EQUATIONS FOR TRACER EXPERIMENTS

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The use of isotopes makes possible quantitative measurements of many biochemical and physiological processes, hitherto secure from such detailed observation. Perhaps the most frequent use of this tool is in the observations of physiology that are concerned with the transfer of fluid from one portion of the body to another, and in the biochemical observations of the formation of one compound from another. It is the purpose of this paper to present in detail some of the mathematical equations that govern such flow and synthesis, and to show incidentally that many of these equations are common to the fields of physics, and chemistry, as well as to the biological disciplines to which they will be applied here. A series of case studies will be presented, including, as examples, the formation of cholesterol from deuterium-marked body water, the uptake of phosphorus by the red blood cell and the flow of sodium out of the plasma into cerebrospinal fluid.

Mathematical analysis of this kind is certainly not novel; indeed, Burton (1) reports that Harcourt and Esson (2) concerned themselves with similar studies in 1866 when chemical kinetics was just beginning. In another connection, Rutherford, Chadwick and Ellis (3) have expressed some equations governing the familial relationship between radioactive parent elements and their daughters; they report that Bateman (4) as early as 1910 developed a theory which covers the general case of radioactive decay and recovery. The mathematics apply so generally that any survey of the literature must necessarily be incomplete.

1. *Basic assumptions*

The basic assumption that underlies all the equations to be developed is that the tracer element follows its unlabeled isotope faithfully in all biological reactions. Qualitatively there is good agreement on this point; quantitatively, evidence is gradually accruing that the rates of reaction are influenced by the isotopic composition of the reacting molecules. However, no allowance has been made for this in the cases to be discussed. Implicit in this primary assumption is the condition that the injection of the tracer element shall not disturb, in any important fashion, the normal metabolic behavior of the system. As a consequence of this initial assumption, the equations to be derived apply to unlabeled systems also; the tracer provides the method of measurement.

The second basic assumption is that in systems of constant volume the rate of flow of isotope out of a compartment is proportional to the amount of labeled isotope present in the compartment. This reduces to the assumption that rate is proportional to *concentration*, given constant volume. The difference is purely formal and can be taken up by suitable adjustments of the constants involved. Thus, the investigator has his choice of expressing the activity as, say, counts per milliliter, or counts per milliliter times the volume of the compartment in milliliters. The equations that follow have been based on amount. In systems in which the volume is not constant, other assumptions are made.

A third basic assumption is that of uniform distribution throughout the compartments. There are many physiological conditions in which this assumption is invalid, as when the time of mixing is long compared to the reaction rate, or when the viscosity of the fluid in the compartment is high. In many cases, the experimental conditions can be adjusted in order to make these factors unimportant; if not, suitable corrections must be applied.

2. *Symbols*

P = total amount of labeled material in compartment A.
P_o = P at time O.
t = time.
k_{ab} = coefficient of transfer from compartment A to compartment B.
p = concentration of labeled material in compartment A.

v_p = volume of compartment A.
Q = total amount of labeled material in compartment B.
k_{ba} = coefficient of transfer from compartment B to compartment A.
q = concentration of labeled material in compartment B.
v_q = volume of compartment B.
k_{ao} = coefficient of transfer from compartment A to outside.
etc.

3. *Case 1—Simple decay*

The decay of a radioactive isotope can be described by an equation which states that the rate of loss of isotope depends upon the amount of isotope remaining. This is perhaps the most common example of simple decay. In chemistry, monomolecular reactions are governed by a similar equation, since the rate of reaction is dependent on the amount of reactant remaining (though not necessarily independent of concentration). Margenau and Murphy (5) point out that the rate of growth of bacterial cultures in an unlimited nutrient medium is dependent on the number of bacteria present; thus the equation is similar to that for radioactive decay, though the sign, of course, is different.

1-1 $$\frac{dP}{dt} = -k_{ao}P$$

1-2 $$P = P_o e^{-k_{ao}t}$$

1-3 $$\ln P = \ln P_o - k_{ao}t$$

1-4 $$t_{\frac{1}{2}} = 0.693/k_{ao}$$

Figure 1 shows the decay of radioactive phosphorus (P^{32}). The amount of this material remaining, P (expressed as relative counts/minute in a unit mass of sample) is plotted on a logarithmic scale against t. The slope of this straight line gives k_{ao}, from which the half-life, $t_{\frac{1}{2}}$, may be derived by equation 1–4. In this case, $t_{\frac{1}{2}}$ measures the time required for half the material to disintegrate.

4. *Case 2—Uptake from a constant source*

This case arises most frequently in stable isotope experiments in which the body fluids are brought to a constant deuterium level, and the uptake of deuterium in various compounds is examined. The basic assumption is that the isotopic content of the circulating fluids remains constant. This includes the implicit assumption that the return of material to the circulating fluids from the compartment examined is unimportant.

This example of Case 2 is shown diagrammatically in Figure 2. Here compartment A represents the body fluids, and P represents the total amount of deuterium in the fluids. The concentration, p, is given by the atom per cent deuterium in the body fluids. Compartment B represents the cholesterol; Q is the total amount of deuterium in the cholesterol, and q is the concentration of deuterium given by atom per cent deuterium in the cholesterol (usually referred to atom per cent D in the body fluid as 100%).

Another common example of this case is the formation of radioactive isotopes in nuclear reactors. The basic assumption in this case may be restated: the flux of neutrons must remain constant, and be unaffected by the products formed in the material being irradiated.

2-1 $$\frac{dQ}{dt} = k_{ab}P - k_{bo}Q, \qquad P = P_o = \text{const.}$$

2-2 $$Q = \frac{k_{ab}}{k_{bo}} P_o(1 - e^{-k_{bo}t})$$

2-3 $$Q_\infty = \frac{k_{ab}}{k_{bo}} P_o, \qquad t = \infty$$

2-4 $$\ln(1 - Q/Q_\infty) = -k_{bo}t$$

2-5 $$t_{\frac{1}{2}} = 0.693/k_{bo}$$

These equations differ materially from those of simple decay. For one thing the half-life is dependent upon the rate of decay of the material in compartment B, not on the rate of its synthesis, unless an equilibrium obtains, in which case the two rates are equal. The dependence of half-life upon decay may be illustrated by considering the formation of isotopes in reactors. For a given rate of decay, that is, a given half-life, the *amount* of material formed depends directly on the intensity of the neutron flux. The *time* it takes to achieve the maximum formation of radioactive material is dependent on the half-life and independent of the neutron flux. That is, more radioactive material can be formed by an increase in neutron flux, but the time it takes to reach the new maximum value is independent of this increase in neutron flux.

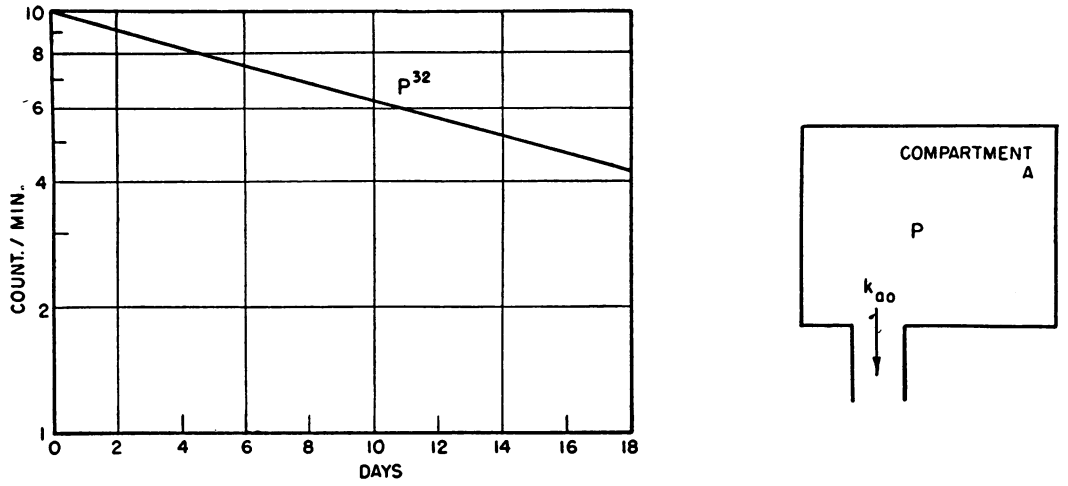

FIG. 1. CASE 1: RADIOACTIVE DECAY OF P^{32}
Relative counts/min. in a unit mass of sample is plotted on a logarithmic scale against time.

In order to determine whether the equation 2–1 holds for conditions in which the volume is not constant, the equation 2–1 has been fitted to experimental results on the uptake of deuterium by maternal and fetal cholesterol reported by Goldwater and Stetten (6). Pregnant rats put on a heavy water regimen in the 18th day of term were sacrificed at quarter-day intervals from the 18th to the 19th day, and at half-day intervals from the 19th to the 20th day. One control was kept on the heavy water regimen for the total 20-day term in order to determine the maximum uptake of deuterium in the fetal cholesterol, and the maternal cholesterol, both liver and carcass. In the two-day period between the 18th and the 20th day, the weight per fetus varied between 2 and 5.2 grams. The tissue content of cholesterol in all three cases showed a slight increase. Thus, the amount of cholesterol, the volume as it were, of compartment B is certainly not constant.

The weight increase in the maternal rat will show whether constant isotope concentration in the body fluids is equivalent to a constant amount of deuterium in the fluids. If one ascribes the weight increase to increase in fetal

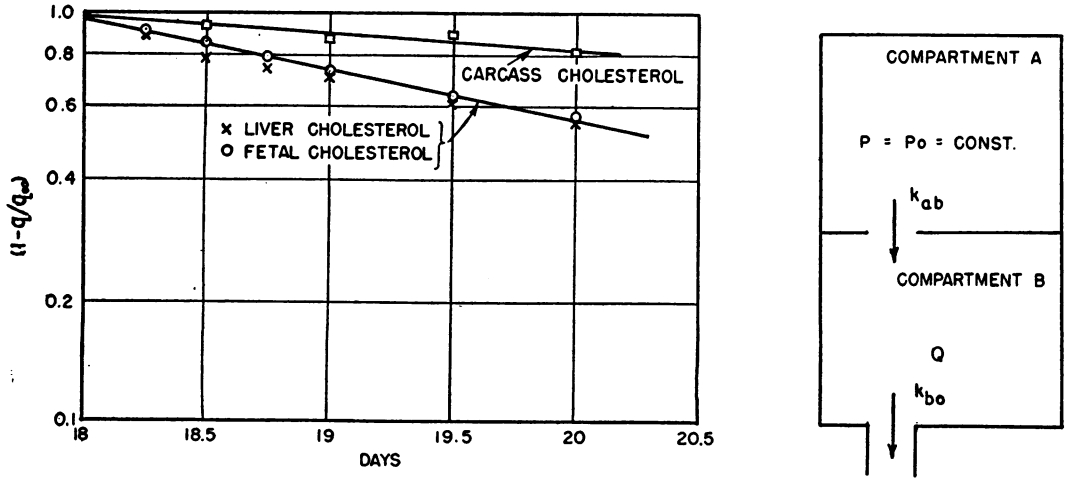

FIG. 2. CASE 2: UPTAKE OF DEUTERIUM BY CHOLESTEROL IN THE MATERNAL RAT AND FETUS
The function $(1 - q/q_\infty)$ is plotted on a log scale against time. The slope of the curve determines the transfer coefficient k_{bo}.

weight alone, Goldwater and Stetten's figures show that the average increase in maternal rat weight in the two-day period is about 11%. This is small compared to the other changes in the system; hence, we will assume that both concentration and amount of deuterium in the body fluids remain constant.

If the assumptions in equation 2–1 are correct, the function derived in equation 2–4 should fall on a straight line when plotted against time. Since it does not, a different assumption must be made. We may test the assumption that the rate of change of the *amount* of labeled cholesterol is proportional to the *concentration* of deuterium in the body fluids, and the *concentration* of deuterium in the cholesterol. Stated analytically, this becomes:

$$2\text{–}6 \qquad \frac{dQ}{dt} = k_{ab}p - k_{bo}q$$

If one assumes that the change in cholesterol content in the fetus varies linearly with time, an assumption supported by the data, this equation can be solved. In this case, too, the equation is seen to fail in describing the data. However, when one assumes that the rate of change of *concentration* of cholesterol is proportional to the *concentration* of deuterium in the body fluids and the *concentration* of deuterium in the cholesterol the equation fits the data. In this case the equation becomes

$$2\text{–}7 \qquad \frac{dq}{dt} = k_{ab}p - k_{bo}q$$

and 2–4 reduces to

$$2\text{–}8 \qquad \ln(1 - q/q_\infty) = - k_{bo}t$$

The calculations are given in Table I, and the function is plotted in Figure 2. It will be seen that all three cases fit the equation nicely, even though the volume in compartment B is greatly changed during the course of the experiment. Many chemical systems can be postulated that can be described analytically by equation 2–7.

5. *Case 3—Closed two-compartment system*

The equilibration of red blood cells with K^{42}-marked plasma is used as an example of this case. The basic assumption is that the total amount of labeled material in the system remains constant. In Figure 3 this case is shown diagrammatically. Compartment A represents the plasma containing an amount P of K^{42}, and compartment B represents the erythrocytes containing an amount Q of K^{42}.

Since Cohn and Cohn (7) investigated the permeability of the red corpuscles of the dog to sodium ions, many experiments have been carried out on cellular permeability. Sheppard (8) has recently reviewed these experiments and reported some additional results of his own. The equations he derives, specific examples from a previously published general treatment (9), correspond with those to be derived below.

$$3\text{–}1 \qquad P + Q = P_o = \text{const.}$$

$$3\text{–}2 \qquad \frac{dP}{dt} = - k_{ab}P + k_{ba}Q$$

$$3\text{–}3 \qquad \frac{dQ}{dt} = k_{ab}P - k_{ba}Q$$

$$3\text{–}4 \qquad Q = \frac{k_{ab}}{k_{ab} + k_{ba}} P_o[1 - e^{-(k_{ab}+k_{ba})t}]$$

$$3\text{–}5 \qquad Q_{equil} = \frac{k_{ab}}{k_{ba}} P_{equil}$$

$$3\text{–}6 \qquad Q_\infty = \frac{k_{ab}P_o}{k_{ab} + k_{ba}}$$

$$3\text{–}7 \qquad \ln(1 - Q/Q_\infty) = - (k_{ab} + k_{ba})t$$

$$3\text{–}8 \qquad t_{\frac{1}{2}Q} = 0.693/(k_{ab} + k_{ba})$$

$$3\text{–}9 \qquad P = \frac{k_{ba}P_o}{k_{ab} + k_{ba}} \left[1 + \frac{k_{ab}}{k_{ba}} e^{-(k_{ab}+k_{ba})t} \right]$$

$$3\text{–}10 \qquad P_\infty = \frac{k_{ba}P_o}{k_{ab} + k_{ba}}$$

$$3\text{–}11 \qquad \ln(P/P_\infty - 1) = - (k_{ab} + k_{ba})t - \ln \frac{k_{ba}}{k_{ab}}$$

The example chosen to illustrate this case has been taken from some unpublished work of Raker, Taylor and Weller (10) on the uptake of K^{42} by human erythrocytes in a dilute solution of K^{42}-marked plasma. Figure 3 shows the function $(P/P_\infty - 1)$ plotted on a logarithmic scale against time. The constants describing the system may be determined as shown in equation 3–11 from the slope and intercept of this line. The calculations have taken account of a slight swelling of the cells towards the end of the experiment, as shown by a 10% increase in the hematocrit. This change in volume of compartment B is so small, however, that it does not affect the fit of the equation. The function $(P/P_\infty - 1)$ in equation 3–11 is plotted on a logarithmic scale against time in Figure 3. A curve drawn on the assumption that the flow of

TABLE I

Uptake of deuterium in pregnant rats whose body fluids were enriched with D_2O
Calculations from data presented by Goldwater and Stetten to obtain the function $(1 - q/q_\infty)$.

Days on D_2O	Fetal cholesterol			Liver cholesterol			Carcass cholesterol		
	D	$q/q_\infty = D/D_{20}$*	$1 - q/q_\infty$	D	$q/q_\infty = D/D_{20}$	$1 - q/q_\infty$	D	$q/q_\infty = D/D_{20}$	$1 - q/q_\infty$
	per cent body water			*per cent body water*			*per cent body water*		
18–18¼	5.0	0.090	0.910	4.8	0.105	0.895			
18–18½	8.2	0.148	0.852	10.5	0.229	0.771	2.5	0.072	0.928
18–18¾	11.3	0.204	0.796	11.3	0.246	0.754			
18–19	15.4	0.279	0.721	13.5	0.294	0.706	4.7	0.135	0.865
18–19½	20.4	0.368	0.632	17.7	0.386	0.614	3.9	0.112	0.888
18–20	24.2	0.438	0.562	19.8	0.432	0.568	6.7	0.193	0.807
1–20	55.4	—	—	45.8	—	—	34.8	—	—

* D_{20} represents the D of the various fractions for the rat which was fed D_2O for the total 20-day period.

K^{42} is determined by its *concentration* in plasma and cells fits the data equally well, and is probably preferable on theoretical grounds.

The symmetry of this system is very interesting. The observations of the time course can be made on either plasma or cells, provided that

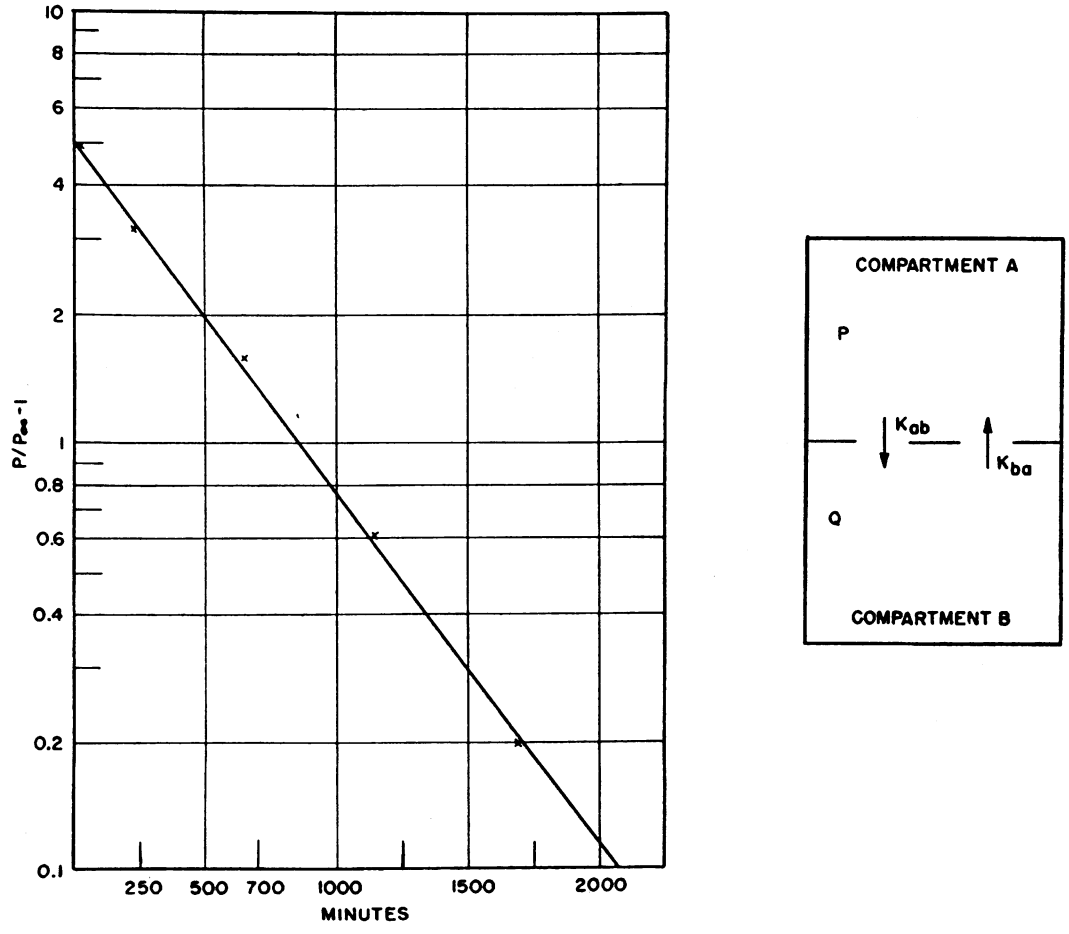

FIG. 3. CASE 3: UPTAKE OF K^{42} BY RED BLOOD CELLS FROM PLASMA

The function $P/P_\infty - 1$ is plotted on a logarithmic scale against time. From the slope and intercept of this curve the transfer coefficients k_{ba} and k_{ab} may be determined.

the equilibrium amounts in both compartments be determined. Indeed, it is theoretically possible, though not experimentally desirable, to characterize this system exactly by only three measurements: the amount of isotope in either component at any time t, prior to equilibrium, and the equilibrium amounts.

6. *Case 4—Diffusion or flow out of a compartment*

An example of flow out of a compartment is the escape of sodium from the blood stream following a single intravenous injection. Merrell, Gellhorn and Flexner (11) have derived equations which describe this process in the guinea pig. In the case of the guinea pig, the process can be described by a simple exponential similar to, but not identical with, that given in Case 3, in which compartment A is the plasma and P is the amount of tracer sodium in the plasma. Merrell *et al.* assume that the process of sodium movement is one of diffusion, that is, that the coefficient of transfer into the plasma equals the coefficient of transfer out when the equation is expressed in terms of concentration of sodium in the various compartments. Apparently, it is assumed implicitly that there is no sodium excretion during the course of the experiments.

In the dog (Gellhorn, Merrell, and Rankin [12]) and in man (Flexner, Cowie, and Vosburgh [13] and Burch, Reaser, and Cronvich [14]), the process is described by at least two separate rates. A two-rate system (neglecting return from extra-cellular fluid to plasma) implies the presence of a split compartment in the extra-cellular fluid, rather than a compartment with two leaks of different size. Two leaks of different size are equal to one leak of larger size, and can be described by a single transfer coefficient. However, as Gellhorn *et al.* point out (12), the situation is far different when the return of Na^{24} to the plasma is taken into account. Assume that the Na^{24} escapes from the plasma into two separate external compartments as shown diagrammatically in Figure 4, entering one quickly and the other slowly. The return from these two compartments to the plasma must be characterized by two different rates, since the amount of tracer in compartment B is different from the amount in compartment C. Thus, the flow of Na^{24} from the plasma will be described by an equation containing at least two exponentials. It is not assumed that the compartments B and C exist as such in the body, but rather that one, say B, represents the average of a number of areas to which the sodium is transferred quickly, and the other, C, the average of a number of areas to which the sodium is transferred more slowly.

In the treatment that follows, three basic assumptions will be made: first, that the volume of the compartments remains constant; and second, that there is no excretion of Na^{24} during the period of observation. For simplification, we will also assume that the coefficient of transfer across the membrane separating the compartments is the same in both directions. That is,

4–1 $$k_{ab} = k_{ba} = k_1; \qquad k_{ac} = k_{ca} = k_2$$

The equations that follow are similar to those derived by Gellhorn *et al.*, and the reader is referred to Appendix 1 of reference 12 for the derivation.

4–2 $$\frac{dP}{dt} = -k_1P + k_1Q - k_2P + k_2R$$

4–3 $$\frac{dQ}{dt} = k_1P - k_1Q$$

4–4 $$\frac{dR}{dt} = k_2P - k_2R$$

4–5 $$P + Q + R = P_o$$

4–6 $$\frac{d^2P}{dt^2} = -2(k_1 + k_2)\frac{dP}{dt} - 3k_2k_1P + k_2k_1P_o$$

4–7 $$P = a_1e^{-b_1t} + a_2e^{-b_2t} + \tfrac{1}{3}P_o$$

where

$$a_1 = \tfrac{1}{3}P_o + \tfrac{1}{6}P_o\frac{(k_1 + k_2)}{\sqrt{k_1^2 - k_1k_2 + k_2^2}}$$

$$a_2 = \tfrac{1}{3}P_o - \tfrac{1}{6}P_o\frac{(k_1 + k_2)}{\sqrt{k_1^2 - k_1k_2 + k_2^2}}$$

$$b_1 = (k_1 + k_2) + \sqrt{k_1^2 - k_1k_2 + k_2^2}$$

$$b_2 = (k_1 + k_2) - \sqrt{k_1^2 - k_1k_2 + k_2^2}$$

4–8 At equilibrium,

$$P = Q = R = \tfrac{1}{3}P_o$$

In the course of studies now in progress to determine the uptake of Na^{24} by cerebrospinal fluid, Sweet, Solomon, and Selverstone (15) have examined the diffusion of this isotope from human plasma. The results have been plotted in Figure 4, and analyzed on the basis of the equation 4–7. For the purpose of this example, a smooth curve drawn through the experimental

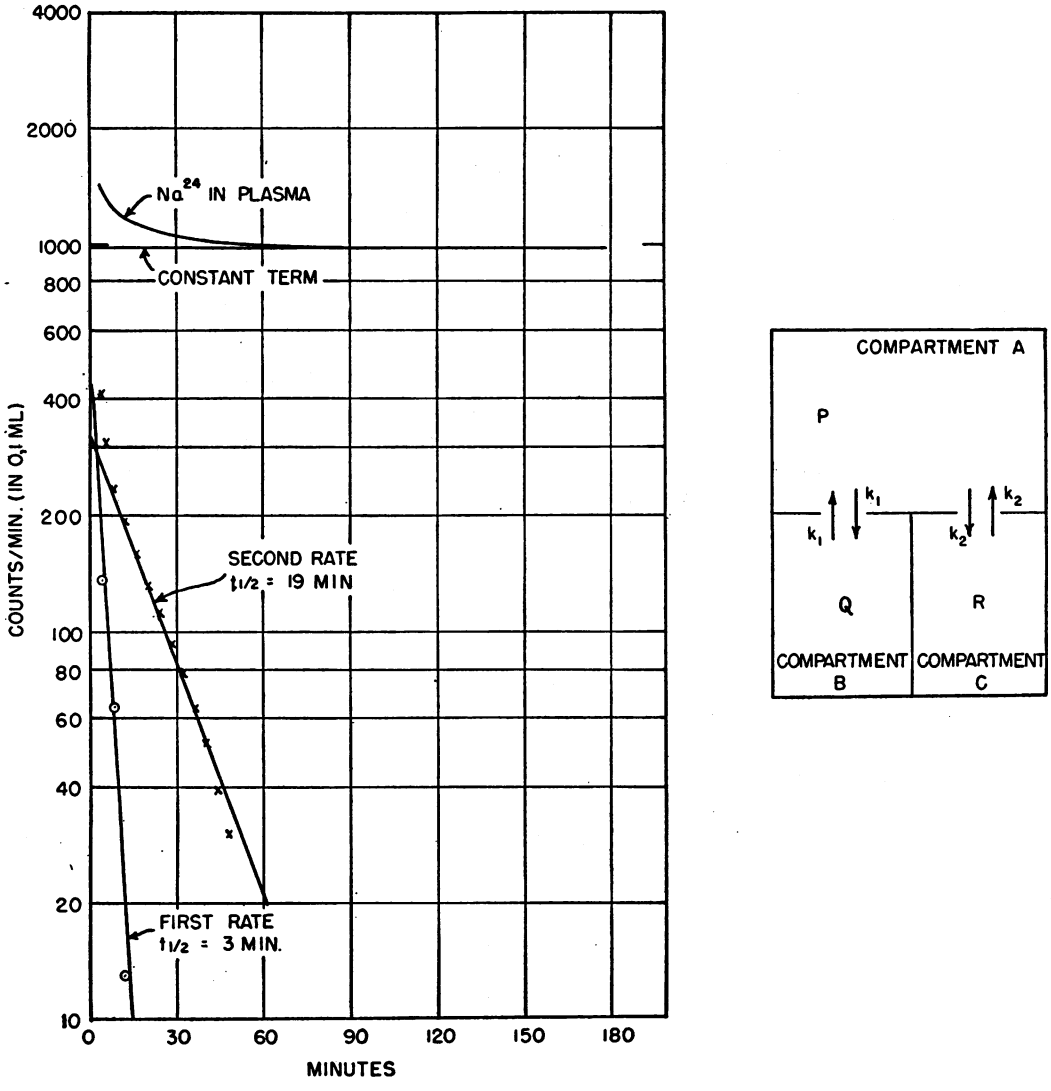

FIG. 4. CASE 4: DISAPPEARANCE OF Na^{24} FROM PLASMA IN MAN

Counts/min. in 0.1 ml in the plasma is plotted on a logarithmic scale against time. The transfer coefficients may be determined from the slopes and intercepts of the first and second rates, and the value of the constant term.

points has been used; the full data will be published elsewhere. In Figure 4, the radioactivity has been expressed as counts per minute in 0.1 ml, plotted on a logarithmic scale against time; since constant volume is assumed, this expression is a valid index of the total amount of radioactivity, P. In order to analyze a curve plotted in this way into its components, the constant term is first subtracted graphically from the curve. The difference between these two curves, shown in Figure 4 as the crosses, is then plotted, and the straight line is drawn which best fits these points. That straight line gives the second rate and has the slope $-b_2$. It, in turn, is subtracted from the points represented by the crosses that do not fall on the line at the beginning of the period of measurement. Thus, the first rate, given by the circles on Figure 4, is obtained. The method of analyzing experimental curves by a sum of exponentials with constant coefficients is so powerful, and the number of adjustable constants so large, that great care must be taken in interpreting such curves physiologically.

Accepting this curve as a formal description of the behavior of the labeled material in the

plasma, one would expect that a knowledge of the total amount of injected material, and the intercept of the curve of concentration of Na^{24} at zero time, would lead to a good value of the total plasma volume. In the dog, Gellhorn *et al.* (12) were unable to obtain reliable results by the use of this method, a conclusion supported by the preliminary experiments in man of Sweet, Solomon, and Selverstone (15). This may be due to incomplete knowledge about the early part of the curve.

7. *Case 5—Uptake of radioactive material in a three-compartment system*

An example of this case is found in the uptake of Na^{24} by ventricular cerebrospinal fluid, following intravenous injection. In order to determine the coefficient of transfer into the cerebrospinal fluid, it is not necessary to accept any hypothesis as to the physiological basis for the time curve of isotope in the plasma; it suffices to express the curve analytically. The equations that follow have been developed on the basis of the plasma equation given in Case 4, as equation 4–7. As can be seen from the diagram in Figure 5, the conditions are not exactly identical with those assumed for Case 4. Then we assumed that the coefficient of transfer from compartment A to compartment B was equal to that of transfer in the reverse direction. As has been shown, an equation can be derived on those assumptions, which describes the time course of the tracer

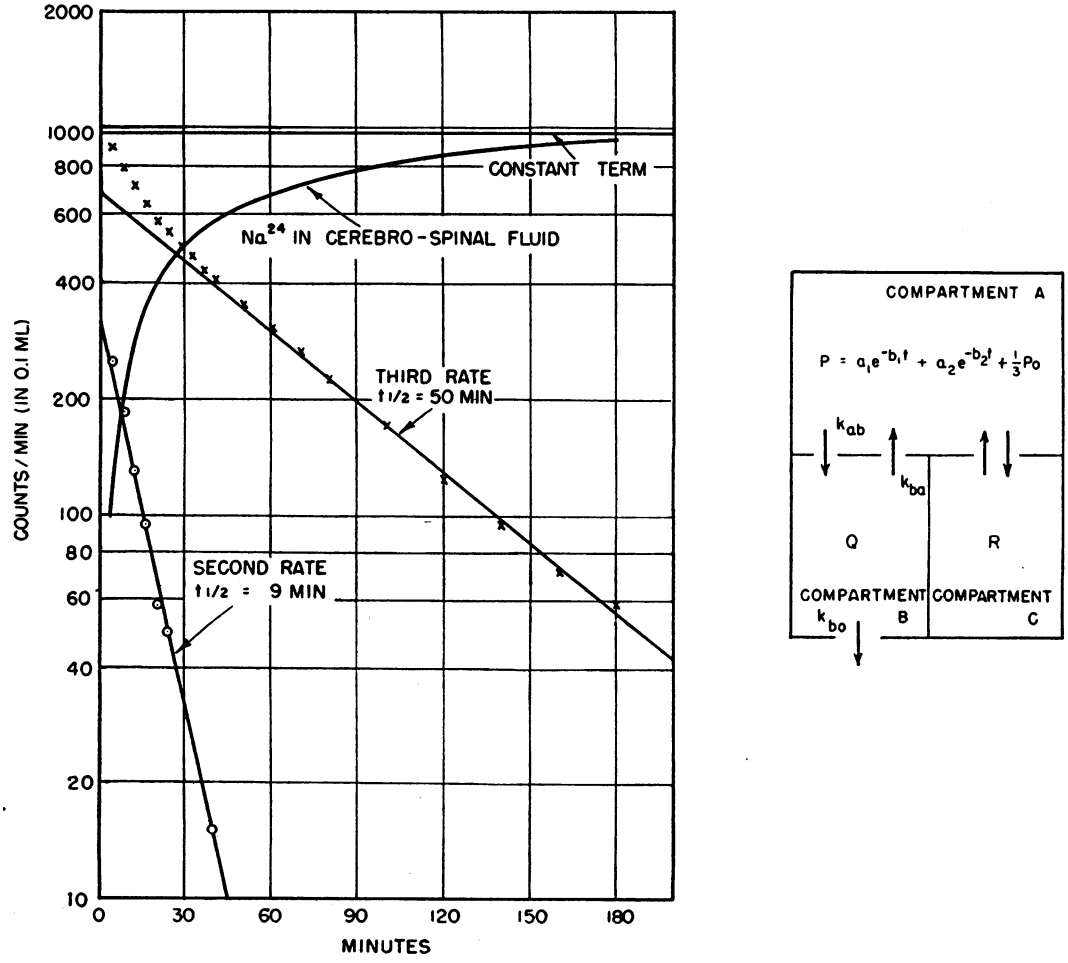

FIG. 5. CASE 5: UPTAKE OF Na^{24} BY CEREBROSPINAL FLUID FROM THE PLASMA

Counts/min. in 0.1 ml in the cerebrospinal fluid is plotted on a logarithmic scale against time. The transfer coefficient k_{ab} and the sum of coefficients ($k_{ba} + k_{bo}$) can be determined from the slopes and intercepts of the second and third rate and the value of the constant term.

content of the plasma. In the case of cerebrospinal fluid, we will assume that the volume of the compartments and their Na concentration is constant.

We will represent the plasma as before by compartment A, and the ventricle as compartment B. The coefficient of transfer k_{bo} will represent the flow of Na^{24} out of the ventricle toward the cistern. Since some of the Na^{24} will find its way back to the plasma by this route, k_{bo} and k_{ba} could, if desired, be represented together as a single constant. The equations to be derived in this case do not permit their individual determination.

4–7 $$P = a_1e^{-b_1t} + a_2e^{-b_2t} + \tfrac{1}{3}P_o$$

5–1 $$\frac{dQ}{dt} = k_{ab}P - k_{ba}Q - k_{bo}Q = k_{ab}P - (k_{ba} + k_{bo})Q$$

5–2* $$Q = \frac{k_{ab}a_1}{(k_{ba} + k_{bo}) - b_1}e^{-b_1t} + \frac{k_{ab}a_2}{(k_{ba} + k_{bo}) - b_2}e^{-b_2t} + (k_{ba} + k_{bo})e^{-(k_{ba}+k_{bo})t} + \frac{k_{ab}}{3(k_{ba} + k_{bo})}P_o$$

5–3 At equilibrium

$$P = \frac{(k_{ba} + k_{bo})Q}{k_{ab}}$$

Figure 5 presents a smoothed curve drawn from the experimental results of Sweet, Solomon, and Selverstone (15). The same method has been applied in plotting and analyzing this curve as has been used with Figure 4. The values of the transfer coefficients can be obtained from the slopes and intercepts of the various segments of the curve, since a_1, a_2, b_1, b_2 are all known, at least empirically, from the curve presented in Figure 4. It will be noted that the analysis in Figure 5 does not include the first rate of Figure 4 with its half-life of three minutes. A further test of the validity of equation 5–2 is available by comparing the slopes of the second term of equation 4–7 ($t_{\frac{1}{2}}$ = 19 mins.) with the same term ($t_{\frac{1}{2}}$ = 9 mins.) in 5–2. It is seen that the agreement is not good. It would perhaps be surprising to obtain good agreement under these conditions, since no account has been taken of the viscosity of the cerebrospinal fluid, nor has any allowance been made for mixing time. In so complex a system, it must be emphasized that the equations give only a formal description. Many experiments must be carried out in order to determine whether such a mathematical description fits the physiological facts.

* For details of the solutions of such differential equations, see the relevant chapter in reference (5).

8. *Ultra-filtration vs. secretion*

Kinsey and Grant (16) in their studies on the uptake of tracers by the aqueous humor have pointed out that the equilibrium values for the components in tracer systems are not in themselves sufficient to decide what mechanism is operating. Consider Case 2 and equation 2–7, as an example of secretion

2–7 $$\frac{dq}{dt} = k_{ab}p - k_{bo}q$$

Here p and q are the concentrations in compartments A and B, and return from compartment B to compartment A is excluded.

If it is assumed, as in ultra-filtration, that the barrier between compartments A and B is a simple membrane, and that the coefficient for transfer across that membrane (with respect to concentration) is the same in both directions, equation 2–7 becomes

6–1 $$\frac{dq}{dt} = k_{ab}p - k_{ab}q - k_{bo}q$$

The solution for 2–7 (secretion) is

6–2 $$q = \frac{k_{ab}}{k_{bo}}p_o(1 - e^{-k_{bo}t})$$

and for 6–1 (ultra-filtration)

6–3 $$q = \frac{k_{ab}}{k_{ab} + k_{bo}}p_o(1 - e^{-(k_{ab}+k_{bo})t})$$

Since $k_{ab} + k_{bo}$ can be represented by another constant, say k_{bb}, it is not, in general, possible to decide between these two hypotheses without additional independent measurements of one of the constants. If, however, the constant in the denominator of equation 6–3 is less than k_{ab}, it is apparent that the mechanism is not one of ultra-filtration.

Another example of this problem is afforded by the uptake of Na^{24} by cerebrospinal fluid. As has been pointed out, the sum of the constants $k_{ba} + k_{bo}$ in equation 5–2 can easily be determined, but the individual values cannot be obtained independently without further measurement.

9. *Precursors*

Zilversmit, Entenman, and Fishler (17) have put forward a criterion that states that one compound, A, is the precursor of a second, B, if, when the specific activity of B has reached its maximum, it equals the specific activity of A. Let us examine this criterion on the basis of our

Case 2 which is similar to that described by Zilversmit *et al.*

Let the specific activity in A be denoted by $P_a = P/P^*$ where P^* is the total amount of unlabeled isotope in compartment A, and similarly, let $Q_a = Q/Q^*$.

From 2–1

$$\frac{dQ}{dt} = k_{ab}P - k_{bo}Q$$

for Q = maximum

10–1 $$\frac{dQ}{dt} = 0 = k_{ab}P_aP^* - k_{bo}Q_aQ^*$$

10–2 $$k_{ab}P_aP^* = k_{bo}Q_aQ^*$$

Since $P_a = Q_a$ to satisfy the criterion

10–3 $$k_{ab}P^* = k_{bo}Q^*$$

Equation 10–3 states that the amount of unlabeled isotope in compartment B is in equilibrium, in agreement with the assumptions initially made by Zilversmit *et al.*

Another criterion may be found by consideration of a chain of reactions. Let $J \rightarrow K \rightarrow L$, in a series of unimolecular reactions which can be described by the basic assumptions in section 1. The rate of growth of K from J will be given by an equation with a single exponential term, as shown in Cases 2 and 3. The rate of growth of L from J, however, will be governed by an equation which requires two exponential terms to describe it, and each succeeding member in the chain, M, N, and O, will require an additional exponential term in the equation (Burton [1]). Thus, we can say, if J is the precursor of K, a single exponential will suffice to describe the growth of K from J. If our basic assumptions describe the reaction correctly, the addition of a second exponential in the expression for growth must mean a two-step process.

10. *Discussion of mathematical methods*

As has already been mentioned, the use of differential equations to describe steady states of this kind is not new. Indeed, Burton (1) derived his description of the steady state in 1936 with no reference to isotopes. The most general treatment of this kind is that given by Sheppard (9). Tobias (18) has derived a system of equations describing the movement of isotopes under conditions of constant volume. Burch, Threefoot, and Cronvich (19) have examined the effect of changes both of compartment volume and amount of unlabeled isotope on the constants involved in such equations.

It is also possible to describe the behavior of tracer systems by the use of integral equations. Shemin and Rittenberg (20) have made use of an integral equation in order to determine the life span of the human red blood cell using glycine-labeled hemin, a case very different from those considered here, since the decrease of labeled activity is a function of the age of the red blood cell in which it is contained. Branson (21) has also used integral equations in order to describe isotope systems.

SUMMARY

Equations have been developed and discussed covering five common cases of the use of isotopes in biological and physiological systems.

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