THE CALCULATION OF TRANSFER RATES IN TWO COMPARTMENT SYSTEMS NOT IN DYNAMIC EQUILIBRIUM*

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ABSTRACT

Dynamic equilibrium in a biological system implies that the compartment under study does not change in size during the period of observation. In many biological systems there are, however, net changes with time and this report deals with the mathematical treatment necessary to calculate unequal rates of inflow and outflow.

A method is presented for the calculation of transfer rates in a two compartment system when the rates of flow between these compartments are unequal but constant. Equations were developed to calculate the amount of material transported per unit time derived from measurements of specific activity and compartment size.

The problems of (1) sampling from the pool and (2) the effects of analytical errors on the estimation of rate have been evaluated. An example has been presented in which the derived equations have been applied to a study of the simultaneous passage of sodium into and out of a permanently isolated loop of bowel.

I

INTRODUCTION

The use of isotopes to measure the passage of material through a metabolic process or through one or more compartments has become well established in the past 10 years. There are two assumptions that are usually part of the mathematics necessary for the calculation of this passage. One is that the size of the metabolic pool does not change during the period of observation; that is to say, that the rate of material entering (inflow) and the rate of its leaving the pool (outflow) are equal. The second assumption is that the rates are constant during the period of observation. These circumstances have been described as the "steady state" or "dynamic equilibrium" (1–3).

In many biological systems there is a net change with time and, in fact, these were the only systems the kinetics of which could be studied prior to the

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advent of isotopes. The mathematical treatment necessary to calculate unequal rates of inflow and outflow has received scant attention (4–7). These systems are in a "non-steady state" and, in this report, the term is used to mean that the metabolic pool or compartment under study is changing in size with time or that rates of inflow and outflow are changing. The non-steady state may be one of two types; either (1) the rates of inflow and outflow are unequal but constant with time,[1] or (2) the rates of inflow and outflow vary with time.[2] In the second type of non-steady state, the rates of inflow and outflow in a single compartment although changing with time, might maintain a constant difference, and in this case the size of the pool would increase or decrease at a constant rate. Thus, the observation of a linear change of pool size with time does not necessarily mean that the rates of inflow and outflow are constant.

	TIME	COMPARTMENT I	COMPARTMENT II
AMOUNT OF MATERIAL (LABELLED PLUS UNLABELLED)	0	A_0	B_0
SPECIFIC ACTIVITY		θ_{A_0}	θ_{B_0}
EXCHANGE FROM I TO II EXCHANGE FROM II TO I	t	$\longrightarrow \alpha$ $\beta \longleftarrow$	
AMOUNT OF MATERIAL		$A=A_0+(\beta-\alpha)t$	$B=B_0+(\alpha-\beta)t$
SPECIFIC ACTIVITY		θ_A	θ_B
EXCHANGE OF MATERIAL		$\longrightarrow \alpha\Delta t$ $\beta\Delta t \longleftarrow$	
EXCHANGE OF TRACER		$-\theta_A\alpha\Delta t\longrightarrow$ $\leftarrow\theta_B\beta\Delta t-$	

FIG. 1. Notations used in mathematical formulations.

As a corollary, a metabolic pool which remains constant in size with time does not necessarily mean constant rates of inflow and outflow as these rates may vary with time but remain equal to each other. The second type of non-steady state presents many alternatives and is not considered further. This report describes a method for the calculation of inflow and outflow from tracer data in a non-steady state system of the first type.

[1] To call attention to the differences in terminology, in Hart's classification (7) this first type of non-steady state is labelled Steady state, class 2.

[2] There are many ways in which rates of inflow and outflow may change with time. One of many possibilities is that the transfer rates may proceed as exponential functions of time. In this trivial case of the non-steady state, a rate of flow of material (labelled plus unlabelled) which itself proceeds as an exponential function of time should not be confused with the "rate constant" of steady state kinetics. In the steady state the rate constant describes again an exponential function but it describes the fraction of total tracer which enters or leaves the pool per unit of time, while the inflow and outflow are themselves constant and equal.

II

Theoretical Treatment

As a model, a closed two compartment system in which the rates of flow between the compartments were unequal but constant was selected. Equations were developed to calculate from tracer measurements the absolute amount of material entering and leaving each compartment per unit of time. The assumptions necessary to develop these equations are (1) there is complete and instantaneous mixing in either compartment and (2) the metabolic process or transport mechanism does not fractionate the isotopes. The condition of equal rates of exchange (the steady state) is treated as a special case.

The following notations are introduced (Fig. 1): A represents the amount of material (labelled plus unlabelled) in compartment I, B represents the amount of material in compartment II, θ_A represents the specific activity (the ratio of labelled material to labelled plus unlabelled material) of the material in compartment I, θ_B represents the specific activity in compartment II, α is the rate of passage of material from compartment I to compartment II (amount per unit time), β is the rate of passage of material from II to I, t represents time.

The initial conditions at $t = 0$ are

$$A = A_0 \tag{1 a}$$

$$B = B_0 \tag{1 b}$$

$$\theta_A = \theta_{A_0} \tag{1 c}$$

$$\theta_B = \theta_{B_0} \tag{1 d}$$

The mass of material (labelled plus unlabelled) in compartment I at time t is

$$A = A_0 + (\beta - \alpha)t. \tag{2}$$

The mass of tracer in compartment I at time t is $\theta_A[A_0 + (\beta - \alpha)t]$. The mass of material at time $t + \Delta t$ is $A_0 + (\beta - \alpha)(t + \Delta t)$. The mass of tracer at $t + \Delta t$ becomes $\theta_A[A_0 + (\beta - \alpha)t] + \theta_B \beta\Delta t - \theta_A \alpha\Delta t$. The specific activity in compartment I at $t + \Delta t$ is $(\theta_A + \Delta\theta_A)$ and equal to

$$\frac{\theta_A[A_0 + (\beta - \alpha)t] + \theta_B\beta\Delta t - \theta_A\alpha\Delta t}{A_0 + (\beta - \alpha)(t + \Delta t)},$$

which may be rearranged to

$$\frac{\Delta\theta_A}{\Delta t} = \frac{\beta(\theta_B - \theta_A)}{A_0 + (\beta - \alpha)(t + \Delta t)}. \tag{3}$$

For $\Delta t \to 0$,

$$\frac{d\theta_A}{dt} = \frac{-\beta(\theta_A - \theta_B)}{A_0 + (\beta - \alpha)t}. \tag{4}$$

Similarly for compartment II,

$$\frac{d\theta_B}{dt} = \frac{\alpha(\theta_A - \theta_B)}{B_0 + (\alpha - \beta)t}. \tag{5}$$

Equation (5) is subtracted from (4) to yield

$$\frac{d(\theta_A - \theta_B)}{(\theta_{A_0} - \theta_{B_0})} = -\frac{\beta\, dt}{A_0 + (\beta - \alpha)t} - \frac{\alpha\, dt}{B_0 + (\alpha - \beta)t}. \tag{6}$$

Equation (6) may be integrated to yield

$$\ln\frac{\theta_A - \theta_B}{\theta_{A_0} - \theta_{B_0}} = \frac{\beta}{\alpha - \beta}\ln\frac{A}{A_0} - \frac{\alpha}{\alpha - \beta}\ln\frac{B}{B_0}. \tag{7}$$

With equation (2), equation (7) may be rearranged to

$$\beta = \frac{A - A_0}{t}\left[\frac{\ln\dfrac{(\theta_A - \theta_B)}{(\theta_{A_0} - \theta_{B_0})} + \ln\dfrac{B}{B_0}}{\ln\dfrac{B}{B_0} - \ln\dfrac{A}{A_0}}\right] \tag{8}$$

and

$$\alpha = \frac{A - A_0}{t}\left[\frac{\ln\dfrac{(\theta_A - \theta_B)}{(\theta_{A_0} - \theta_{B_0})} + \ln\dfrac{B}{B_0}}{\ln\dfrac{B}{B_0} - \ln\dfrac{A}{A_0}} - 1\right] \tag{9}$$

The condition $\alpha \neq \beta$ is necessary for the solution of equation (7). For the special case in which $\alpha = \beta$ the differential equation has the form

$$\frac{d(\theta_A - \theta_B)}{(\theta_{A_0} - \theta_{B_0})} = -\left[\frac{\beta}{A_0} + \frac{\alpha}{B_0}\right]dt, \tag{10}$$

the solution of which is

$$\ln\frac{\theta_A - \theta_B}{\theta_{A_0} - \theta_{B_0}} = \left[-\frac{\beta}{A_0} + \frac{\alpha}{B_0}\right]t \tag{11}$$

and

$$\beta = \alpha = \frac{-\ln\dfrac{\theta_A - \theta_B}{\theta_{A_0} - \theta_{B_0}}}{\left[\dfrac{1}{A_0} + \dfrac{1}{B_0}\right]t}. \tag{12}$$

Equations (8) and (9) have general application to the non-steady state in which rates of flow between two compartments are unequal but constant with time. Equation (12) applies to the special case in which rates of flow between compartments are equal. Transfer rates (α and β) are in terms of amount of

material per unit time and are determined from measurements of specific activity (θ_A, θ_B) and compartment size (A, B).

III

DISCUSSION

The need for calculation of transfer rates in a system not in dynamic equilibrium arose in experiments in which the object was to measure the simultaneous passage of sodium into and out of an isolated loop of bowel in the dog. A blind end Thiry fistula was prepared and the continuity of the bowel reestablished (8, 9). In a pair of experiments on the ileum radiosodium was first placed in the lumen of the bowel and in the second experiment it was administered intravenously. In both these experiments there was little change in the amount of sodium in the lumen and the rate of fall of specific activity with radiosodium placed in the lumen approximated the rate of rise of specific activity with radiosodium administered intravenously (Fig. 2). When the same pair of experiments was repeated on a loop of colon, the fall in specific activity in the lumen again approximated the rate of rise (Fig. 3), albeit in this pair of experiments there was a decrease in the total amount of sodium in the colonic lumen and flow out from the lumen had to be faster than flow into the lumen. This seeming discrepancy was the stimulus for the development of the preceding equations.

When radiosodium is administered intravenously, the rise in specific activity in the bowel lumen reflects the entry of radiosodium and sodium into the lumen. If, when radiosodium is placed in the bowel lumen, radiosodium and sodium leave in the same ratio that exists in the lumen, their outflow *per se* does not alter the specific activity of the sodium remaining in the bowel lumen. If the specific activity of sodium in the bowel lumen does decrease, it is because sodium has entered to reduce the existing ratio of radiosodium to sodium. Thus whether radiosodium is administered intravenously or placed in the bowel, either a rise or fall of specific activity of sodium in the bowel lumen reflects only the inflow of sodium and is not related to outflow (except for correction for the net change, *vide infra*).

Mathematical confirmation of these observations was developed from equation (7). Compartment I is considered the sodium in the bowel lumen, compartment II the extraluminal sodium spaces of the dog in exchange with the lumen and $B/B_0 \leqq 1$. When $B/B_0 = 1$ equation (7) can be considered to describe inflow and outflow in a single compartment. If equation (7) is now expanded in the form of a power series, the effect of inflow on specific activity may be distinguished from that of outflow. Substituting for A from equation (2), the first three terms of the expanded form are

$$\ln \frac{\theta_A - \theta_B}{\theta_{A_0} - \theta_{B_0}} = -\frac{\beta t}{A_0} + \frac{\beta(\beta - \alpha)t^2}{2A_0^2} - \frac{\beta(\beta - \alpha)^2 t^3}{3A_0^3} \tag{13}$$

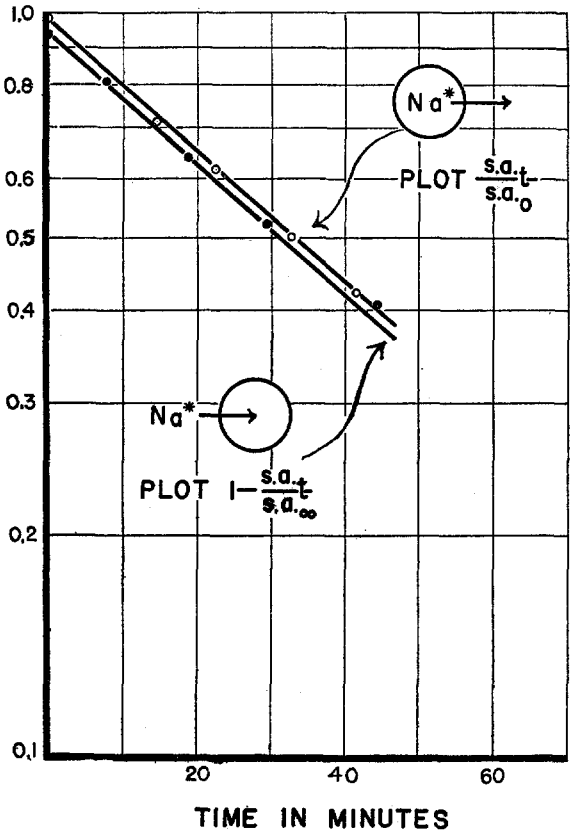

FIG. 2. A modified Ringer's solution containing radiosodium (Na^{24}) was placed in a loop of ileum and samples were removed at intervals. The decrease in specific activity was measured and plotted on a log scale against time, assigning a value of one to the first sample (open circles) (in the notations of Fig. 1 θ_A/θ_{A_0} plotted against t). For the second experiment, the loop was cleansed and radiosodium administered intravenously. When plasma specific activity became constant, the loop was cleansed again, fresh Ringer's solution (without radioactivity) was placed in it, and the rise of specific activity measured with time. In order to compare the rate of rise of specific activity in the second experiment with the rate of fall in the first experiment, the rate of rise has been plotted as one minus the ratio of "bowel" specific activity to "plasma" specific activity (solid circles) (in the notations in Fig. 1, $1 - \theta_A/\theta_B$ plotted against t). Inulin or methyl cellulose was included in the Ringer's solution and from measurement of inulin (or methyl cellulose) and sodium concentrations, the total amount of sodium in the lumen could be estimated.

(in this form the condition $\beta = \alpha$ is no longer a special case). In a single compartment in the non-steady state, the second and subsequent terms of the right side of equation (13) may be considered a correction for the net change $(\beta - \alpha)$. Except for this correction for the net change, either a rise or fall in specific activity is the result of inflow (β). In a single compartment in the steady

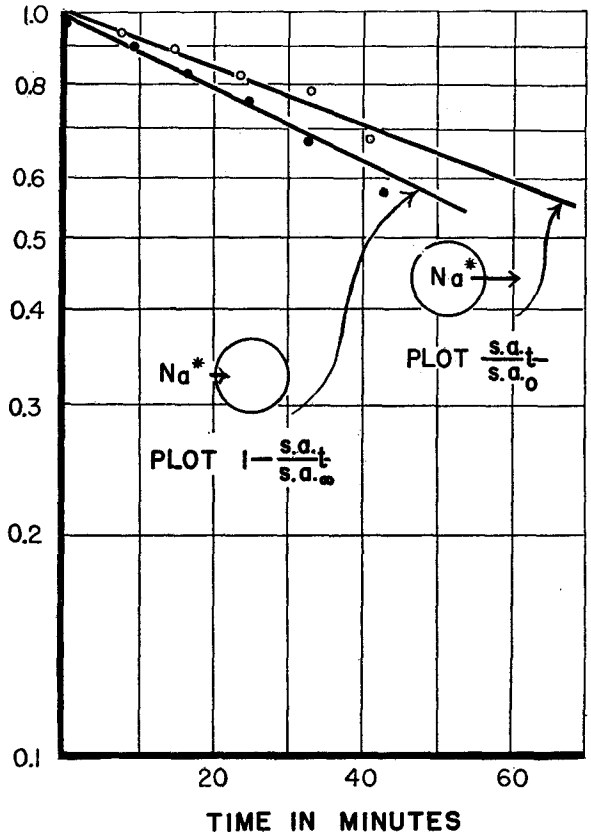

FIG. 3. The fall and rise in specific activity are compared in a pair of experiments on the colon, identical with the pair of experiments described for the ileum (Fig. 2).

state in which rates of inflow and outflow are equal $(\beta = \alpha)$ the second and subsequent terms of the power series are indeterminate and only the first term applies. Although in the derivation of equation (13) no assumptions were made as to whether specific activity varied as inflow or outflow, equation (13) indicates that either a rise or fall in specific activity in a single compartment in the steady state is purely the result of inflow into that compartment.

There are two points to be made. (1) Changes in specific activity are to be

distinguished from changes in total mass of tracer in the pool,[3] as outflow of material from the labelled pool *per se* clearly decreases the total mass of tracer but does not alter the ratio of tracer to non-labelled material. Therefore care must be taken in relating a change in the total mass of radioactivity to a change in specific activity. (2) When inflow and outflow are equal (observations on the ileum, Fig. 2), it makes no difference mathematically whether a change in specific activity is considered inflow or outflow, but caution must be exercised in interpreting a fall in specific activity as outflow. In a study concerned with the formation and degradation of a compound, a falling specific activity does not reflect degradation, but reflects the incorporation of unlabelled components into the compound.

IV

An Experimental Application of the Derived Equations

The conditions and assumptions for the calculations of inflow and outflow in a two compartment system may of course be modified to conform to particular experimental circumstances of the non-steady state. What follows is a physiological study which illustrates the application of the equations to the measurement of the simultaneous passage of sodium into and out of an isolated loop of bowel.

It was first necessary to decide whether the inflow and outflow were constant (or a function of time) during the period of observation. The net change of sodium in the bowel with time furnished a partial answer. Inulin was used to follow the volume of fluid in the bowel and the amount of sodium in the bowel was calculated from the known amount of inulin in the bowel, and from measurements of the inulin and sodium concentrations (Table I).[4] When the amount of sodium in the bowel was plotted on (1) an arithmetic scale and

[3] Visscher in 1944 conducted experiments similar to those described above (9). In his calculations he used the decrease in the total number of labelled ions as a measure of the rate out of the bowel. By dividing the total number of labelled ions lost by the mean specific activity he arrives at a measure of the outflow of sodium from the bowel lumen. The difficulty, however, lies in estimating the mean specific activity. Visscher used the arithmetic mean whereas the correct mean is a function of (1) the integral of the change in specific activity and (2) the effects of the net change. With relatively small changes in specific activity, up to 40 per cent, there is little difference between the arithmetic and correct means. With larger changes in specific activity, the arithmetic mean overestimates the correct mean at a rapidly increasing rate.

[4] After December, 1952, methyl cellulose was substituted for inulin as it was found that in some of the experiments inulin was degraded by the bowel contents. Methyl cellulose was found to be stable for as long as 3 years in bowel samplings kept at room temperature.

(2) a logarithmic scale against time, the slope of these values did not definitely establish either an arithmetic or logarithmic process as the total change was relatively small in relation to the accuracy of the analytical procedures (Fig. 4, open circles). However following the administration of desoxycorticosterone there was a rapid net decrease in the amount of sodium in the bowel, and in this instance when the net change was plotted against time the progression was an arithmetic one over a fourfold decrease in the amount of sodium in the bowel (Fig. 4, half-solid circles). The experiment was repeated again 24 hours after a second dose of desoxycorticosterone (Fig. 4, solid circles).

TABLE I

Calculation of the Amount of Sodium in the Bowel from Sample Analyses
Dog SKP, October 15, 1952

Time	Sample size	Inulin concentration	Residual inulin	Volume	Sodium concentration	Total sodium	Sodium previously removed	Sodium corrected	Relative amounts of sodium
min.	*gm.*	*mg./ml.*	*mg.*	*ml.*	*μeq./ml.*	*μeq.*	*μeq.*	*μeq.*	
0	0.454	4.18	100.0	23.9	140.0	3349	0	3349	1.000
7.5	0.371	4.21	98.1	23.3	140.0	3262	64	3326	0.993
15.8	0.457	4.64	96.5	20.8	139.1	2894	115	3010	0.899
23.0	0.523	4.78	94.4	19.8	138.0	2725	179	2904	0.867
30.5	0.447	5.15	91.9	17.8	137.5	2454	251	2705	0.808
37.8	12.680	5.52	89.6	16.2	124.2	2016	313	2328	0.695

The amount of inulin in the bowel is calculated from that originally placed in it less that lost in sampling. Sodium corrected is the amount of sodium that would have been in the bowel had there been no sampling (the sodium previously removed in sampling is added to the actual amount in the bowel). Relative amounts of sodium is the corrected sodium expressed as a proportion of the sodium present at zero time. Relative amounts of sodium are plotted on both an arithmetic and logarithmic scale against time (Fig. 4, open circles).

These data are consistent with the interpretation that the net change was an arithmetic constant over the period of observation and independent of the amount of sodium or the volume of fluid in the bowel within the range of values observed. (Obviously, when the sodium in the lumen is completely resorbed there must be a shift in transfer rates.) The possibility remains that the rates in and out may be increasing or decreasing while maintaining a constant difference, but if this were the case experimental data from consecutive samplings would quickly reveal this possibility when substituted in equations (8) and (9). Observations of this type and the subsequent calculation of flow rates in many experiments over a 5 year period form the evidence for the assumption that the rates of inflow and outflow are constant within the period of observation.

In the present experiments the sodium outside the loop of bowel is of the order of 400 to 800 m.eq. and the sodium in the bowel lumen is of the order

of 1 to 3 m.eq. Thus when compartment I is the bowel lumen and compartment II is the extraluminal exchangeable sodium of the dog, B/B_0 can be considered unity and equations (7), (8), and (9) may be simplified to

$$\ln \frac{\theta_A - \theta_B}{\theta_{A_0} - \theta_{B_0}} = \frac{\beta}{\alpha - \beta} \ln \frac{A}{A_0}, \tag{14}$$

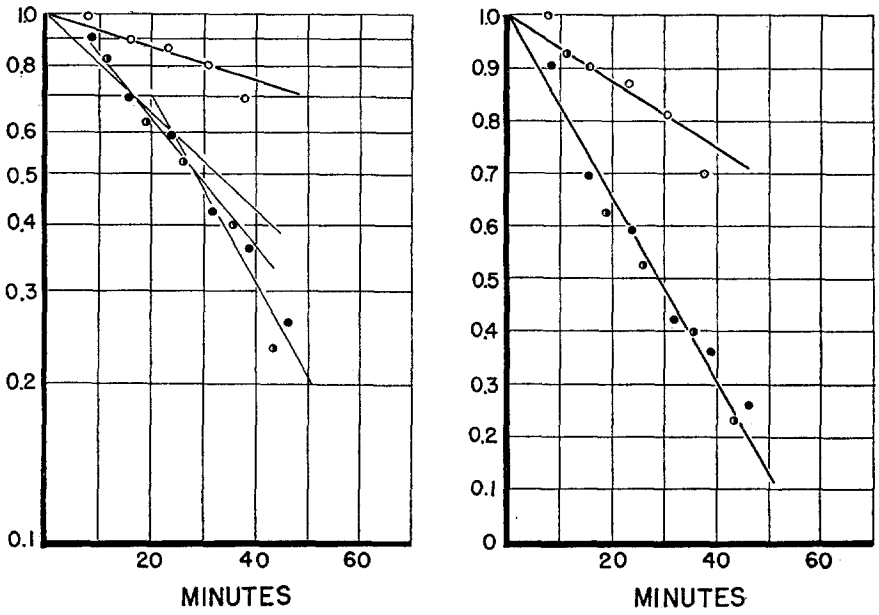

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FIG. 4. Three experiments have been conducted on successive days on the same loop of colon, a control observation and two observations 24 hours after an intramuscular dose of 2 mg. per kg. of desoxycorticosterone acetate in oil. The decrease in sodium in the bowel in each of these experiments has been plotted on both a logarithmic and arithmetic scale against time. The sodium in each experiment is plotted as the Relative amount of sodium as illustrated for the control experiment (Table I).

$$\beta = \frac{A - A_0}{t} \left[\frac{\ln [(\theta_A - \theta_B)/(\theta_{A_0} - \theta_{B_0})]}{-\ln A/A_0} \right], \tag{15}$$

and

$$\alpha = \frac{A - A_0}{t} \left[\frac{\ln [(\theta_A - \theta_B)/(\theta_{A_0} - \theta_{B_0})]}{-\ln A/A_0} - 1 \right]. \tag{16}$$

For the special case in which $\alpha = \beta$, α has a value of 0.03 to 0.15 m.eq. per minute (10) and with a value of 400 to 800 m.eq. for B_0, $\alpha/B_0 \leqq 0$ and equations (11) and (12) may be modified to

$$\ln \frac{\theta_A - \theta_B}{\theta_{A0} - \theta_{B0}} = -\frac{\beta_t}{A_0} \tag{17}$$

and

$$\beta = \alpha = -\frac{A_0}{t} \ln [(\theta_A - \theta_B)/(\theta_{A0} - \theta_{B0})] \tag{18}$$

When $B/B_0 \leqq 1$, complete mixing in compartment II need not be assumed and the assumption of complete and instantaneous mixing need be made only for compartment I.[5]

Sampling needs to be considered in arriving at values for inflow and outflow. When methyl cellulose is used to follow the volume of fluid in the lumen, it may be shown (Appendix) that

$$\beta = \frac{A - A_0}{t} \left[\frac{\ln [(\theta_A^n - \theta_B^n)/(\theta_{A1} - \theta_{B1})]}{-\ln \frac{[\mathrm{Na}]^n/[\mathrm{MC}]^n}{[\mathrm{Na}]_1/[\mathrm{MC}]_1}} \right] \tag{22}$$

and

$$\alpha = \frac{A - A_0}{t} \left[\frac{\ln [(\theta_A^n - \theta_B^n)/(\theta_{A1} - \theta_{B1})]}{-\ln \frac{[\mathrm{Na}]^n/[\mathrm{MC}]^n}{[\mathrm{Na}]_1/[\mathrm{MC}]_1}} - 1 \right], \tag{23}$$

in which θ_{A1}, $[\mathrm{Na}]_1$, and $[\mathrm{MC}]_1$, are respectively the specific activity, sodium concentration, and methyl cellulose concentration in the first sample, and $\theta_A{}^n$, $[\mathrm{Na}]^n$, and $[\mathrm{MC}]^n$ are their values at any subsequent sampling from the bowel lumen. θ_{B1} and $\theta_B{}^n$ are respectively the plasma specific activities at the first and subsequent sampling.[6] A is calculated as illustrated by Sodium corrected (Table I). A representative experiment on the colon illustrating the application of equations (22) and (23) is presented in Table II.

Thus, with measurements of specific activity, sodium concentration, and methyl cellulose concentration, equations (22) and (23) permit calculation of

[5] In practice, the contents of the lumen are churned by the bowel and at the time of sampling the entire contents are removed, quickly mixed in a syringe, a sample taken, and the contents returned.

[6] θ_{B1} and θ_B^n may be neglected in equations (22), (23), and (24) in which radiosodium is placed in the lumen (as opposed to intravenous administration), where inflow is relatively slow and where the period of observation is relatively short (under 1 hour). In such experiments the measured plasma specific activity is quite small compared to the specific activity of the sodium in the bowel lumen ($\theta_A \gg \theta_B$). In experiments in which inflow is relatively rapid and the period of observation of long duration when θ_A falls to a value which is an appreciable approach towards θ_B, it is necessary to account for plasma specific activity.

the simultaneous flow of sodium (and with proper substitutions other ions or molecules) into and out of an isolated loop of bowel.

TABLE II

Measurement of Sodium Exchange across the Colon
Sample Experiment: Dog SKP, February 25, 1953

Analytical data					Derived data					
Time	Sample weight	Na^{24}	Na^{22}	Methyl* cellulose	A‡	$A - A_0/t$	θA	$\ln \frac{\theta_A{}^n}{\theta_{A_1}} \Big/ \ln \frac{[Na]^n/[MC]^n}{[Na]_1/[MC]_1}$§	Rate into bowel	Rate out of bowel
min.	*gm.*	c.p.m./*ml.* $\times 10^{-3}$	*μeq./ml.*	*mg./ml.*	*μeq.*	*μeq./min.*	c.p.m./*μeq.*		*μeq./min.*	*μeq./min.*
0	0.924	60.87	153.2	3.65	3358		397			
8.3	0.932	52.50	149.7	3.87	3106	−30.4	351	−1.53	46.5	76.9
18.5	1.131	44.98	149.0	4.26	2835	−28.3	302	−1.51	42.7	71.0
28.5	1.243	37.55	145.8	4.70	2565	−27.8	258	−1.43	39.9	67.7
35.6	1.181	31.56	142.7	5.07	2386	−27.3	221	−1.47	40.0	67.3
42.4	4.318	25.41	134.9	5.31	2232	−26.6	188	−1.49	39.5	66.0
Mean									41.7	69.8

* 80 mg. methyl cellulose in bowel to start.

‡ A, corrected for sample removal as in Table I.

§ In terms of equations (22) and (23).

APPENDIX

Problem of Sampling

Sampling needs to be considered in arriving at values for inflow and outflow in equations (8) and (9) as these equations presume the labelled pool to be left undisturbed. With the removal of a sample and with a smaller labelled pool as a result, there is a more rapid fall (or rise) in specific activity with a constant inflow than would have been the case had no sample been removed. In order to compute inflow and outflow, the theoretical variables (A and θ) in equations (8) and (9) are determined from actual measurements through corrections. It turns out, however, that for mathematical reasons these corrections can be greatly simplified.

For clarity, the problem of sampling is dealt with in terms of the experiment on the bowel in which the removal of 0.5 to 1 ml. samples appreciably alters the size of the labelled pool (A) which usually starts at 20 ml. and may increase or decrease.

The following notations are introduced in Table III, A of equations (8) and (9) is to be distinguished from Na_n. A is the amount of sodium in the pool had there been no sampling. Na_n is the actual amount of sodium in the pool immediately after the n^{th} sampling and, therefore, partially determined by the $(n - 1)$ previous sam-

plings. Similar remarks apply to θ_n, MC_n, and $[MC]_n$. Na^n is the amount of sodium at the end of the n^{th} period, etc.

From equation (14), with the notation introduced in Table III:
for the first period

$$\ln \frac{(\theta_A^1 - \theta_B^1)}{(\theta_{A_1} - \theta_{B_1})} = \frac{\beta}{\alpha - \beta} \ln \frac{Na^1}{Na_1}, \tag{19 a}$$

TABLE III

Sampling Notations

	Placed in pouch prior to sampling	Sample	Begin first period	End first period	Sample	Begin second period	End second period	Sample	Begin nth period	End nth period	Sample
Time			t_0			t_1			t_{n-1}		
Bowel contents											
Sample size, *ml.*		S_1			S_2			S_n			S_n
Specific activity, c.p.m./*μeq.*			θ_{A1}	θ_A^1		θ_{A2}	θ_A^2		θ_{An}	θ_A^n	
Amount of sodium, *μeq.*			Na_1	Na^1		Na_2	Na^2		Na_n	Na^n	
Concentration of sodium, *μeq./ml.*			$[Na]_1$	$[Na]^1$		$[Na]_2$	$[Na]^2$		$[Na]_n$	$[Na]^n$	
Amount of methyl cellulose, *mg.*	MC_0		MC_1	MC^1		MC_2	MC^2		MC_n	MC^n	
Concentration of methyl cellulose, *mg./ml.*			$[MC]_1$	$[MC]^1$		$[MC]_2$	$[MC]^2$		$[MC]_n$	$[MC]^n$	
Plasma											
Specific activity, c.p.m./*μeq.*			θ_{B1}	θ_B^1		θ_{B2}	θ_B^2		θ_{Bn}	θ_B^n	

for the second period

$$\ln \frac{(\theta_A^2 - \theta_B^2)}{(\theta_{A_2} - \theta_{B_2})} = \frac{\beta}{\alpha - \beta} \ln \frac{Na^2}{Na_2}, \tag{19 b}$$

for the n^{th} period

$$\ln \frac{(\theta_A^n - \theta_B^n)}{(\theta_{A_n} - \theta_{B_n})} = \frac{\beta}{\alpha - \beta} \ln \frac{Na^n}{Na_n}. \tag{19 c}$$

From the beginning of the 1st period to the end of the n^{th} period,

$$\ln \frac{(\theta_A^1 - \theta_B^1)}{(\theta_{A_1} - \theta_{B_1})} \cdot \frac{(\theta_A^2 - \theta_B^2)}{(\theta_{A_2} - \theta_{B_2})} \cdots \frac{(\theta_A^n - \theta_B^n)}{(\theta_{A_n} - \theta_{B_n})} = \frac{\beta}{\alpha - \beta} \ln \frac{Na^1}{Na_1} \cdot \frac{Na^2}{Na_2} \cdots \frac{Na^n}{Na_n}, \tag{20}$$

in which sodium is measured as follows:—

$$\mathrm{Na}_1 = \frac{\mathrm{MC}_0 - S_1[\mathrm{MC}]_1}{[\mathrm{MC}]_1} \cdot [\mathrm{Na}]_1,$$

$$\mathrm{Na}^1 = \frac{\mathrm{MC}_0 - S_1[\mathrm{MC}]_1}{[\mathrm{MC}]^1} \cdot [\mathrm{Na}]^1,$$

$$\mathrm{Na}_2 = \frac{\mathrm{MC}_0 - S_1[\mathrm{MC}]_1 - S_2[\mathrm{MC}]_2}{[\mathrm{MC}]_2} \cdot [\mathrm{Na}]_2,$$

$$\mathrm{Na}^2 = \frac{\mathrm{MC}_0 - S_1[\mathrm{MC}]_1 - S_2[\mathrm{MC}]_2}{[\mathrm{MC}]^2} \cdot [\mathrm{Na}]^2$$

$$\mathrm{Na}_n = \frac{\mathrm{MC}_0 - S_1[\mathrm{MC}]_1 - S_2[\mathrm{MC}]_2 \ldots - S_n[\mathrm{MC}]_n}{[\mathrm{MC}]_n} \cdot [\mathrm{Na}]_n,$$

$$\mathrm{Na}^n = \frac{\mathrm{MC}_0 - S_1[\mathrm{MC}]_1 - S_2[\mathrm{MC}]_2 \ldots - S_n[\mathrm{MC}]_n}{[\mathrm{MC}]^n} \cdot [\mathrm{Na}]^n;$$

in which

$$[\mathrm{MC}]^1 = [\mathrm{MC}]_2, \quad [\mathrm{MC}]^{n-1} = [\mathrm{MC}]_n, \quad [\mathrm{Na}]^1 = [\mathrm{Na}]_2, \quad [\mathrm{Na}]^{n-1} = [\mathrm{Na}]_n;$$

and in which

$$(\theta_A^1 - \theta_B^1) = (\theta_{A_2} - \theta_{B_2}) \quad \text{and} \quad (\theta_A^{n-1} - \theta_B^{n-1}) = (\theta_{A_n} - \theta_{B_n}).$$

Equation (20) may be reduced to

$$\ln \frac{(\theta_A^n - \theta_B^n)}{(\theta_{A_1} - \theta_{B_1})} = \frac{\beta}{\alpha - \beta} \ln \frac{[\mathrm{Na}]^n/[\mathrm{MC}]^n}{[\mathrm{Na}]_1/[\mathrm{MC}]_1}, \tag{21}$$

or

$$\beta = \frac{A - A_0}{t} \left[\frac{\ln \dfrac{(\theta_A^n - \theta_B^n)}{(\theta_{A_1} - \theta_{B_1})}}{-\ln \dfrac{[\mathrm{Na}]^n/[\mathrm{MC}]^n}{[\mathrm{Na}]_1/[\mathrm{MC}]_1}} \right] \tag{22}$$

$$\alpha = \frac{A - A_0}{t} \left[\frac{\ln \dfrac{(\theta_A^n - \theta_B^n)}{(\theta_{A_1} - \theta_{B_1})}}{-\ln \dfrac{[\mathrm{Na}]^n/[\mathrm{MC}]^n}{[\mathrm{Na}]_1/[\mathrm{MC}]_1}} - 1 \right], \tag{23}$$

which are in terms of measurable quantities. $(A - A_0)/t$ is corrected for sampling as illustrated by Sodium corrected (Table I).

For the special case $\alpha = \beta$ and $\mathrm{Na}_1 = \mathrm{Na}^1$, $\mathrm{Na}_2 = \mathrm{Na}^2$, etc., sampling may be corrected for by the equation

$$\beta = \alpha = \frac{-\ln \dfrac{(\theta_A^n - \theta_B^n)}{(\theta_{A_1} - \theta_{B_1})}}{\dfrac{t_1 - t_0}{\mathrm{Na}_1} + \dfrac{t_2 - t_1}{\mathrm{Na}_2} \cdots \dfrac{t_n - t_{n-1}}{\mathrm{Na}_n}} \tag{24}$$

Equation (24) is developed from equation (17) in a manner similar to the development of equations (22) and (23) from equation (14). Na_1, Na_2 ... Na_n may be calculated in a manner similar to Total sodium (Table I[6]).

Effect of Analytical Errors on Estimation of Rate

With these involved formulations, the question naturally arises as to the effects of technical and analytical errors on the calculated values of β and α. If the logarithm of each side of the basic equations (8) and (9) is taken and each side is then differentiated with respect to θ, A, and t (11), the following equations are obtained

$$\frac{\Delta\beta}{\beta} = \left[\frac{1}{\ln\frac{\theta_A - \theta_B}{\theta_{A_0} - \theta_{B_0}} + \ln\frac{B}{B_0}}\right]\frac{\Delta\theta_A}{\theta_A - \theta_B} \tag{25 a}$$

$$\frac{\Delta\alpha}{\alpha} = \left[\frac{1}{\ln\frac{\theta_A - \theta_B}{\theta_{A_0} - \theta_{B_0}} + \ln\frac{A}{A_0}}\right]\frac{\Delta\theta_A}{\theta_A - \theta_B} \tag{25 b}$$

$$\frac{\Delta\beta}{\beta} = \left[\frac{A}{A - A_0} + \frac{1}{\ln\frac{B}{B_0} - \ln\frac{A}{A_0}}\right]\frac{\Delta A}{A} \tag{25 c}$$

$$\frac{\Delta\alpha}{\alpha} = \left[\frac{A}{A - A_0} + \frac{1}{\ln\frac{\theta_A - \theta_B}{\theta_{A_0} - \theta_{B_0}} + \ln\frac{A}{A_0}} + \frac{1}{\ln\frac{B}{B_0} - \ln\frac{A}{A_0}}\right]\frac{\Delta A}{A} \tag{25 d}$$

$$\frac{\Delta\beta}{\beta} = -\frac{\Delta t}{t} \tag{25 e}$$

$$\frac{\Delta\alpha}{\alpha} = -\frac{\Delta t}{t} \tag{25 f}$$

In equation (25 *a*) a 2 per cent error in the measurement of specific activity would affect β by a factor of

$$\frac{1}{\ln\frac{\theta_A - \theta_B}{\theta_{A_0} - \theta_{B_0}} + \ln\frac{B}{B_0}}$$

The order of magnitude of these coefficients may be seen in Table IV where they have been calculated for two hypothetical experiments, one in which $\beta = 35$, $\alpha = 60$, the other in which $\beta = 105$, $\alpha = 100$; in both experiments $A_0 = 2800$ and 1 ml. samples were removed every 10 minutes. In Table IV, a 2 per cent error in the estimate of the specific activity of the 10 minute sample of the first experiment would effect a (2×7.64) or 15 per cent error in β. Under the circumstances when the estimates of A from concentrations of methyl cellulose and sodium vary within analytical errors among successive periods, $\beta \cong \alpha$, equation (24) gives a better estimate of β and α than equations (22) or (23).

In large part these errors expressed precisely in equations (25 *a*) to (25 *f*) reflect the fact that the estimate of rate may in some time intervals depend upon a small

difference between two large numbers, each of which is subject to analytical errors [$(\theta_A - \theta_{A_0})$, $(A - A_0)$]. Thus, more rapid transfers, smaller pools, and longer time intervals all tend to minimize errors (Table IV).

TABLE IV

Error in Estimation of Rate Relative to Error in Measurement of Specific Activity, θ; or Amount of Sodium in Pouch, A

A_0 = 2800 μeq. θ_0 = 1.0

Time	Error in β *versus* error in θ	Error in α *versus* error in θ	Error in β *versus* error in A	Error in α *versus* error in A
	β = 35 μeq./min.		α = 60 μeq./min.	
min.				
10	−7.64	−4.46	+0.49	−3.96
20	−3.52	−2.05	+0.32	−1.73
30	−2.13	−1.24	+0.25	−0.99
40	−1.42	−0.83	+0.19	−0.64
50	−0.98	−0.57	+0.14	−0.44
60	−0.66	−0.39	+0.06	−0.32
	β = 105 μeq./min.		α = 100 μeq./min.	
10	−2.69	−2.82	+0.50	−2.32
20	−1.32	−1.39	+1.21	−0.18
30	−0.87	−0.91	+1.50	+0.59
40	−0.64	−0.67	+1.57	+0.90
50	−0.50	−0.53	+1.64	+1.12
60	−0.41	−0.43	+1.70	+1.26

(Sodium concentration in bowel maintained at 140 m.eq. per liter.)

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$$\frac{dA}{dt} = -\alpha + \beta \tag{I}$$

$$A = A_0 - \alpha t + \beta t \tag{II}$$

$$\frac{d(A\theta_A)}{dt} = -\alpha\theta_A + \beta\theta_B \tag{III}$$

$$A\frac{d\theta_A}{dt} + \theta_A\frac{dA}{dt} = -\alpha\theta_A + \beta\theta_B \tag{III a}$$

Multiplying all terms in Equation I by θ_A

$$\theta_A\frac{dA}{dt} = -\alpha\theta_A + \beta\theta_A \tag{IV}$$

Subtracting IV from III *a*

$$A\frac{d\theta_A}{dt} = \beta\theta_B - \beta\theta_A \tag{V}$$

With Equation II and rearranging

$$\frac{d\theta_A}{dt} = \frac{-\beta(\theta_A - \theta_B)}{A_0 + (\beta - \alpha)t} \tag{VI}$$

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