was reduced to less than one-third of its magnitude in oxygen, the lag time was unchanged at about 3 min. (Table 1). The same result was obtained when the K-transport was reduced by lowering the K concentration in the blood-side solution. When the K concentration was reduced from the control value of 32 mM to low K values by replacing part of the potassium by sodium, the K-transport was correspondingly reduced to 0·8–37% of its value in 32 mM-K, but the lag time again remained unchanged (Table 2). Similarly, when the K concentration was reduced to 2 mM (leaving no cation to exchange with cell K) simply by replacing part of the potassium by sucrose, the K transport was reduced to 5·2–22% of its value in 32 mM-K and again the lag time remained unchanged (Table 3). Finally, the same independence of K-flux and lag time is observed when the flux is inhibited by the natural potential (Table 3). The results can be summarized by saying that the K transport can be reduced as much as 100-fold with only minor effects on the lag time.

The exchange found by directly labelling gut K with ^{42}K from the blood-side in experiments lasting around 12 min. was sometimes as high as 70 % in 32 mM-K and it would be necessary to correct for the high specific activity of K being transported through the gut to find the exchange per minute. However, in similar experiments

Table 2. *Influence of inhibition of K-transport by low K concentration on K-flux and lag time of short-circuited midguts*

Control experiments were performed in S 1 (32 mM-K). For the low-K experiments part of the blood-side K was substituted by Na.

	Control (32 mM-K)		Low K		
Date	K-influx (μ-equiv./hr.)	Lag time (min.)	K (mM/l.)	K-influx (μ-equiv./hr.)	Lag time (min.)
12 May	48	1·5	3·2	9	2·2
13 May	59	1·9	3·2	22	2·2
17 May	71	4·3	0·4	0·6	4·2
18 May	98	2·4	3·0	14	1·4
26 May	117	2·7	2·0	7	1·8
2 June	53	4·1	1·0	0·8	3·2
3 June	42	3·6	0·8	1·1	3·5
Mean	—	2·9	—	—	2·7

Table 3. *Influence of inhibition of K-transport by low K concentration on K-flux and lag time of isolated midguts*

Control preparations were bathed in S 1 (32 mM-K). The low K-flux was obtained by bathing the preparations in a solution containing 2 mM-$KHCO_3$ and 256 mM sucrose.

	Control (32 mM-K)			Low K (2 mM-K)		
Date	K-influx (μ-equiv./hr.)	Lag time (min.)	E (mV)	K-influx (μ-equiv./hr.)	Lag time (min.)	E (mV.)
30 June	(1) 32	2·2	100	5·8	4·2	42
1 July	32	3·8	125	7·0	3·8	65
30 June	(2) 119	3·4	0	6·2	4·0	60
	51	3·4	0	—	—	—
	(3) 73	2·7	0	9·7	3·2	80
	(4) 59	1·1	0	4·5	3·6	50
Mean	58	2·8	—	6·6	3·8	—

Table 4. *K concentration in isolated midguts equilibrated with solutions of different K concentrations*

Column 3 shows that Na in the bathing solutions does not influence the K content of the gut.

No. of determinations	[K^+] Blood-side (mM/l.)	[Na^+] Both sides (mM/l.)	Gut K ($\frac{\mu\text{-equiv. K}}{\text{g. wet wt. gut}}$)	Gut K reduced for adherent solution* ($\frac{\mu\text{-equiv. K}}{\text{g. wet wt. gut}}$)
5	32	0	54	65
4	20	12	35	45
11	10	22	38	48
5	6	26	35	47
5	2·4	0	34	46

* From determination of sucrose ^{14}C or sulphate ^{35}S space.

with the gut in 2 mM-K, the exchange was 18% in 12 min. and one can directly estimate its value to be around 2% per minute (Table 5).

As mentioned under Methods, gut K does not change significantly with change in blood-side K concentration, particularly in the range from 20 to 2·4 mM-K (Table 4).

Table 5. *Specific activity of midgut after 12 min., and time for attaining steady state with the blood-side K*

The blood-side solution was 2·4 mM-$KHCO_3$ plus 255 mM sucrose. The lumen solution was 260 mM sucrose in Expt. A and 260 mM sucrose plus 2 mM-$NaHCO_3$ in the other experiments.

Date	Equilibration time (min.)*	Specific activity of gut K (% of blood-side)	K-flux (μ-equiv./hr.)
13 July	(A) 6	9·5	1·7
	(B) 4	16	2·9
	(C) 6	26	7·5
	(D) 6	23	5·5
	(E) 3	17	2·4
Mean	5	18	4·0

The mean specific activity of the gut after 5 min. is $\frac{5}{12} \times 18 = 8\%$ of the specific activity of the blood-side solution.

* The time to actually reach the steady state, with accuracy 15%; in general about twice the lag time.

DISCUSSION

Several pathways may be considered for the active transport of potassium through the one-cell-thick midgut epithelium. The ions being transported might mix with the ions in both columnar and goblet cells, with those in just one type of cell, or they might not mix with cellular ions at all.

The lag time between introducing ^{42}K on the blood-side of the isolated midgut and its attainment of a steady rate of appearance in the lumen solution has a constant value of about 2–4 min. whether the full transport is taking place or is inhibited (Tables 1–3). The change of K-flux recorded in these three tables is not governed by a change in gut K, which is almost constant as seen in Table 4. Several simple models can be suggested for the movement of K through the gut; the lag time can be calculated according to the assumptions given; and a comparison of measured and calculated lag times can be used to choose which models are consistent with the experimental findings.

(Models 1 and 2). Labelled K enters the gut from the blood-side solution at the rate $a+\Delta$, mixes completely with gut K, and leaves the gut from this side at the rate a, where Δ is the K-flux toward the lumen, and a is defined in model 2 below (see Fig. 3). The

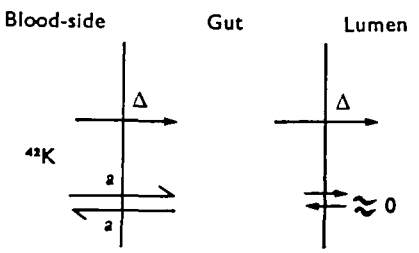

Fig. 3.

flux from the lumen to the gut cells is assumed to be so small that it can be neglected. Assume no change in the amount of K in the gut, S_0(μ-equiv.). The amount of ^{42}K in the gut at time t is S_t (μ-equiv.). The following equation can be written for the flux of labelled K from blood-side to lumen (Ussing & Zerahn, 1951; Solomon, 1964).

$$dS_t = (a+\Delta)\,dt - \frac{S_t}{S_0}(a+\Delta)dt, \tag{1}$$

$$\frac{dS_t}{S_0 - S_t} = \frac{(a+\Delta)\,dt}{S_0}, \tag{2}$$

$$\frac{d(S_0 - S_t)}{S_0 - S_t} = -\frac{(a+\Delta)\,dt}{S_0} \tag{3}$$

$$\ln (S_0 - S_t) = -(a+\Delta)\frac{t}{S_0} + \mathcal{J}, \tag{4}$$

for $t = 0$, $S_t = 0$, so $\mathcal{J} = \ln S_0$ and

$$\ln (S_0 - S_t) = \ln S_0 - \frac{a+\Delta}{S_0}t. \tag{5}$$

In *model* 1, assume that a is zero. Equation (5) becomes

$$\ln (S_0 - S_t) = \ln S_0 - \frac{\Delta}{S_0}t. \tag{6}$$

Calculating the lag time for 75% mixing of cell K with blood-side K, i.e. taking $S_t = 0{\cdot}75S_0$, we obtain:

$$\ln (S_0 - 0{\cdot}75S_0) = \ln S_0 - \frac{\Delta}{S_0}t, \tag{7}$$

so

$$t = (\ln 1\,S_0 - \ln \tfrac{1}{4}\,S_0)\frac{S_0}{\Delta} = \ln 4\,\frac{S_0}{\Delta} = 1{\cdot}4\frac{S}{\Delta}. \tag{8}$$

Calculating t in minutes for $S_0 = 5$ μ-equiv. (the amount of K contained in a gut weighing 100 mg. wet weight) and $\Delta = 5$ μ-equiv./hr. (the K flux for 2 mM-K on the blood-side),

$$t = 1{\cdot}4 \times \frac{5}{5/60} = 84 \text{ min.} \tag{9}$$

The lag time predicted from this model is therefore much longer than that observed. Furthermore, the model predicts that the lag time should be inversely proportional to Δ if S_0 is constant, whereas the measured lag time is independent of Δ. Therefore, this simple model must be discarded.

In *model* 2, a can be obtained by exchange of labelled K for unlabelled gut K or other gut cation, or by simple diffusion of K. In the steady state the exchange will be equal in both directions. We know that such an exchange exists because after 12 min. in S 1 (32 mM-K), the gut is 70% or more equilibrated with the labelled blood-side K. The flux from lumen to the cells is assumed to be so small that it can be neglected. This assumption is valid too, because if this flux were large, there would be no net K-flux to the lumen or too little to account for the observed net K-transport. Under this circumstance, the assumptions we have made regarding K-movement through the cells and active transport of K to the lumen from the cells would not be fulfilled. The ex-

change actually measured between lumen and gut was 0·1 %/min. Again, for 75 % mixing of cell K with blood-side ^{42}K, for $S_0 = 5$ equiv. and $\Delta = 5$ μ-equiv./hr., and taking the measured lag time, $t = 3$ min., we obtain for a, in μ-equiv./min.:

$$a = 1{\cdot}4(S_0/t) - \Delta = 1{\cdot}4 \times \frac{5}{3} - \frac{5}{60} = 2{\cdot}25 \ \mu\text{-equiv./min.} \qquad (10)$$

Thus when Δ is only $\frac{1}{12}$ μ-equiv./min., a would have to be 2·2 μ-equiv./min. to yield 75 % mixing in 3 minutes. In other words, the exchange with the blood-side would have to be 27 times the influx to obtain a lag time of 3 min. This value of a is faster than the exchange which we can determine from Table 5 to be 2 %/min. × 5 μ-equiv. K which amounts to 0·1 μ-equiv./min. On this kinetic evidence, model 2 must also be rejected. The rejection of models 1 and 2 is supported by determinations of the specific activity of the gut K as follows:

Specific activity of gut K and lumen K

Harvey & Nedergaard (1964) found that the short circuit current and the transport of ^{42}K agreed within 15 %. When the steady state is obtained after about 5 min., the agreement between current and ^{42}K flux shows that the K in transport appearing in the lumen must have a specific activity approaching 100 % of that of blood-side K. To obtain such a high specific activity, the source should have at least as high a specific activity. From Table 5 we see that the specific activity of the gut after 5 min. is only 8 % of the blood-side K, far from the specific activity of K appearing at the lumen. The K-flux at the low concentration (2 mM) agreed well with the flux expected by comparison with short-circuited guts in 32 mM-K. There was no indication of any appreciable loss of label during transport, which would be the case if the K in transport were exchanging with gut K.

These results confirm those from the kinetic study in rejecting models 1 and 2. Even though there is an appreciable exchange of blood-side ^{42}K with gut K, it is not great enough to account for the constant lag time; and it does not label the gut fast enough to provide a K-source with sufficiently high specific activity to account for the agreement between K-flux and current.

Model 3. So far, we have assumed that all the epithelial cells behave in the same way. However, there is a possibility that the number of cells transporting K is proportional to the K-flux. If this were the case, we would obtain a constant lag time even though the flux varied. However, all cells of the same type seem to be structurally equivalent, are the same age, and are surrounded by the same medium. Furthermore, no difference in electrical potential of the individual midgut cells is found when the gut is punctured with microelectrodes, whether under normal conditions or with the flux inhibited by lack of oxygen or low K concentration (Wood, Farrand & Harvey, 1969). Although these arguments do not allow us to discard model 3, they render it rather implausible.

Model 4. The possibility remains that only a fraction of the epithelial K is taking part in the transport process. For example, the goblet cells alone might perform the transport of K, as suggested by the structure of the gut (Anderson & Harvey, 1966). There is only about one goblet cell for every two columnar cells, and the amount of cytoplasm in the goblet cells is less than half of the amount in the columnar cells. The concentration of K in the goblet cells is not known, but it could be somewhat lower than the mean K concentration of the gut, even if it is difficult to conceive it to be close

to zero. The actual S_0, if restricted to the goblet cells, would be much smaller than the total gut K so that a treatment like that in model 2 might be valid for the goblet cells alone. Although Table 5 shows that low blood-side K does not decrease gut K significantly, the K in the goblet cells may be so small that a change may escape detection. With Na present, the K in the goblet cells may be substituted to a great extent by Na and in this case the lack of change in lag time might be due to a diminished goblet cell K. However, when the solution contains only 2 mM-$KHCO_3$ and sucrose, such an exchange cannot take place. A cell may lose some K, but not more than a minor fraction, because the large anions will not leave the cell, and K will be the only available cation to neutralize them. Nevertheless, a large decrease in S_0 would make the lag time small, and changes in lag time hard to measure. Therefore restricting the transport to the goblet cells would both satisfactorily explain the constant lag time and account for the low specific activity of the gut. However, this model would require that the goblet cells consume oxygen much faster than the columnar cells for the K/O ratio measured by Harvey *et al.* (1967; see page 246) to be satisfied. The oxygen consumption of the total gut is already very high but a large multiplication factor for the goblet cells is still acceptable. The oxygen uptake of the goblet cells would approach, but remain under, the high values found for the flight muscle of insects such as bees (Weis-Fogh, 1964). Moreover, the goblet cells might get energy for transport from the columnar cells across cell junctions (Loewenstein, 1966), although this supply would have to be arranged without any simultaneous mixing of the K in the cells.

Model 5. These models are all rather speculative; the assumptions made are possible but without experimental evidence. The potential measurements with microelectrodes are all more or less alike with no indication that the two types of cell which make up the epithelium differ electrically (Wood *et al.* 1969). The remaining possibility is to abandon the assumptions of a trans-membrane K transport from any cell to lumen, and postulate that the K-transport is performed along an unknown route between cells, or along intracellular cell surfaces in such a way that the K undergoing transport does not mix appreciably with cell K.

SUMMARY

(1) The time lag for attaining a steady state of ^{42}K movement from blood-side to lumen was 2–4 min.

(2) The flux of ^{42}K was changed as much as 100-fold with but minor effects on the lag time.

(3) The specific activity of the gut did not approach 100% of the blood-side value in this short time although the specific activity of K arriving in the lumen from the transport mechanism must be 100%.

(4) From these results we conclude: I that the K being transported through the gut cannot be mixing with the total cell K of the gut, and therefore II that the route of K transport may be through just the goblet cells, or III that the K may pass through special pathways between or through epithelial cells.

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