ACTIVE TRANSPORT OF POTASSIUM BY THE CECROPIA MIDGUT; TRACER KINETIC THEORY AND TRANSPORT POOL SIZE

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SUMMARY

1. Tracer influx kinetics have been analysed theoretically to determine the size of the transport pool with no assumptions regarding the transport pathway.

2. For a calculation of the size of the transport pool to be made, the following six conditions are required by the theory: tracer steady state attained, tissue steady state attained, I_{sc} measures net flux, small magnitude and constant time-course of efflux, and correction for decay in pumping rate.

3. The size of the pool, S_I, is given by the steady state influx, J^∞, divided by the mixing-time constant, α.

4. Some experimental results are analysed by three different graphical methods, and it is shown that these three methods are equivalent. Specifically, α is equal to the reciprocal of the 75 % mixing time, t_{75}, divided by ln 4 and is equal to the reciprocal of the lag time, X.

5. The tracer kinetic theory is applied to active potassium transport across the isolated short-circuited midgut: the transport meets the six conditions required by the theory.

6. The size of the transport pool of potassium in one midgut is calculated to be 80·5 μequiv./g wet weight under high-K steady-state conditions. A value as high as this suggests that the pool is intracellular.

INTRODUCTION

The size of the transport pool in the route of potassium transport across the silkworm midgut has become controversial. The pool size is a function of the delay between adding tracer to one side of a transporting epithelium and its appearance at the same specific activity on the other side. Harvey & Zerahn (1969) expressed the delay in terms of the lag time (defined in Appendix A), found it to be short and constant and argued that the pool size is small in low K (2 mM). Wood (1972) and Harvey & Wood (1972) expressed the delay in terms of the mixing time (defined in footnote on page [304]), found it to be long and argued that the pool size is large. Zerahn (1973) found a fast change-over from potassium to caesium transport and argued that the pool must be very small. We will now present a formal treatment of tracer influx kinetics, and using this theory we will show that the potassium pool in the Cecropia midgut is large under steady-state conditions in high K (16 mM K and 16 mM Rb).

THEORY

The analysis of influx kinetics developed here allows the determination of the size of the pool which causes a delay between the time that a tracer is added to a compartment on one side of an epithelial tissue and the time at which it reaches a steady state (defined below) in the compartment on the other side. The only data required are the tracer-measured influx, the tracer-measured efflux, and the short-circuit current. It should be emphasized that this theoretical treatment does not assume any models for the transport pathway: on the contrary, the theory involves only parameters that can be determined from flux and short-circuit current measurements which must satisfy six simultaneous conditions, as pointed out below. Finally, the analysis developed here applies strictly only to the simple influx kinetics obtained from the K-transport system of the midgut, but it could be expanded to accommodate more complicated epithelial transport systems (see Wood, 1976).

The key to this analysis is the criterion used to determine the steady state of the tracer, which depends in turn on the criterion used to determine the steady state of the tissue. In most cases the active transport rate across isolated preparations of epithelial tissue continues to decay even after the tissue apparently has reached an ionic and osmotic steady state (Fig. 1). This decay in the transport rate must be corrected if the true time-course of the tracer-measured influx is to be determined. The time-constant for the tracer mixing can be obtained from the time-course of the corrected influx plotted by the influx logarithmic method (Fig. 2; after Hoshiko & Ussing, 1960). Finally, the size of the influx pool can be calculated from this time-constant and the steady-state influx. These steps in the analysis of the tracer-influx kinetics will now be developed in the order just given.

Tracer steady state

The criterion for the tracer steady state is that the time-course of the labelled influx reaches that of the theoretical steady state influx. At this time the specific activity in the pool has become equal to that on the hot side and samples from the cold side now measure the influx. Obviously, an experiment must be continued until the tracer steady state is attained (condition 1). In cases in which the tissue reaches a true steady state (defined below) the criterion for the tracer steady state is simply that the tracer-measured influx reaches a constant value.

Tissue steady state

The tissue steady state is attained when both the size of the pool and the rate at which it is labelled become constant; the usual criterion is a constant transport rate, as measured by a constant tracer-measured influx and under certain conditions (see conditions 3 and 5 below) by a constant I_{sc}. In cases in which the transport rate decays, a true tissue steady state is never attained and the simple criterion for the tracer steady state cannot be applied directly. To evaluate the tracer steady state in such a case the decay in the I_{sc} must be minimized and the residual I_{sc} decay must be used to correct the influx for the decay in the transport rate. The criterion for the tracer steady state in a preparation which attains such a pseudo tissue steady state is that the *corrected* tracer-measured influx reach a constant value.

Corrections for I_{sc} decay

The tracer-measured influx can be corrected for the I_{sc} decay by adding back the I_{sc} decrement from the time when the isotope was added, multiplied by the relative specific activity of the ion arriving in the cold-side compartment, provided that the I_{sc} decay is small enough not to affect the size of the pool or the rate at which it becomes labelled (condition 2). Formally, we have:

$$\mathcal{J}^n = \mathcal{J}_u^n + (I_{so}^0 - I_{sc}^n) r^n, \tag{1}$$

where $\mathcal{J}$ is the corrected influx, $\mathcal{J}_u$ is the uncorrected influx, I_{sc} is the short-circuit current, and r is the specific activity of the ionic species arriving in the cold-side compartment relative to that introduced in the hot-side compartment. The superscripts refer to the time elapsed after adding the isotope.

The relative specific activity of the ion arriving in the cold-side compartment is given by definition as the labelled influx divided by the total influx. We have:

$$r^n = \frac{\mathcal{J}_u^n}{\mathcal{J}_T^n}, \tag{2}$$

where $\mathcal{J}_T$ is the total influx.

Condition 3 is that the I_{sc} is a direct measure of the net flux of the ion whose influx is being measured; the total influx of that ion is given by the sum of the I_{sc} and the simultaneous tracer-measured efflux*. We have:

$$\mathcal{J}_T^n = I_{so}^n + \mathcal{J}_e^n, \tag{3}$$

where $\mathcal{J}_e$ is the tracer-measured efflux.

Substituting equation (3) into equation (2) into equation (1), the current-correction equation becomes:

$$\mathcal{J}^n = \mathcal{J}_u^n + (I_{sc}^0 - I_{so}^n) \frac{\mathcal{J}_u^n}{I_{so}^n + \mathcal{J}_e^n}. \tag{4}$$

Time-constant for mixing

Condition 4 is that the efflux is small or that the passive component of the influx has the same delay as the active component of the influx. Condition 5 is that the efflux is constant so that the current-corrected influx plotted by the influx time-course method (Fig. 1) reaches a constant level, $\mathcal{J}^\infty$. Condition 6 is that the corrected influx labels at a single exponential rate†, so that the results plotted by the influx logarithmic method (Fig. 2) are a straight line after a small delay ($t = \gamma/\alpha$).

* In the case where the efflux is constant – a condition required by the theory but not by the correction equation – its value would be given by the difference between the tracer-measured influx and the I_{sc} after the tracer steady state is attained. In this case only the I_{sc} and the influx need be measured and Equation (4) would reduce to:

$$\mathcal{J}^n = \mathcal{J}_u^n + (I_{sc}^0 - I_{sc}^\infty) \frac{\mathcal{J}_u^n}{I_{sc}^n + \mathcal{J}_e^n}, \tag{1'}$$

where p is any time after the tracer steady state is attained.

† In the case where the influx labels at a double exponential rate with no delay ($\gamma = 0$), the results would be fitted by the following equation:

$$\mathcal{J}^n = \mathcal{J}^\infty [1 - (A e^{-\alpha t} + B e^{-\beta t})], \tag{2'}$$

where A and B are the fractions of the influx labelling at each rate ($A + B = 1$). The size of the pool would be given by

$$S_I = \mathcal{J}^\infty \left(\frac{A}{\alpha} + \frac{B}{\beta} \right). \tag{3'}$$

This logic could be extended to include any number of exponentials.

The time-constant for mixing is given by the slope of the straight line whose equation is:

$$\ln\left(1 - \frac{\mathcal{J}^n}{\mathcal{J}^\infty}\right) = -\alpha t + \gamma \tag{5}$$

where the slope (α) is given by:

$$\alpha = \left[\ln\frac{1 - (\mathcal{J}^n/\mathcal{J}^\infty)}{1 - (\mathcal{J}^{n+1}/\mathcal{J}^\infty)}\right]\left[\frac{1}{t^{n+1} - t^n}\right] \tag{6}$$

and the y-intercept (γ) is given by:

$$\gamma = \ln\left(1 - \frac{\mathcal{J}^0}{\mathcal{J}^\infty}\right). \tag{7}$$

The time-constant for mixing[*], α, will be used to calculate the size of the influx pool below.

Size of influx pool

The size of the influx pool, S_I, is given by the area between the influx time-course and the theoretical, instantaneous, steady state time-course (Fig. 1). The equation for the influx time-course is given by solving equation (5) for $\mathcal{J}^n$:

$$\mathcal{J}^n = \mathcal{J}^\infty(1 - e^{-\alpha t + \gamma}) \tag{8}$$

and the area required is then:

$$S_I = A_0^\infty = \int_0^\infty \{[\mathcal{J}^\infty] - [\mathcal{J}^\infty(1 - e^{-\alpha t + \gamma})]\}\, dt. \tag{9}$$

However, the γ-component of the area is probably due to non-instantaneous mixing of the tracer in the bulk solution in the hot-side compartment[†], and the true size of the influx pool is given by the α-component only. Moreover, the integral is improper

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[*] Previous investigators (Harvey & Zerahn, 1969; Wood, 1972; Harvey and Wood, 1972, 1973) have used the 75 % mixing time (t_{75}) rather than the mixing-time constant (α) to analyse influx kinetics. The 75 % mixing time is defined as the time to reach the 75 % level of mixing. Formally, we have

$$t_{75} = t^b - t^a, \tag{4'}$$

where t^b is the time when $\mathcal{J}^b = (\mathcal{J}^a + 3\mathcal{J}^\infty)/4$. Substituting these values into equation (6), and solving for $t^b - t^a$, we obtain

$$t_{75} = \frac{\ln 4}{\alpha}. \tag{5'}$$

Therefore, the mixing-time constant is related to the 75 % mixing time by the constant, ln 4.

[†] The area between the two curves corresponding to the γ-component can be approximated by a rectangle bounded by $\mathcal{J}^\infty$ and $t = \gamma/\alpha$ as follows:

$$S_{I\gamma} = \mathcal{J}^\infty \frac{\gamma}{\alpha}. \tag{6'}$$

The total amount of substance involved in the delay before the attainment of the tracer steady state would be the sum of the α- and γ-components as follows:

$$S_{I\alpha+\gamma} = \frac{\mathcal{J}^\infty}{\alpha}(1 + \gamma). \tag{7'}$$

because the two curves never meet. As a consequence, the equation for the true size of the pool becomes:

$$S_{I_\alpha} = \int_{\gamma/\alpha}^{0} \mathcal{J}^\infty (e^{-\alpha t + \gamma})\, dt. \tag{10}$$

After the integration ($\mathcal{J}^\infty$ must be constant), we obtain:

$$S_{I_\alpha} = \frac{\mathcal{J}^\infty}{-\alpha} (e^{-\alpha c + \gamma} - e^{-\alpha(\gamma/\alpha) + \gamma}). \tag{11}$$

Since as $c \to \infty$, $e^{-\alpha c + \gamma} \to 0$, we obtain:

$$S_{I_\alpha} = \frac{\mathcal{J}^\infty}{\alpha}. \tag{12}$$

Therefore, the size of the influx pool is given by equation (12).

This theory of tracer influx kinetics will now be applied to the results obtained from isolated midgut preparations very close to a tissue steady state.

METHODS

Mature, fifth-instar larvae of *Hyalophora cecropia* (L.) were reared on artificial diet (Riddiford, 1968). Each larva was immobilized by chilling and its midgut was isolated and mounted as a flat sheet in the chamber described by Wood (1972, 1975) which is similar to the one described by Ussing & Zerahn (1951). Both sides of the isolated midgut were bathed in a solution (pH = 8·3) containing KCl (16 mM), RbCl (16 mM), CaCl₂ (5 mM), Trisma base (5 mM) and HCl (1·5 mM), sucrose (166 mM), mannitol (0·05 mM) and inulin (10⁻⁷ M). MgCl₂ was omitted from the bathing solution since magnesium is actively transported from lumen-side to blood-side (Wood, Jungreis & Harvey, 1975), so that the I_{sc} directly measures the net flux of potassium (condition 3; Wood & Harvey, in preparation). The midgut was short-circuited continuously using a three-bridge system and a negative feedback amplifier (Wood, 1972, 1975) and the I_{sc} was monitored by a Triplett ammeter (Model 630) and recorded on a Servoscribe recorder (Model RE 541). The I_{sc} decay was minimized (condition 2) by delaying the pool-size determination until after the fast component of the double exponential decay had run to completion (> 90 min; Wood, 1972, 1975). All radioactive isotopes were obtained from New England Nuclear, Boston, Mass. The K + Rb influx (i.e. from blood-side to lumen-side) was measured with ⁸⁶Rb and the K + Rb efflux was measured with ⁴²K. Radioactivity from ⁸⁶Rb and ⁴²K in the samples was counted by the Cerenkov effect in a Packard liquid scintillation counter (Model 3380). The ⁸⁶Rb was re-counted after 10 days and the ⁴²K was calculated by difference after correcting for ⁸⁶Rb decay. The ⁸⁶Rb and ⁴²K were then corrected for decay during counting. The extracellular spaces were marked with 10 min pulses of [¹⁴C] mannitol on the blood-side and [³H] inulin on the lumen-side. Radioactivity from the ¹⁴C and ³H was counted after adding Packard Insta-Gel to the same samples. Calculations were made on a Monroe programmable calculator (Model 1785).
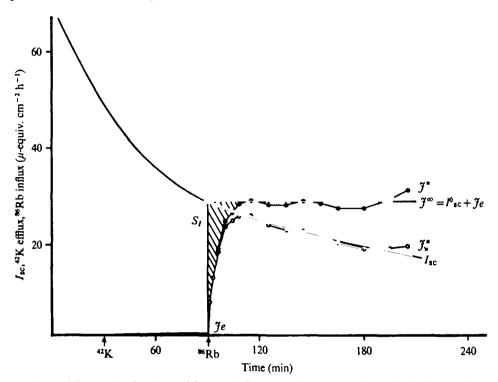
Fig. 1. The results of the best of four pool-size determinations are plotted by the influx time-course method. The midgut was mounted as a flat sheet in a chamber and bathed in Mg-free 16 K, 16 Rb-S-Tris. The midgut was continuously short-circuited and the time-course of the short-circuit current (I_{sc}) is shown by the smooth curve. ^{42}K was added to the lumen-side at 30 min and 2 ml samples were removed from the blood-side at 15 min intervals; the time-course of the ^{42}K-measured efflux (J_e) is just perceptible. ^{86}Rb was added to the blood-side at 90 min and 1 ml samples were removed from the lumen-side at either 2, 4 or 10 min intervals; the time-course of the uncorrected ^{86}Rb-measured influx (J_u^n) is shown by the open circles. The time-course of the ^{86}Rb-measured influx corrected for I_{sc} decay (J^n) is shown by the closed circles. The time-course of the theoretical, instantaneous, steady-state influx (J^∞) is shown by the horizontal line ($J^\infty = I_{so}^0 + J_e = 0.473$ μ-equiv. cm^{-2} min^{-1} for this experiment). The tracer steady state is attained at approximately 30 min after adding ^{86}Rb when the *corrected* ^{86}Rb-measured influx becomes constant. The 75% mixing time is 7.7 min for this experiment. The size of the pool (S_I) is shown by the shaded area and given by equation (12) to be 2.74 μ-equiv. for this experiment.

RESULTS

The results of the best of four pool-size determinations are shown in Figs. 1–3. The results are plotted by the influx time-course method in Fig. 1. The short-circuit current (I_{sc}, smooth curve) decayed (0.11 μ-equiv. cm^{-2} min^{-1}; 0.38% min^{-1}) during the 120 min determination. The ^{42}K-measured efflux (J_e) is virtually constant. The uncorrected ^{86}Rb-measured influx (J_u^n, open circles) approaches the I_{sc}. The influx corrected for I_{sc} decay (J^n, closed circles) approaches a constant value (J^∞). The size of the pool (S_I, shaded area) is given by the area between the current-corrected influx curve and the theoretical, instantaneous, steady state influx curve.

The results of this same experiment are plotted by the influx logarithmic method in Fig. 2. Both the curve calculated from the uncorrected influx and the curve calculated from the influx corrected for I_{sc} decay (closed circles) follow the same straight line
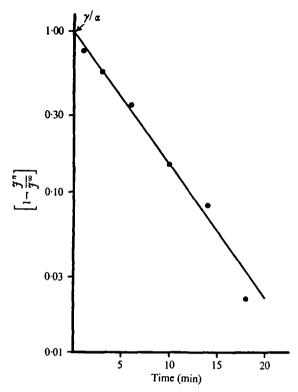
Fig. 2. The results of this same experiment are plotted by the influx logarithmic method, where $\ln(1 - \mathcal{F}^*/\mathcal{F}^\infty)$ is plotted against linear time. The curve calculated from the uncorrected influx and the curve calculated from the influx corrected for I_{sc} decay (closed circles) follow the same straight line after no delay in this experiment ($\gamma/\alpha = 0$ min; normally about 1 min; Wood, 1976). The curve calculated from the uncorrected influx, which normally falls downward, does not fall because the I_{sc} decay is very low. The slope of the straight line is the mixing-time constant (α) and is 0.191 min^{-1} for this experiment.

after no delay ($\gamma/\alpha = 0$ min) meaning that the pool labels at a single exponential rate. The slope of the straight line is the mixing-time constant, α, and its y-intercept is γ.

The results of this same experiment are plotted by the amount time-course method (see Appendix A) in Fig. 3. The curve for the uncorrected influx (open circles) is S-shaped, curving upwards for about 20 min, dominated by the labelling of the pool, and downwards thereafter, dominated by the I_{sc} decay, whereas the curve for the influx corrected for the I_{sc} decay (closed circles) rises to an asymptote (S_{C_a}, equation 15) whose time-axis intercept is the lag time, X, and whose amount-axis intercept is the size of the pool, S_{I_a}.

The size of the influx pool for the experiment shown in Figs. 1, 2 and 3 calculated from equation (12), where $\mathcal{F}^\infty = 0.473$ μ-equiv. cm^{-2} min^{-1} and $\alpha = 0.191$ min^{-1}, is 2.48 μ-equiv. Of the three other determinations, two had an increasing efflux (violates condition 5) and one had a high efflux (violates condition 4), both of which are characteristic of Mg-free bathing solutions (Wood & Harvey, unpublished). These experiments cannot be analysed by the present influx kinetic theory (but see Wood, 1976), but they show the long mixing time-constant and the large pool size.
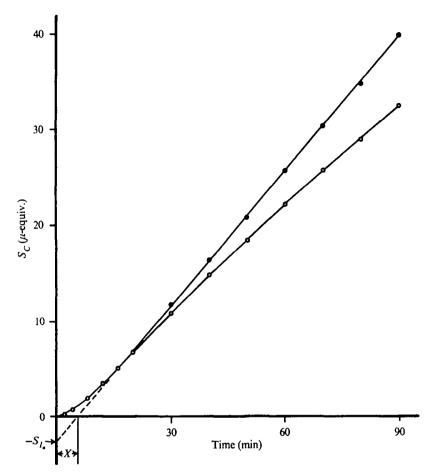
Fig. 3. The results of this same experiment are plotted by the amount time-course method, where the amount of tracer-measured K + Rb accumulated in the lumen-side is plotted against time. The curves for the uncorrected and corrected amounts are the same for the first 20 min. Thereafter the curve for the uncorrected influx (open circles) is S-shaped, whereas the curve for the influx corrected for the I_{sc} decay (closed circles) rises to an asymptote (dotted line). The lag time (X) is 5·3 min and the amount-axis intercept $(-S_{I_a})$ is 2·47 μ-equiv. for this experiment.

DISCUSSION

The kinetics of K-influx across the midgut under high K (16 mM K, 16 mM Rb), steady-state conditions meets all six of the conditions required by the theory for determining the size of the influx pool. The tracer steady state is attained (condition 1) because the current-corrected influx becomes constant (Fig. 1). The decay in the short-circuit current is sufficiently small that the size of the pool and the rate at which it becomes labelled cannot change very much (condition 2, see Fig. 1). The I_{sc} is a direct measure of the net flux of potassium (condition 3) since the influx minus the efflux is equal to the short-circuit current (Fig. 1). The efflux is sufficiently small that whether the passive influx has the same delay as the active influx or not is inconsequential (condition 4, see Fig. 1). The efflux is virtually constant and the current-corrected influx reaches a constant level (condition 5, see Fig. 1). The labelling of the influx is at a single exponential rate after a short delay (condition 6, see Fig. 2).

Since the kinetics of K-influx across the midgut under high K, steady-state conditions meets the conditions required by the theory, the size of the influx pool may be computed by using equation (12). It is found to be large under high K, steady-state conditions. The size of the pool for the experiment on 25 September 1974 calculated from equation (12) is 2·48 μ-equiv. The t_{75} is 7·7 min, versus a calculated value from equation (18) of 7·3 min; the lag-time, X, is 5·3 min, versus a calculated value from equation (18) of 5·2 min; and the amount-axis intercept, S_{I_a}, is 2·47 μ-equiv., versus a calculated value of 2·48 μ-equiv.

The pool size for this experiment is equivalent to 80·5 μ-equiv./g wet weight. The present results are supported by seven experiments using a spherical preparation[*] (Table II of Harvey & Wood, 1973) and by more than 50 other experiments using a flat sheet (Wood & Harvey, unpublished; see also Wood, 1976). Therefore, the size of the influx pool is equivalent to a large percentage of the total tissue K in the midgut.

The large percentage of tissue K involved in the transport pool suggests that the transported K mixes with cellular K (see Harvey & Wood, 1972, 1973). This large pool size was derived from the data using no prior assumptions of any model for the transport route. Therefore, the pool size will be used to deduce the precise location of this pool, in further publications (Wood & Harvey, in preparation). The small pool size deduced by Zerahn (1973) from the fast change-over from potassium to caesium transport under non-steady-state conditions is contradicted by the results found under these steady-state conditons. This clear demonstration of a large pool under high K, steady-state conditions should resolve the controversy regarding the size of the midgut K-transport pool.

APPENDIX A

Andersen & Zerahn (1963) proposed a method for analysing tracer influx kinetics in which the amount of tracer-measured substance accumulated in the cold-side compartment, S_c, is plotted against time and the straight portion of the resulting curve is extrapolated back to the time-axis to give the lag time for tracer mixing, and extrapolated back to the amount-axis to give the size of the pool. This graphical method was used by Harvey & Zerahn (1969) in the original study of tracer influx kinetics in the midgut. Since this method has been criticized recently by Harvey & Wood (1972) and by Curran (1973) it is worth while to analyse it in some detail.

The equation for the influx data plotted by this amount time-course method is obtained by integration of equation (8) to give:

$$S_O = \int d\mathcal{J}^n = \mathcal{J}^\infty t + \frac{\mathcal{J}^\infty}{\alpha} e^{-\alpha t + \gamma} + C, \tag{13}$$

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[*] The technically complicated simultaneous determination of the influx and efflux across a spherical preparation in a closed chamber (Fig. 2 of Harvey & Zerahn, 1972) was not attempted. Rather, the efflux was assumed to be constant before p, since it was constant after p, and the size of the pool was calculated by the method described in the footnote p. 303.

when $t = 0$, $S_C = 0$ and the constant of integration is $C = (-\mathcal{J}^\infty e^\gamma)/\alpha$. Therefore, the equation for the results plotted by the amount time-course method (Fig. 3) is:

$$S_C = \mathcal{J}^\infty \left[t + \frac{1}{\alpha} (e^{-\alpha t + \gamma} - e^\gamma) \right]. \tag{14}$$

Moreover, the curve described by this equation reaches an asymptote, since as $t \to \infty$, $e^{-\alpha t + \gamma} \to 0$, and the equation for the asymptote (Fig. 3) is:

$$S_{C_a} = \mathcal{J}^\infty \left(t - \frac{e^\gamma}{\alpha} \right). \tag{15}$$

From this asymptote equation, the lag time, X, is the time-axis intercept ($S_{C_a} = 0$), given by:

$$X = \frac{e^\gamma}{\alpha} \tag{16}$$

and the size of the pool, S_{I_a}, is the amount-axis intercept ($t = 0$), given by:

$$S_{I_a} = -\frac{\mathcal{J}^\infty}{\alpha} e^\gamma. \tag{17}$$

In the case where $\gamma = 0$, equation (16) reduces to (compare with equation 5'):

$$X = \frac{1}{\alpha} = \frac{t_{75}}{\ln 4} \tag{18}$$

and equation (17) reduces to:

$$S_{I_a} = -\frac{\mathcal{J}^\infty}{\alpha}, \tag{19}$$

which is equivalent to equation (12). Therefore the amount time-course method is a valid method for analysing tracer influx kinetics when it is used correctly.

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NOTE ADDED IN PROOF

Recently active calcium transport from lumen-side to blood-side was demonstrated across the isolated and short-circuited Cecropia midgut. The presence of this small (1μ-equiv. $cm^{-2} h^{-1}$) calcium transport leads to a small underestimation of the size of the K-influx pool in the present paper.