Effect of glucose feeding on net transport of plasma free fatty acids

NOME BAKER and HOJAT ROSTAMI

Radioisotope Research, Veterans Administration Center, Los Angeles, California 90073, and Department of Biological Chemistry, University of California at Los Angeles School of Medicine, Los Angeles, California 90024

ABSTRACT The effect of a single glucose feeding upon the net inflow and outflow transport of plasma free fatty acids (FFA) has been studied in 75 unanesthetized rats. The animals were fasted for 22 ±2 hr; then 50 rats were refed 2 ml of 50% glucose by gastric intubation. At 0, 10–15, and 30–35 min after glucose refeeding, the rats were injected with palmitate-1-^{14}C complexed to rat serum. The tracer dose included ^{131}I-labeled albumin.

Plasma FFA concentration, ^{131}I concentration, and FFA-^{14}C were measured at five time intervals after injection of the tracer dose. From these data the irreversible disposal rate, or *net outflow transport*, and the net inflow transport of plasma FFA were calculated. Estimations were based upon a special case of a general solution for measuring net inflow and outflow transport of a circulating metabolite. The general solution is independent of the number of compartments, how they are interconnected, the number of nonradioactive inflows, and where the inflows enter the system.

Net inflow = net outflow transport = 7.6 μeq/min in the fasted state and 3.5 μeq/min in the new steady state that is reached 30–40 min after glucose refeeding. A very slight imbalance between the rates of net inflow and outflow transport could account for the rapid fall in plasma FFA concentration that results from a single glucose feeding. Theoretical and practical problems associated with studying inflow and outflow transport by means of the technique using a single injection of racer are discussed.

SUPPLEMENTARY KEY WORDS irreversible disposal rate · inflow–outflow transport · nonsteady state · unanesthetized rats · palmitate turnover

MANY INVESTIGATORS who are interested in the hormonal and dietary control of lipid metabolism have utilized radioisotopes to measure turnover rates of circulating lipids and of lipolytic products such as glycerol. In many instances, complicated experimental techniques, such as constant infusion of tracer, or overly simplified models which are inconsistent with the data have been used in order to obtain the desired rates. In other cases the rates have not been calculated even though all of the necessary data were available. Some investigators may be unfamiliar with the simple mathematical relationship between the specific activity–time curve of a circulating metabolite after a single injection of tracer and the rate of irreversible disposal or turnover of the substance.

The irreversible disposal rate (mass/time) of a substance in the steady state is equivalent to net inflow and outflow transport from a system. Other workers have used the term "turnover rate" to describe the same parameter. Recently an international study group has recommended (2) that the term "turnover rate" only be used to express fractional rates (units of reciprocal time). We have used both the original expression, *irreversible disposal rate*, and the recommended nomenclature of the international study section (2), *net outflow transport*, in the present paper.

Baker, Shipley, Clark, and Incefy (1) showed that for several three pool models, the irreversible disposal rate (R) may be calculated from the following relationship:

$$R = \frac{Q_1 (g_1 g_2 g_3)}{H_1 g_2 g_3 + H_2 g_1 g_2 + H_3 g_1 g_2} \qquad \text{Eq. 1}$$

where the radioactivity, $y(t)$, in compartment 1 is given by the equation

$$y(t) = \Sigma H_i e^{-g_i t}$$

and Q_1 = the pool size of the traced constituent. Thus H_i are the intercepts and g_i are the slopes of the curves which

Abbreviation: FFA, free fatty acids.

comprise the function of $y(t)$ in compartment 1. This equation may also be expressed as follows:

$$R = Q_1/\Sigma(H_i/g_i). \qquad \text{Eq. 2}$$

We now wish to show (see Appendix) that this relationship holds for any model regardless of the number of compartments or how they are interconnected, provided that the tracer is injected into the circulating blood, that the substance being traced is replaced by entry of the substance directly into the circulation, and that (fractional) turnover rates are constant during the experiment. Although this relationship has been utilized by several workers who have indicated that it is perfectly general (3–5), formal proof of the general relationship (Eq. 2) and the theoretical and practical limitations associated with its use have not been presented.

As an example of this approach, we have studied the net rate of FFA production in fasted and in glucose-refed rats. We have found that the rate of irreversible disposal of FFA is directly proportional to the plasma FFA concentration is unanesthetized, glucose-refed rats throughout the time that plasma FFA is rapidly decreasing (6).

METHODS

75 Sprague–Dawley male rats (Charles River Breeding Laboratories, approximately 210 g) were used. They were fasted for 20–24 hr. 25 rats were studied in the fasted state; 50 were refed 2 ml of 50% glucose in water by gastric intubation. All animals were unanesthetized. The experimental protocol was as follows: 25 fasted rats were injected intravenously with tracer (palmitate-^{14}C and albumin-^{131}I, see below for preparation). 5 rats were decapitated at each of the following times after injection: 0.5, 1, 2, 5, and 10 min. 25 glucose-fed rats were injected with tracer at 10–15 min after glucose ingestion and another 25 at 30–35 min after ingestion. They were then decapitated in groups of 5 at 0.5, 1, 2, 5, and 10 min after tracer injection. Time intervals after glucose feeding were chosen on the basis of a previous study (6) in which it was shown that plasma FFA levels decreased to half the control levels by 30 min after glucose ingestion and stayed constant thereafter. Time intervals for the tracer studies were based upon an earlier study of FFA turnover in fasted rats (3). Blood was collected in plastic heparinized tubes.

Plasma was separated and analyzed for ^{131}I, plasma FFA (7), and plasma FFA-^{14}C. The latter measurements were made on isopropanol–isooctane extracts of plasma. The method included deproteinization of plasma and splitting of the FFA–albumin complex in acidified isopropanol. FFA were extracted with isooctane. The lower aqueous isopropanol phase was used for the assay of albumin-^{131}I.

One portion of the upper phase was taken to dryness in a counting vial and then assayed for FFA-^{14}C by liquid scintillation counting. Another portion of the isopropanol–isooctane phase was titrated to yield the FFA content (7). ^{131}I was counted in a gamma scintillation counter. Aliquots of plasma were counted both directly (5 and 10 min after tracer injection) and after extraction of FFA with acidified isopropanol–isooctane (all samples). Insufficient plasma was available for direct ^{131}I assays at early times since the bleeding period was limited to 20 sec. Longer bleeding times were allowed when animals were killed at 5 and 10 min after injection. Plasma volumes were about 10% higher in the samples counted directly. No ^{131}I was found in the lipid extracts.

The radioactive dose was prepared as follows: 0.01 mmole of palmitic acid-1-^{14}C (New England Nuclear Corp., lot No. 31-200A-15; specific activity, 10 mc/mmole) was dissolved in 0.5 ml of 95% ethanol. Radiochemical purity of the labeled fatty acid was established by thin-layer chromatography. 0.04 ml of 2% KOH in ethanol was added and the solution warmed to 40°C. About 41 ml of rat serum was added to form a complex with the labeled potassium palmitate. 3 ml (approximately 45 μc) of dialyzed serum albumin ^{131}I dissolved in physiological saline (human; Abbott Laboratories, North Chicago, Ill.) was then added. 0.50 ml of the dose was injected into a lateral tail vein of each rat. The animals were wrapped in towels to restrain them during the injection. Separate experiments established that the small amount of ethanol included in the dose does not influence the rate of FFA turnover in rats.

RESULTS

Plasma FFA Pool Size

Assuming that the plasma FFA is distributed in the same volume as albumin-^{131}I, the plasma FFA pool size (Q_1) may be calculated as follows:

$$Q_1 = [\text{FFA}] \times PV \times (\text{bw} \div 100)$$

where bw = body weight (g), [FFA] = plasma FFA concentration (μeq/ml), and PV = plasma volume as % (v/w) of body weight. PV was calculated from Fig. 1 as follows:

$$PV = [\text{radioactivity injected (albumin-}^{131}\text{I)}]/(\text{radioactivity/ml of plasma at } t = 0)$$

$$= 2.6 \times 10^5/6.6 \times 10^4 = 4.0\% \text{ of body weight.}$$

The concentration of plasma FFA after the gastric administration of glucose in previously fasted rats is shown in Fig. 2. In confirmation of an earlier study (6), average plasma FFA levels decreased to half the initial values 30 min after glucose feeding. Although a linear decrease is

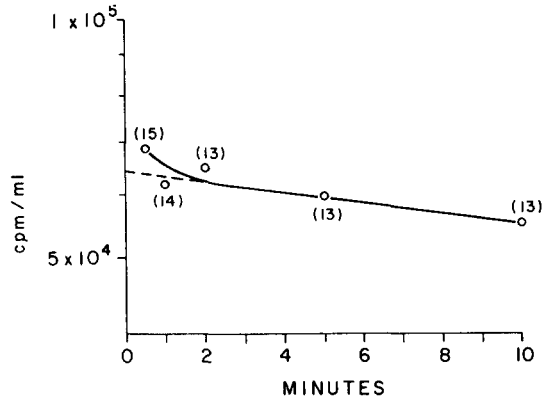

FIG. 1. Disappearance of albumin-^{131}I from plasma of fasted and glucose-refed rats. Data were grouped together because no effect of glucose feeding on ^{131}I concentrations was observed. The zero-time intercept gives 6.6×10^4 cpm/ml (corrected to 100 g body weight) for initial concentration of radioactivity in plasma. The number of animals in each group is indicated. The injected dose was 2.63×10^5 cpm.

shown in Fig. 2, the change in pool size may also be described by the following functions which take into account the observation that no statistically significant decrease in plasma FFA concentration was observed 10 min after glucose feeding, and the previously published finding (6) that after 40 min the plasma FFA concentration levels off:

$$[\text{FFA}(t)] = 0.73 \text{ when } 0 < t \leqslant 10 \text{ min};$$
$$[\text{FFA}(t)] = 0.73 - 0.013\,(t - 10) \text{ when } 10 \leqslant t \leqslant 40;$$
and
$$[\text{FFA}(t)] = 0.33 \text{ when } t \leqslant 40.$$

The total plasma FFA pool size Q_1 (t) of a fasted 210 g rat $= 210 \times 0.040 \times 0.73 = 6.1$ μeq, whereas 30 min after glucose refeeding, $Q_1 = 2.8$ μeq.

Plasma FFA-^{14}C

The mean percentage of the injected FFA-^{14}C per ml of plasma in each of the three groups of rats is shown in Fig. 3. Each set of data was fitted to the sum of two exponentials and the value for $\Sigma\, H_i/g_i$ calculated (see Appendix).[1] The values for this sum were: fasted, 0.80; fasted, glucose-refed (18 min), 0.82; and fasted, glucose-refed (36 min), 0.82.

DISCUSSION

In the Appendix to this paper formal proof is presented that a simple equation may be used to calculate the irreversible disposal rate—the net outflow transport (2)—of a circulating metabolite. The simple equation is independent of the number of compartments in a model[1] or how they are arranged (with one important exception, discussed below). The experiments described demonstrate how this theoretical approach may be used to study lipid metabolism in unanesthetized animals.

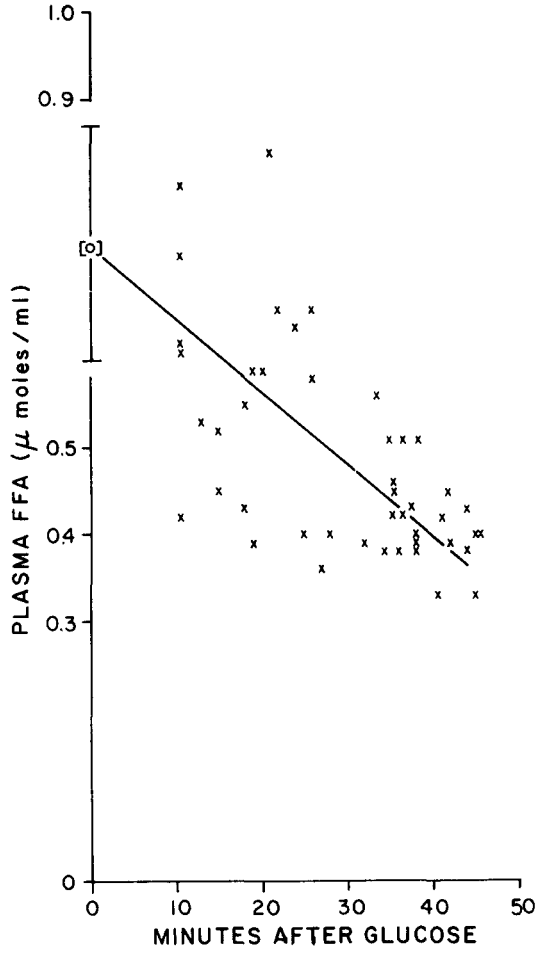

FIG. 2: Plasma FFA concentration in fasted and glucose-refed rats. The zero-time (fasted) value = 0.73 ± 0.14 μeq/ml (mean ± SD; n = 25). Individual values for glucose-refed rats are shown.

The example we have chosen is that of FFA transport in fasted and in glucose-refed rats. The plasma FFA

[1] The curves were each considered to be the sum of two exponentials, one of which was defined by the last two points of the curve. The latter exponential function was extrapolated to zero time and subtracted from the observed curve. This gave the second exponential function.

As shown in the Appendix, the theoretical basis for calculating irreversible disposal rates is relatively independent of the model; however, in practice, it is quite likely that an experimental curve will not be completely or accurately defined. If an experiment is terminated before all the late components of a complex exponential function have been defined, the irreversible disposal rate, as calculated here, will be in error. In the present case, the experimental data have been treated as the sum of two exponentials; therefore, we have, in a sense, assumed a 2-pool model. In fact, had the present experiment been of longer duration, additional components probably would have been observed. To the extent that the additional H_i/g_i influence $\Sigma H_i/g_i$, the original calculation would be in error. Thus, while the theoretical basis of our calculation is independent of model, the practical application of the theory has serious limitations that should be recognized.

concentration is relatively constant and elevated in the fasted state. It falls to a new steady-state level 30 min after a single glucose feeding (6). Considerable evidence, obtained both in vivo and in vitro, indicates (6, 8–10) that the fall in plasma FFA levels is due to a decreased rate of FFA transport into the circulation. However, Stoner and Mathews (11) have emphasized that the in vitro evidence supporting this view is weak and that simple physiological methods are needed to study such phenomena. The single injection technique provides such a method.

Our experimental data define both the plasma FFA compartment size (Q_1) as a function of time and the disappearance of tracer FFA-^{14}C at two steady-state levels (fasted and 36 min after glucose refeeding) and during the nonsteady state when Q_1 was falling rapidly. From these data, the irreversible disposal rate, R, can be calculated by equation 2. The calculation is based upon the following assumptions. (*a*) All newly formed plasma FFA enters the circulation directly. (*b*) All (fractional) turnover rates remain constant throughout the isotopic experiments. (*c*) The tracer reflects the kinetic behavior of all plasma FFA. (*d*) The total titratable acid extracted by isooctane is FFA. (*e*) Estimation of plasma volume using albumin-^{131}I is valid. No additional assumptions need be made with respect to the number or sizes of FFA and non-FFA compartments which may interchange fatty acid moieties, and no model need be drawn in order to calculate R (μeq of FFA per min).[1]

Hence, the irreversible disposal rate in unanesthetized, fasted rats: $R = Q_1/0.80$. After a glucose load the value for $\Sigma H_i/g_i$ remains constant (0.80) even though Q_1 falls rapidly. Therefore we may conclude that under these experimental conditions the irreversible disposal rate is directly proportional to the plasma FFA compartment size: $R = 1.25\ Q_1$. A similar conclusion had been reached by Armstrong et al. (8), who used the constant infusion technique to study FFA turnover in unanesthetized dogs under a variety of experimental conditions; of course, the constants of proportionality in dogs and rats are different.

The values for R in the two steady-state periods (fasted and 35 min after glucose refeeding) were, respectively, 7.6 (1.25 × 6.1) μeq/min and 3.5 (1.25 × 2.8) μeq/min. Between 10 and 35 min after glucose refeeding R is a function of time; at 20 min, for example, when $Q_1 = 5.0$ μeq, $R = 6.3$ μeq/min.

In the steady-state conditions, the *net* rate of plasma FFA formation must have equaled the irreversible disposal rate. Therefore, a single oral load of glucose lowered the net rate of plasma FFA production to 46% (3.5/7.6) of the fasted rate. However, during the nonsteady state period when the plasma FFA levels were decreasing,

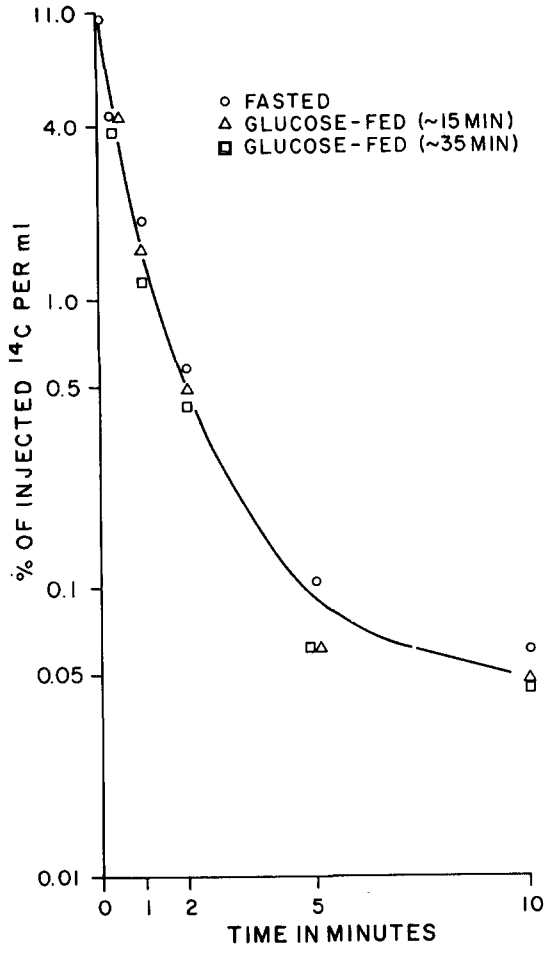

FIG. 3. Disappearance of palmitate-^{14}C (complexed to rat serum) from the circulation of fasted and glucose-refed rats. The ordinate shows the radioactivity per ml of plasma remaining at any time after the injection, which took place either 15 or 35 min after glucose refeeding. 25 rats were in each of the three groups.

the *net* rate of plasma FFA formation must have been less than the net outflow transport of FFA from plasma. The net inflow transport of plasma FFA cannot be calculated in the nonsteady state unless additional information regarding the extraplasma FFA compartments (3) is available. If no extraplasma FFA were to exchange with plasma FFA, then one may estimate from the following relationship that the net inward transport of plasma FFA would have to be only about 0.16 μeq/min less than the rate of irreversible disposal during the period $t = 10$ to $t = 35$ min in order to account for the observed fall in plasma FFA concentration:

$$Q_1(t_1) - Q_1(t_2) = \int_{t_1}^{t_2} R(t)\,dt - \int_{t_1}^{t_2} I(t)dt$$

where R = net outflow transport and I = net inflow transport of plasma FFA. However, in the n-pool model, in which all newly formed plasma FFA enters plasma directly and pools 1 to m (for meaning of m see Appendix)

contain FFA, a correction factor must be applied to the above equation as follows:

$$\left(1 + \sum_{i=2}^{m} k_i\right)[Q_1(t_1) - Q_1(t_2)] = \int_{t_1}^{t_2} R(t)dt - \int_{t_1}^{t_2} I(t)dt$$

where k_i = the ratios of extraplasma FFA pool sizes to plasma FFA pool size (Q_i/Q_1). This equation holds true only if the ratios k_i remain constant. If we assume that the ratios k_i were constant in our experiment even though the pool sizes were varying, we may make some estimates. First, if the total exchangeable FFA pool size were twice as large as the plasma FFA pool, then the net inflow transport of plasma FFA would be about 0.32 μeq/min (instead of 0.16 μeq/min, see above) less than the irreversible disposal rate; and if the (total exchangeable FFA): (plasma FFA) = 10, the net inflow transport, $I(t)$, would be about 1.6 μeq/min less than the net outflow transport $R(t)$ during t = 10–35 min. 20 min after glucose feeding the net inflow rate would have decreased from 7.6 μeq/min to 6.1, 6.0, or 4.7 μeq/min depending upon whether the (total exchangeable FFA):(plasma FFA) were 1.0, 2.0, or 10.

We began this discussion by stating that a simple equation may be used to study the net outward transport of a circulating metabolite and that this approach does not require the formation of a model, with one important exception. All newly formed metabolite must enter the circulation directly, without prior dilution in an extraplasma pool. Furthermore, we tried to show that when one proceeds from the study of irreversible disposal rate to the measurement of net inflow transport in the non-steady state, it becomes necessary to know the amount of the exchangeable metabolite in extraplasma compartments as well as the compartment size in plasma. These exceptions to the simple approach deserve special emphasis, for they have not been discussed by other investigators who have used similar expressions to calculate irreversible disposal rate (1, 4, 5). The reader should also be aware of the fact that general equations were derived in the Appendix (equations 13–16) which do not require assumptions regarding the sites of net inflow of nonradioactive substance S, the circulating metabolite. However, if these general equations are used without any added restrictions, then the relationship between irreversible disposal rate Q_1 and the area ($\Sigma H_i/g_i$) under the radioactivity–time curve, $q_1(t)$, no longer holds true as a general rule.

Nevertheless, the technique as we have applied it provides a great deal of information regarding metabolic processes in vivo. Our data show that the irreversible disposal rate is directly proportional to the compartment size in unanesthetized rats; that a single feeding of glucose lowers the irreversible disposal rate to half the fasting level within 30 min; that a very slight imbalance between the rates of net inflow and outflow transport could account for the rapid fall in plasma FFA concentration which resulted from a single glucose feeding; and that the fall in plasma FFA level occurs as a result of the inhibition of the net inflow transport of FFA.

We wish to thank Mrs. Arlene Garfinkel and Mr. Robert J. Huebotter for their excellent experimental assistance.

This study was supported in part by PHS research Grant No. AM 4705 and No. AM 4706 (Dr. M. C. Schotz) from the National Institutes of Health, U.S. Public Health Service.

APPENDIX

(Mathematical derivation by Mr. Hojat Rostami.)

General Calculation of Irreversible Disposal Rate of Circulating Metabolites

The mathematical approach in the above paper on plasma FFA transport is similar to that described in an earlier study by Baker, Shipley, Clark, and Incefy (12) of glucose metabolism in rats. In the glucose study, the term "irreversible disposal rate" was introduced in order to distinguish between total turnover rate [now called inward and outward transport (2)] and net (irreversible) outward transport of glucose in a complex system that involved recycling of the substance that was being studied. The irreversible disposal rate is equal to net outward transport of the substance (formerly called "turnover rate") as measured by the technique of constant infusion of tracer.

Baker et al. reported (12) that, in each of several different models of glucose turnover, the irreversible disposal rate, R, of glucose could be calculated by the following equation:

$$R = Q_a(g_1g_2g_3)/(H_1g_2g_3 + H_2g_1g_3 + H_3g_1g_2) \qquad (1)$$

where R = irreversible disposal rate; Q_a = plasma glucose compartment size; g_i = slopes; and H_i = intercepts of the components comprising the complex activity–time curve for plasma glucose-^{14}C ($= H_1e^{-g_1t} + H_2e^{-g_2t} + H_3e^{-g_3t}$) after a single injection of tracer glucose into the blood stream.

Equivalent equations have been used by several other workers to define the net inward or outward transport of circulating metabolites; relatively simple models were used in each case (3–5, 13).

At the suggestion of Dr. Heath (MRC Toxicology Research Unit, Carshalton, Surrey, England) we have attempted to determine how general this solution is. We shall try to show that no matter how many compartments interchange a substance, S, in plasma, as long as all the newly formed substance S enters the plasma directly (i.e., without first mixing with a large pool of similar material out of the circulation), and as long as (fractional) turnover rates remain constant during an experiment, the irreversible disposal rate, R, is given by equation 2:

$$R = Q_1/\Sigma(H_i/g_i) \qquad (2)$$

which is equivalent to equation 1. Q_1 is the compartment size of substance S in the compartment into which the tracer is injected. We shall also consider the case in which most of substance S enters a large diluent pool of S before it passes into plasma.

General Solution

The model which serves to define the general case, which we shall treat first, is given in Fig. 4. Pools containing substance S are shown giving rise to non-S material by reversible pathways. No limitation to the number of pools of S and non-S materials is implied. Irreversible disposal is defined in the legend to the figure. It may also be defined as follows:

$$R = \sum_{i=1}^{m} \lambda_{oi} Q_i + \sum_{i=m+1}^{m} \lambda_{oi} f_i Q_i \qquad (3)$$

where f_i represents the fraction of non-S which is derived from S by direct and indirect routes, and λ_{oi} is the unidirectional (fractional) turnover rate of the ith compartment out of the system.

Differential Equations and Solution

The differential equations and their solution follow conventional steps. The rate of change of total radioactivity in each compartment with respect to time ($\dot{q}_i$) is given by the following equation:

$$\dot{q}_i(t) = \sum_{\substack{j=1 \\ i \neq j}}^{m} \lambda_{ij}\, q_j\,(t) - \lambda_{ii}\, q_i\,(t)$$

where λ_{ij} is the fractional rate of flow of substance in compartment j into compartment i.

Using matrix notation (14), these flow equations may be written as:

$$[\dot{q}] = -[\lambda]\,[q], \qquad (4)$$

where

$$[\lambda] = \begin{bmatrix} \lambda_{11} & -\lambda_{12} & \dots & -\lambda_{1n} \\ -\lambda_{21} & & \dots & \\ -\lambda_{n1} & & & \lambda_{nn} \end{bmatrix}$$

and

$$[q(t)] = \begin{bmatrix} q_1 \\ \cdot \\ \cdot \\ \cdot \\ q_n \end{bmatrix}$$

Similarly steady-state equations may be written in matrix form as follows:

$$[\lambda]\,[Q] = [I] \qquad (5)$$

where I_i = nonradioactive inflow into compartment i and

$$[Q] = \begin{bmatrix} Q_1 \\ \cdot \\ \cdot \\ \cdot \\ Q_n \end{bmatrix} \text{ and } [I] = \begin{bmatrix} I_1 \\ \cdot \\ \cdot \\ \cdot \\ I_n \end{bmatrix}$$

Using Laplace transforms, equation 4 may be solved (15) to give

$$[q(t)] = [H]\,[e^{-\theta t}] \qquad (6)$$

where

$$[H] = \begin{bmatrix} H_{11} & H_{12} & \dots & H_{1n} \\ \cdot & & & \\ \cdot & & & \\ \cdot & & & \\ H_{n1} & & & H_{nn} \end{bmatrix}$$

and

$$[e^{-\theta t}] = \begin{bmatrix} e^{-\theta_1 t} \\ e^{-\theta_n t} \end{bmatrix}$$

Irreversible Disposal Rate

A general solution for disposal rate may be derived from the flow equation 3 and the steady-state equation 5.

Equation 5 may also be written as follows:

$$\begin{bmatrix} A & B \\ C & D \end{bmatrix} \begin{bmatrix} Q' \\ Q'' \end{bmatrix} = \begin{bmatrix} I' \\ I'' \end{bmatrix} \qquad (7)$$

where each matrix, A, B, C, D, is a submatrix of $[\lambda]$ defined as:

$$A = \begin{bmatrix} \lambda_{11} & -\lambda_{12} & \dots & -\lambda_{1m} \\ \cdot & & & \\ \cdot & & & \\ \cdot & & & \\ -\lambda_{m1} & & & \lambda_{mm} \end{bmatrix} \qquad m < n$$

$$B = \begin{bmatrix} -\lambda_{1,m+1} & \dots & -\lambda_{1,n} \\ \cdot & & \\ \cdot & & \\ \cdot & & \\ -\lambda_{m,m+1} & & -\lambda_{m,n} \end{bmatrix} \qquad (8)$$

$$C = \begin{bmatrix} -\lambda_{m+1,1} & \dots & -\lambda_{m+1,m} \\ \cdot & & \\ \cdot & & \\ \cdot & & \\ -\lambda_{n,1} & & \lambda_{n,m} \end{bmatrix}$$

$$D = \begin{bmatrix} \lambda_{m+1,m+1} & \dots & -\lambda_{m+1,n} \\ \cdot & & \\ \cdot & & \\ \cdot & & \\ -\lambda_{n,m+1} & & \lambda_{nn} \end{bmatrix}$$

and where

$$[Q'] = \begin{bmatrix} Q_1 \\ \cdot \\ \cdot \\ \cdot \\ Q_m \end{bmatrix} \qquad [Q''] = \begin{bmatrix} Q_{m+1} \\ \cdot \\ \cdot \\ \cdot \\ Q_n \end{bmatrix} \qquad (9)$$

and

$$[I'] = \begin{bmatrix} I_1 \\ \cdot \\ \cdot \\ \cdot \\ I_m \end{bmatrix} \qquad [I''] = \begin{bmatrix} I_{m+1} \\ \cdot \\ \cdot \\ \cdot \\ I_n \end{bmatrix} \qquad (10)$$

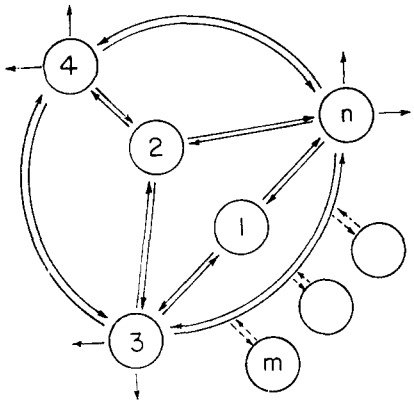

FIG. 4. General n-pool model. Compartments 1 to m, inclusive, contain substance S. Compartments $m + 1$ to n contain non-S substances. Tracer is injected into compartment 1 at zero time. Nonradioactive inflows may enter all compartments; however, they are now shown. Irreversible disposal of substance S equals the net outflow transport of substance S from compartments 1 through m plus that part of the net outflow transport from all non-S compartments which is derived directly or indirectly from substance S.

$[I']$ is the vector of inflows of substance S into compartments 1 through m, and $[I'']$ is the vector of inflows of substances non-S into compartments $m+1$ through n. $[Q']$ is the vector of compartment sizes of substance S, and $[Q'']$ is the vector of compartment sizes of non-S. The steady-state equation 7 will take a new form with these notations:

$$\begin{aligned}[A]\ [Q'] + [B]\ [Q''] &= [I'] \\ [C]\ [Q'] + [D]\ [Q''] &= [I'']\end{aligned} \tag{11}$$

In equation 3 we expressed irreversible disposal rate as a function of pool sizes, unidirectional turnover rates out of the system, and the fraction of non-S that is derived from S. The latter term (f_i) is defined as

$$f_i = \frac{\text{Inflow of substance S into compartment } i}{\text{Total inflow into (or outflow from) compartment } i}.$$

The inflow of substance S into compartment i is composed of two parts: a part which comes directly from compartments containing substance S and which is given by

$$\lambda_{i1}\, Q_1 + \lambda_{i2} Q_2 + \ldots \lambda_{im}\, Q_m;$$

and a part which comes from compartments containing substances non-S, where a fraction, f_j, of non-S substances is derived from substance S. The inflow of substance S in this case is given by

$$\lambda_{i,m+1} f_{m+1}\, Q_{m+1} + \ldots + \lambda_{in} f_n Q_n.$$

Therefore,

$$f_i = [(\lambda_{i1}\, Q_1 + \lambda_{i2}\, Q_2 + \ldots \lambda_{im}\, Q_m) + (\lambda_{i,m+1} f_{m+1}\, Q_{m+1} + \ldots + \lambda_{in} f_n\, Q_n)]/\lambda_{ii}\, Q_i \tag{12}$$

where $i = m + 1, n$.

Using the matrix definitions given previously (equations 8 and 9) the above equations may be rearranged and written as follows:

$$[D]\ [fQ''] = -\ [C]\ [Q'] \tag{13}$$

where

$$[fQ''] = \begin{bmatrix} f_{m+1}\ Q_{m+1} \\ \cdot \\ \cdot \\ \cdot \\ f_n\, Q_n \end{bmatrix}$$

and $f_i Q_i$ is the amount of non-S substance that is derived from substance S in compartment i.

We may now obtain two perfectly general expressions for irreversible disposal rate, R. The first expression (equation 14) relates R to the λ submatrices, A, B, C, and D (see equation 8) and to the pool sizes of all compartments that contain only substance S. The second expression (equation 15) relates R to the λ submatrices and to the inflows of substance S into each compartment that contains S.

$$R = \sum_{i=1}^{m} \sum_{j=1}^{m} ([A] - [B][D]^{-1}[C])_{ij}\, Q_j \tag{14}$$

or, in terms of inflows of substances,

$$R = \sum_{i=1}^{m} ([I'] - [B][D]^{-1}\,[I''])_i \tag{15}$$

The above two equations are obtained simply by solving equation 13 for $[fQ'']$, substituting this expression into equation 3, and by using equations 8 and 9.

If we express the inverse matrix of λ as

$$\lambda^{-1} = \begin{bmatrix} A' & B' \\ C' & D' \end{bmatrix} \text{ where } A', B', C', \text{ and } D'$$

are submatrices with the same dimensions as A, B, C, and D, the submatrix A' is given by

$$A' = (A - BD^{-1}\, C)^{-1}$$

Then the expression for the irreversible disposal rate (equation 14) may be written as follows:

$$R = \sum_{i=1}^{m} \sum_{j=1}^{m} (A'^{-1})_{ij}\, Q_j \tag{16}$$

where $(A'^{-1})_{ij}$ is the ith row and jth column of the $[A']^{-1}$ matrix.

Equation 16 represents the final general solution that we have derived. One may show that the following three relationships follow from equation 16 by expressing $(A'^{-1})_{ij}$ in terms of other known parameters, e.g. slopes and intercepts (15).

I. If all of the newly formed substance S enters compartment 1, and total activity in compartment 1 and $q_1(t)$ are measured, then $m = 1$ and equation 16 may be written as

$$R = (A'^{-1})_{11}\, Q_1.$$

Moreover, using Laplace transform we can show

$$(A'^{-1})_{11} = \frac{1}{(\Delta^{11}/\Delta)} = 1/\Sigma(H_{1i}/g_i)$$

where Δ^{11} is the cofactor of λ_{11} of the λ matrix, and Δ is the determinant of the matrix. Therefore,

$$R = \frac{Q_1}{\Sigma H_{1i}/g_i} = \frac{Q_1}{\text{Area under } q_1(t)} \quad (17)$$

where H_{1i} and g_i are, respectively, the intercepts and the slopes of the total activity curve. The intercepts are subject to the following condition

$$\sum_{i=1}^{n} H_{1i} = 1.$$

and Q_1 is the compartment size for compartment 1.

II. If all of the newly formed substance S enters compartment 1, and specific activity in compartment 1, $a_1(t)$, is measured,

$$R = \frac{1}{\sum_{1}^{n} (H_{1i}'/\infty_i)} \quad (18)$$

where H_{1i}' and g_i are, respectively, the intercepts and slopes of the specific activity curve. The intercepts are subject to the following condition

$$\sum_{i=1}^{n} H_{1i}' = 1/Q_1.$$

III. If some of newly formed substance S enters into compartments 2 to m before entering compartment 1, and total activity in compartment 1, $q_1(t)$ is measured, relationship I (equation 17) is no longer generally true. It becomes necessary to multiply the ratio, $Q_1/[\text{area under } q_1(t)]$, by a factor which is a function of the relative compartment sizes.

Manuscript received 10 June 1968; accepted 15 August 1968.

References

1. Baker, N., R. A. Shipley, R. E. Clark, and G. E. Incefy. 1959. *Amer. J. Physiol.* **196:** 245.
2. Brownell, G. L., M. Berman, and J. S. Robertson. 1968. *Int. J. Isotopes* **19:** 249.
3. Baker, N., and M. C. Schotz. 1967. *J. Lipid Res.* **8:** 646.
4. Shipley, R. A., E. B. Chudzik, A. P. Gibbons, K. Jongedyk, and D. O. Brummond. 1967. *Amer. J. Physiol.* **213:** 1149.
5. Stern, M. P., J. W. Farquhar, A. Silvers, and G. M. Reaven. 1968. *J. Clin. Invest.* **47:** 1947.
6. Baker, N., A. S. Garfinkel, and M. C. Schotz. 1968. *J. Lipid Res.* **9:** 1.
7. Schotz, M. C., G. M. C. Masson, and I. H. Page. 1959. *Proc. Soc. Exp. Biol. Med.* **101:** 159.
8. Armstrong, D. T., R. Steele, N. Altszuler, A. Dunn, J. S. Bishop, and R. C. De Bodo. 1961. *Amer. J. Physiol.* **201:** 9.
9. Fredrickson, D. S., and R. S. Gordon. 1958. *J. Clin. Invest.* **37:** 1504.
10. Laurell, S. 1959. *Acta Physiol. Scand.* **47:** 218.
11. Stoner, H. B., and J. Matthews. 1966. *Quart. J. Exp. Physiol.* **51:** 42.
12. Baker, N., R. A. Shipley, R. E. Clark, and G. E. Incefy. 1959. *Amer. J. Physiol.* **196:** 245.
13. Segal, S., M. Berman, and A. Blair. 1961. *J. Clin. Invest.* **40:** 1263.
14. Sheppard, C. W. 1962. Basic Principles of the Tracer Method. John Wiley & Sons, Inc., New York. 47–70.
15. Berman, M., and R. Schoenfeld. 1956. *J. Appl. Physics.* **27:** 1361.