TRACER METHODS FOR IN VIVO KINETICS

THEORY AND APPLICATIONS

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ACADEMIC PRESS New York and London 1972

ACADEMIC PRESS, INC.
111 Fifth Avenue, New York, New York 10003

United Kingdom Edition published by
ACADEMIC PRESS, INC. (LONDON) LTD.
24/28 Oval Road, London NW1

LIBRARY OF CONGRESS CATALOG CARD NUMBER: 73-187233

PRINTED IN THE UNITED STATES OF AMERICA

PREFACE

The purpose of this book is to bring together for explanation and evaluation the variety of working formulas called upon in applying tracer methods for kinetic studies *in vivo*. Examination reveals that many formulations, in what at first glance may appear a confusing array, in reality rest upon relatively few related basic concepts, even though the intended use may be for such dissimilar purposes as measurement of cardiac output or estimation of rate of production of a hormone. Readers encountering the wide range of formulas expressed in varying terms and symbols may, understandably, fall victim to a sense of confusion bordering on disenchantment or, at the least, be reduced to uneasy acceptance, on faith, of mathematical development and argument. In order to alleviate this confusion we define the basic concepts, derive the pertinent equations, and evaluate each working formula to ensure its proper application within the set of experimental conditions at hand. Included is an appraisal of inherent potential sources of error encountered in the various applications.

In the interest of the biomedical scientist who as often as not may be uncomfortable when confronted with involved algebra, let alone calculus, we have attempted to maintain as simple and informal an approach as possible in deriving equations. Rigorous and complex qualifying generalities with

attending proliferation of symbols have been avoided when the practical purpose—an orderly and straightforward development—is not compromised. We believe that nonmathematicians should welcome such an explanation of concepts supporting various formulations and also appreciate the simple representative numerical examples which are included. Many of the sequences of steps included in the text, whether algebraic or numerical, would ordinarily be omitted from research reports or mathematical treatises; yet we feel certain that many manipulations which are perhaps inelegant and obvious to mathematicians may be helpful to others. In this spirit they are retained here.

Although considerable attention is given stochastic or probabalistic methods as applied to "black box" systems, explicit compartmental models are regularly invoked if for no other reason than to illustrate working formulas in a tangible context and to provide a framework for illustrative arithmetic. We believe that the generous assortment of model systems depicted in the illustrations will assist in the understanding of concepts which may be elusive if sole reliance is placed on abstract mathematical argument.

LIST OF SYMBOLS

A	Area under a curve for a given function from t_1 to t_2, or $t = 0$ to $t = \infty$, e.g., $\int_0^\infty f(t)\,dt$.
a, b, c, d, etc.	Designation of specific pools, usually as subscripts. Pool a ordinarily is the labeled pool and the others are secondary pools. Where double labeling requires that other pools also be labeled, this is noted in the text.
α (alpha)	Specific activity of pooled excreted material collected over a period of time sufficiently long to permit recovery of nearly all tracer destined to leave by this route.
C	A constant (except electric capacitance (Chapter 4)).
c	A general symbol for concentration in water (e.g., blood or plasma) or in tissue. As indicated in the text it may apply to either solute (tracee) or tracer. In case both types of concentration appear in the same formula, that for tracer is designated c', c_a', etc. The symbol c usually appears with a subscript. See the next four entries.
c_a, c_b, etc.	Concentration in pool a, pool b, etc.
c_{a0}	Concentration in pool a at zero time.

c_b^a, c_a^b, etc.	Concentration of tracer in pool b when dose was to pool a; in pool a when dose was to pool b, etc.
c_A, c_V	Concentration of tracer in arterial or in venous blood, respectively.
Cl	Clearance, expressed as volume nominally cleared of solute per unit time. Cl′ is used for clearance of tracer when that for solute appears in the same formula.
D	Total activity in a single dose of tracer.
D^a, D^b	Dose *to* pool a, dose *to* pool b.
Δt (delta t)	A very small interval of time approaching zero duration.
DR	Irreversible disposal rate of a particular species from a system, e.g., DR_a is disposal rate of species a.
E	Subscript denoting equilibrium.
E	Electric charge, Chapter 4.
e	Natural log base.
F	Rate of transfer (flow) of unlabeled material, e.g., in milliliters per minute or milligrams per minute.
F_{ba}, F_{oa}, F_{ao}, etc.	Rate of transfer *to* pool b from a; to the outside of the system from pool a; to pool a from the outside, etc.
f	Rate of blood flow as milliliters per gram of tissue.
$f(t)$	Any mathematical function of the independent variable, t.
g_1, g_2, etc.	Separate exponential slopes comprising a complex exponential curve.
H_1, H_2, etc.	Coefficients (intercepts) of the separate terms of a complex exponential curve from the labeled pool after observed intercepts (I) are normalized in terms of fraction of their total. Example $H_1 = I_1/(I_1 + I_2 + \cdots I_n)$.
$h(t)$	Transport function: Fraction of dose of tracer lost from a system per unit time as a function of time.
I	An intercept of an extrapolated slope of a complex exponential curve. It also is one of the coefficients in the equation for the curve.
i, j	Subscripts of generality, e.g., A_i means *any given* area; F_{ij} means the rate of movement to any pool, i, from any pool, j.
K_1, K_2, etc.	Coefficients (intercepts) of the separate terms of the complex exponential curve for quantity of tracer in pool b when amount is expressed as fraction of dose to labeled pool a.
k	A rate constant of transfer from a pool in terms of fraction of total content moving per unit time.
k_{ba}, k_{bc}, etc.	See F for definition of subscripts.

k_{aa}, k_{bb}, etc.	Sum of all rate constants of output from pool a, pool b, etc.
L_1, L_2, etc.	Coefficients for curve from pool c (as defined for pool b).
ln	Log to the base e.
$\mathscr{L}\{\ \}$	A Laplace transform.
λ (lambda)	Partition coefficient for a gas: quantity per gram of tissue versus quantity per milliliter of blood.
M_1, M_2, etc.	Coefficients for a curve from pool d (as defined for pool b).
m	Mass (weight) of tissue.
N, or n	Any integer such as the nth member of a series, or the number in a series.
0	Subscript denoting zero time, e.g., t_0 ; q_{a0} is quantity of tracer in pool a at zero time.
o	Outside the system, e.g., F_{ao} is rate of transfer of tracee to pool a from the outside.
P	Quantity of tracer in a priming dose preceding constant infusion.
PR	Production rate of a specific species, e.g., PR_a is production rate of species a (new to the system).
p	A "dummy variable" in a Laplace transform.
q	Quantity of tracer, e.g., counts per minute in a pool or specified space denoted by subscript.
q_a, q_b, etc.	Quantity of tracer in pool a, pool b, etc.
q_{a0}	Quantity of tracer present in pool a at zero time. (Numerically the same as D introduced to pool a.)
$q_b{}^a$, $q_a{}^b$, etc.	Quantity of tracer in pool b when a single dose was introduced to pool a; quantity of tracer in pool a when dose was to pool b, etc.
Q	Quantity of unlabeled material (tracee) in a pool or space, e.g., Q_a is quantity in pool a.
R	Reading of a radiation detector. (Also electrical resistance, Chapter 4).
r	Rate of movement (or infusion) of tracer as units per unit time.
$S(t)$	Probability function of arrival time of tracer.
SA	Specific activity as units of tracer per unit weight of natural atoms of the same species.
SA_a	SA of pool a, etc.
T	A specific interval of time.
$T_{1/2}$	Half time. Time required for q or SA to decline by half when the curve is of simple exponential type.

T_{mean}	Mean time, e.g., mean time for loss of tracer.
t	Time, as an independent variable; a specific point in time.
(t)	A "function of time," e.g., $q_a(t)$ is amount of radioactivity in pool a (dependent variable) as a function of time (independent variable). (Frequently omitted when a variable is obviously a function of time.)
t_0	Zero time.
t_{max}	Point in time where a curve is at maximum height.
τ (tau)	A time interval on a special subscale in the convolution integral (Chapter 12).
U	A complex denominator of specified constants.
V	Units of liquid volume.
ω (omega)	Subscript (e.g., t_ω, q_ω denoting a value at a point in time terminating an interval during which observations are made).
x, y, z	Variables as defined when used.
∞	At infinite time.

Operator Signs

$\approx$	Approximately equal to.
$\sum$	Summation of.
xy or $(x)(y)$ or $[x][y]$ or $x \cdot y$	x multiplied by y, except that (t) is always "as a function of time" and $(t - \tau)$ is "as a function of $t - \tau$."
!	Factorial. For example 3! is $3 \cdot 2 \cdot 1$, and 4! is $4 \cdot 3 \cdot 2 \cdot 1$.

TRACER METHODS FOR IN VIVO KINETICS

CONTENTS

CHAPTER 1

COMPARTMENT ANALYSIS: A SINGLE POOL, REAL OR BY LUMPING

Compartments or Pools

1. Tracers such as radioactive atoms are assumed to behave chemically and physiologically exactly like their natural counterpart atoms save for slight effects of difference in mass. Because the dose can be very small in terms of the number of existing natural atoms, the added material does not perturb the system under observation. Tracers have several potential uses in the intact animal. One of these is to study pathways of chemical conversion by identifying tracer in product after introduction into a precursor. If such a pathway is already known, tracer may serve to assess *rate* of conversion. The animal body may be viewed as an assortment of pools or compartments each made up of identical molecules which tend, more or less, to be enclosed by anatomic boundaries. For example, glucose resides for the most part in extracellular fluid. Body pools tend to remain constant in size while undergoing replacement by input equal to output. This dynamic equilibrium is known as *steady state*. Such a state will be assumed for all analyses presented in this book unless otherwise noted. (See Chapter 10 for nonsteady state and more explicit definitions.) *Compartment analysis* is based on the assumption that specific pools can be identified and that discharge of tracer therefrom

can be described by exponential equations. Tracer can be delivered to a pool system as a single, abruptly administered dose, or delivered over an extended period as by continuous infusion at a constant rate. For compartment analysis the single dose technique is the most useful. Except in Chapter 9 and where otherwise noted the analytic approach will be that for a single dose.

2. In addition to measuring rates of chemical transfer or of physical transport such as blood flow or molecular diffusion, compartment analysis also is concerned with assessment of *pool size*, i.e., the mass of natural material (or volume where appropriate) which constitutes the pool. A very important concept is that of rate of *fractional loss* from a pool. The fraction of tracer lost per unit time is known as a fractional rate constant or *rate constant*. If glucose is assumed to constitute a body pool and this pool is labeled with ^{14}C-glucose given as a single dose intravenously, then a curve of specific activity (SA) as units of radioactivity per milligram of glucose carbon is plotted against time, how can this SA curve of declining activity be used to measure the rate constant of glucose loss or the *rate* of loss (and replacement) as milligrams per minute? What is the weight of glucose carbon in the pool? In the sections which follow, these questions will be considered for a *pure* single pool of homogeneous atoms. A pure single pool means that a specifically defined compartment has no side connections to other pools which participate in interchange of tracer with the pool under observation. The truth is that no such pool exists in the animal body, although a compromise sometimes will permit this assumption in a specific instance. In any case, single-pool kinetics must be understood before more complex systems can be examined.

Single-Pool Kinetics

Rate versus Rate Constant

Natural (tracee) atoms and rate constant

3. A pool of fluid (Figure 1A) will serve as the first illustration. It has a fixed volume (V) of 100 ml and inflow–outflow rate (F) of 50 ml/min. By definition, *rate* is units of volume moving per unit time, but the kinetic behavior of the system also may be assessed in another sense. What *fraction* of the content of the pool is being replaced per unit time? In one minute this obviously is 50 ml/100 ml or 50%/min. Such fractional loss is known as a *rate constant*. The rate constant is 0.5 (or more explicitly 0.5/min or 0.5 min^{-1}). It will be assigned the symbol k. Thus in a pool of liquid undergoing volume flow,

$$k = F/V \tag{1a}$$

Figure 1B is strictly comparable to A save that it represents mass rather than

volume. Thus size (Q) is in mass units (here milligrams), and F now represents input–output rate in milligrams per minute. The rate constant now is fraction of 100 mg removed (and replaced) per minute,

$$k = F/Q \tag{1b}$$

If multiple exits should exist (Figure 1C) each will have a separate k value, and the rate constant for the pool as a whole will be the *sum of all rate constants*.

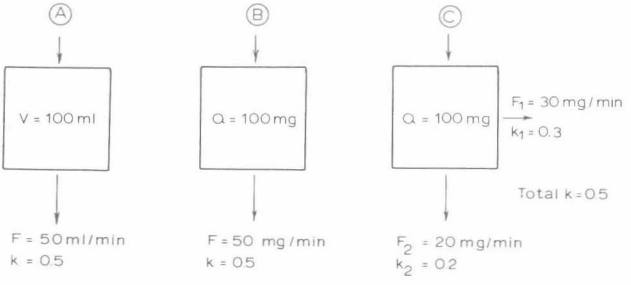

FIG. 1. Single pool systems wherein F is input–output rate, and k is the output rate constant. Model A is for liquid comprising volume, V, and B and C are for mass comprising weight, Q.

Tracer atoms and rate constant

4. One prime purpose of the tracer method is to calculate a flow rate when it cannot be measured directly. If the rate constant is known, this rate is calculable via a rearrangement of Eqs. (1a) and (1b). For mass,

$$F = kQ \tag{2a}$$

or, if size is to be calculated,

$$Q = F/k \tag{2b}$$

Consider that the purpose of adding tracer to the pool is to determine k. Tracer is assumed to mix with tracee almost instantaneously and remain continually mixed. Consequently, probability dictates that during any given time the chance of loss of a tracer atom at the site of output is the same as that for an unlabeled (tracee) atom. Therefore one may predict that loss of tracer should be 50%/min, and that the rate constant for tracer should be 0.5, as was the case for tracee atoms. But, as an experiment, add 1000 units of tracer to the pool. The initial concentration is 10 units per milliliter. At one minute the content of tracer will be 600 and its concentration 6. This is a 40% decline per minute rather than the 50% as predicted for accompanying tracee by Eq. (1a). The discrepancy arises because in one instance the fraction lost is the

ratio of amount lost to a *fixed* amount of tracee during one minute, whereas for tracer the reference amount progressively declines. The true value for fraction lost per unit time can be approached arithmetically by making the time so short that the reference value undergoes minimal change. A general expression for estimation of such fractional rate of loss is

$$\text{estimate of } k \approx \frac{(q_0 - q)/q_0}{t} \tag{3}$$

The symbol q_0 is starting amount of tracer in the pool at zero time, and q is the amount observed later at time t. Direct measurement would give the following for expression (3):

t	q	k
0	1000	—
1	607	0.39
0.5	778	0.45
0.2	905	0.48
0.1	951	0.49

The approached limit of 0.5 is predicted directly by the calculus of Appendix I, which leads to the following equation for the time curve for quantity:†

$$q = q_0 e^{-kt} \qquad \text{or} \qquad q = De^{-kt} \tag{4a}$$

At the beginning the amount in the pool (q_0) is the whole dose (D). The symbol e is the base for natural logarithms. Equation (4a) is converted to one for *concentration* (c) simply by dividing by the constant volume (V) to give units of tracer per milliliter,

$$\frac{q}{V} = \frac{q_0}{V} e^{-kt} \qquad \text{or} \qquad c = c_0 e^{-kt} \tag{4b}$$

Likewise, if pool units are for mass, a division by weight of contained material (Q) converts total units present to units per milligram, i.e., specific activity (SA):

$$\frac{q}{Q} = \frac{q_0}{Q} e^{-kt} \qquad \text{or} \qquad \text{SA} = \text{SA}_0 e^{-kt} \tag{4c}$$

† A more formal notation would be

$$q(t) = q_0 e^{-kt}$$

The parenthetic t means "as a function of time." To keep clutter to a minimum, it will be omitted in this chapter when t is obviously the independent variable.