Tracer Kinetics in Biomedical Research

From Data to Model

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Chapter 2

FUNDAMENTALS OF TRACER KINETICS

2.1 INTRODUCTION

As defined in Chapter 1, the kinetics of a substance in a biological system are its spatial and temporal distribution in that system. The kinetics are the result of several complex events including circulatory dynamics, transport into cells, and utilization. Utilization usually requires biochemical transformations which are characteristics of the substance. The substance can be an element such as calcium or zinc, or a compound such as amino acids, proteins or sugars. All exist normally in the body, and can be of endogenous or exogenous sources, or both. The primary goal of the kinetic events characterizing the metabolism of a substance is to maintain specific levels of the substance in the various components of its systems. The maintenance of these levels is achieved by internal control mechanisms, and involves input into the system to balance the loss which occurs through utilization and excretion.

One wishes to understand the kinetics of a substance under normal circumstances in order to better understand pathophysiological conditions since these may be a result of abnormal kinetics. A fundamental problem in biology and medicine, therefore, is to describe quantitatively the kinetics of substances existing in the body. Among the tools that are available, tracers have been extensively used. Tracers are substances introduced externally into the system to provide data from which quantitative estimates of events characterizing the kinetics of the substance can be made. Tracers can be substances such as dyes or, as described in more detail below, substances labeled with radioactive or stable isotopes.

In this text, the focus will be on characterizing the kinetics of substances already present in the body by using isotopic tracers as probes.

A naturally occurring substance is called a **tracee**. The tracers will be assumed to be ideal where an **ideal tracer** is a substance with the following characteristics:

a. it is detectable by an observer,

b. its introduction into a system does not perturb the system being studied, and

c. it is indistinguishable with respect to the properties of the tracee system being studied.

The first requirement, that of detectability, means that there must be some method by which the amount of tracer in a sample can be quantified. The second requirement means that the introduction of a tracer into the system has no effect on the ongoing metabolic processes which characterize the system under study. This requirement is usually met by introducing an extremely small amount of tracer compared with the amount of tracee already existing, and arguing this small perturbation does not disturb the system. The third requirement means that the system being studied is not able to distinguish between the tracer and tracee, i.e. both follow the same processes with equal probabilities. These requirements are usually met, but the investigator should be aware that problems associated with them can arise.

By definition, the tracer has its own kinetics. The goal of a tracer kinetic study is to infer from the tracer kinetics information on the tracee kinetics. If the three requirements are met, this goal can be attained.

2.2 THE TRACER-TRACEE SYSTEM

2.2.1 Concepts and Definitions

A convenient scheme to illustrate the kinetics of a substance is shown in Figure 2.2.1. In this figure, the circles represent the masses of two interacting substances in specific forms at specific locations, and the arrows represent the transport or flux of material and/or biochemical transformations. This figure shows two specific substances, A and B, to make the point that kinetics includes both transport between different locations, and biochemical transformation. The goal of the tracer study is to determine the masses and fluxes, i.e. transport and biochemical transformation, in this system.

A fundamental assumption in using tracers is that there is at least one component in the system under study which is accessible for tracer administration, and tracer and tracee sampling. This special component is called the **accessible pool**. Examples of accessible pools are a sub-

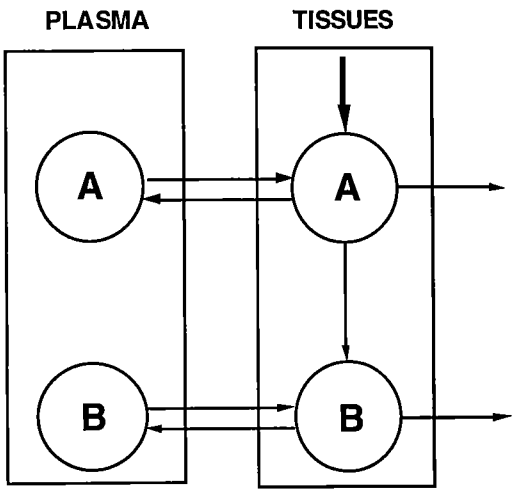

Figure 2.2.1. A schematic of the kinetics of a substance. The circles represent masses and the arrows the fluxes of the substance. The bold arrow into circle A in the tissue represents de novo synthesis. From this pool, it can (i) be irreversibly removed, (ii) exchange with a plasma pool, or (iii) be transformed into form B. In turn, B can exchange with a plasma pool, or irreversibly removed.

stance in physiological spaces such as plasma or a tissue, or a substance in expired air.

Suppose in the system shown in Figure 2.2.1, the plasma component for A is accessible. This means measurements of A can be obtained from plasma. One can redraw this system to emphasize the accessibility of this component for tracee measurement; this is shown in Figure 2.2.2. Notice that while B also exists in plasma, it may not be possible to sample and measure it. Thus plasma B is not accessible, even though it is in plasma. If B could be measured, then this system would have two accessible pools, one for A and one for B. This simple observation will have profound consequences when multiple input-multiple output experimental designs are discussed later.

Suppose the kinetics of the tracee substance described in Figure 2.2.2 is to be studied. The characterization of the system by identifying the components and interconnections, and the availability of at least one accessible pool, set the stage for using a tracer to characterize these

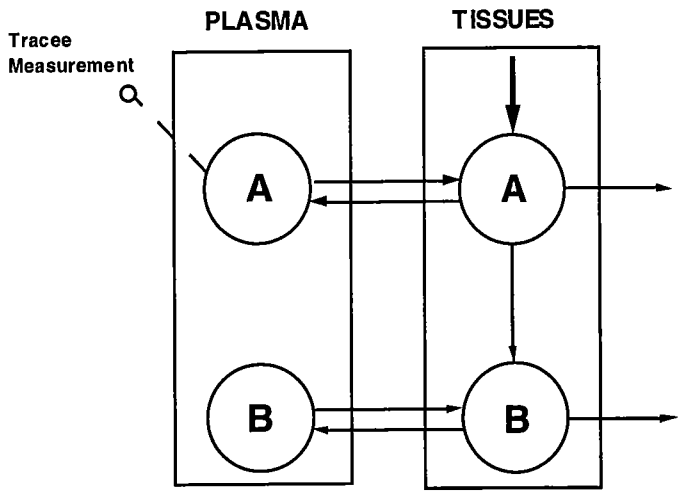

Figure 2.2.2. The system depicted in Figure 2.2.1 with an accessible pool identified and highlighted by the dotted line with the bullet which indicates tracee measurement. The bold arrow into tissue pool A represents de novo entry of material into the system.

kinetics. By appealing to the definition of an ideal tracer, one can assume that the system described in Figure 2.2.2 for the tracee is the same as that for the tracer. Therefore, superimposing the tracer system on that shown in Figure 2.2.2, one has the system shown in Figure 2.2.3.

These two figures emphasize that the two systems for the tracee and tracer are structurally identical, and demonstrate the need for an accessible pool into which tracer can be introduced and from which measurements of tracer and tracee can be made. The main difference between the two is in the inputs. In the tracee system shown in Figure 2.2.2, the input is endogenous into a nonaccessible component of the system. In the tracer system shown above, the input is exogenous, and is into the accessible pool.

Using these figures as representative of tracee and tracer systems, the following will be discussed: (i) the tracee system, (ii) the tracer experiment and the tracer system, (iii) the relationship between the tracee and tracer systems, and (iv) the quantitation of the tracee system from the tracer data. Following a general discussion, the notions will then be applied to radioactive and stable isotopic tracers where, to pass from

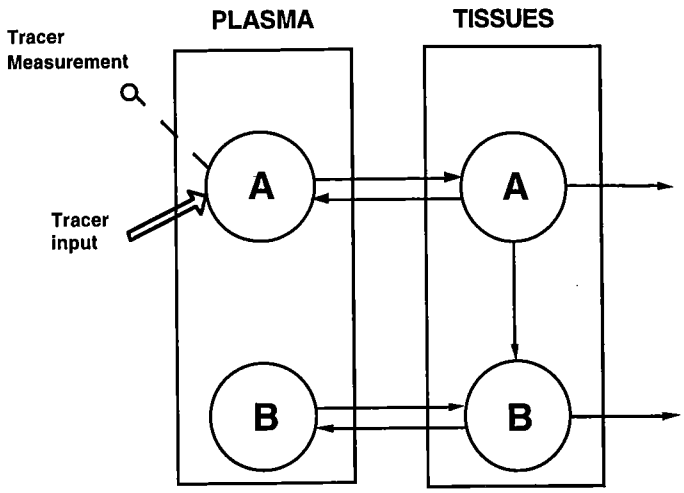

Figure 2.2.3. The tracer system which corresponds to the tracee system depicted in Figure 2.2.2. The administration of the tracer into and sampling from the accessible pool is indicated by the tracer input arrow and tracer measurement sample respectively. The figure implies that once in the system, the tracer is assumed to follow the same pathways the tracee follows.

theory to practice, the measurement of the tracer will be discussed in detail. This strategy will serve to emphasize similarities and differences between using radioactive and stable isotopic tracers, and will form the basis for the analysis of the tracer data with the concomitant inferences about the metabolism of the tracee.

In this Chapter, only the single pool steady-state system will be discussed as a vehicle to introduce the necessary terminology. The precise analyses and the extension to multipool systems will be discussed in subsequent chapters.

2.2.2 The Tracee System

The tracee system to be discussed in this section is given in Figure 2.2.4. The system described in Figure 2.2.4 is a single pool system which is accessible for measurement and in which it is further assumed that the tracee is uniformly distributed. The accessible pool and the system coincide in this particular situation.

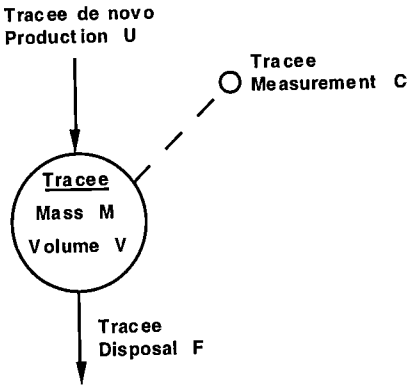

Figure 2.2.4. The tracee system, depicted as a circle, consists of a single pool of volume V containing tracee mass M. Tracee de novo production, U, and disposal F occurs from this pool; they are indicated by the arrows into and leaving the system respectively. The dotted line with the bullet indicates tracee measurement. The symbols given in this figure are summarized in Table 2.2.1

The notation introduced in Figure 2.2.4 which will be used for the tracee system is given in Table 2.2.1. U is sometimes called de novo synthesis, and F utilization, elimination or excretion. Concentration C is defined below in (2.2.3).

Table 2.2.1. Notation for tracee variables

Symbol	*Definition and Units*
V	volume
M	mass
C	concentration (mass/volume)
U	de novo production (mass/time)
F	disposal (mass/time)

Assume the tracee system is in the steady-state case. A **steady state** is an experimental situation where de novo production U and disposal F are equal and constant. This means that the tracee mass M remains constant. To formalize this assumption in mathematical terms, one applies the mass balance principal to the tracee system, i.e. at any point in time the rate at which the tracee mass changes is the difference between

de novo production and disposal. Remembering that U and F are equal, the desired formalism can be expressed in the following equation:

$$\frac{dM(t)}{dt} = U - F = 0 \tag{2.2.1}$$

where t denotes time. In other words, as a result of $U = F$, the rate of change of the tracee mass as a function of time, $\frac{dM(t)}{dt}$, is equal to zero. This means $M(t)$ does not change with time, hence

$$M(t) = M = \text{constant} \tag{2.2.2}$$

For the tracee, the measured value is usually concentration C where

$$C = \frac{M}{V} \tag{2.2.3}$$

In the steady state, C, as a result of the balance between U and F, is a constant. However, from a knowledge of C alone, it is not possible to estimate the fluxes U and F; to do this, a tracer must be used.

2.2.3 The Tracer System

The tracer system to be discussed in this section is given in Figure 2.2.5. As in the previous case, this is single pool system which is accessible for measurement and in which the tracer is assumed to distribute uniformly. Because of tracer-tracee indistinguishability, the volume V is equal to the volume of distribution of the tracee. The notation used in this figure is summarized in Table 2.2.2 below. Note in this table, unlike Table 2.2.1, the dependence of some variables such as mass on time t is explicitly noted, i.e. $m(t)$.

Table 2.2.2. Notation for tracer variables

Symbol	*Definition and Units*
V	volume
$m(t)$	mass
$u(t)$	rate of input (mass/time)
$f(t)$	disposal (mass/time)
d	total input (mass)

The analogue for (2.2.1) for the tracer can be written by again appealing to the mass balance principal, i.e. the rate of change of tracer mass is the difference between the rate of tracer input $u(t)$ and tracer disposal $f(t)$:

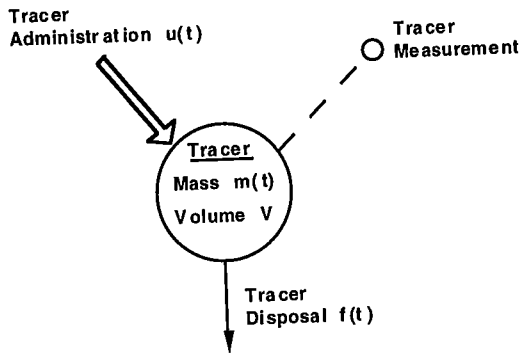

Figure 2.2.5. The tracer system, depicted as a circle, is a single pool of volume V containing tracer mass $m(t)$. Tracer is introduced into the system at a rate $u(t)$; the rate of disposal from this pool is given by $f(t)$. The dotted line with the bullet indicates tracer measurement.

$$\frac{dm(t)}{dt} = u(t) - f(t) \qquad m(0) = 0 \tag{2.2.4}$$

In (2.2.4), $m(0) = 0$ means that when the experiment starts at $t = 0$, there is no tracer mass in the system. (In mathematical terms, $m(0)$ is called the initial condition). In this situation, unlike the previous case where M is constant, $m(t)$ changes with time and hence $\frac{dm(t)}{dt}$ is no longer equal to zero.

While (2.2.4) is written in terms of tracer mass $m(t)$, the manner in which the amount of tracer is actually quantified depends upon the tracer chosen. As discussed in §2.4, the radioactive tracer is usually quantified in terms of tracer concentration $c(t)$, i.e. tracer mass per unit volume:

$$c(t) = \frac{m(t)}{V} \tag{2.2.5}$$

In contrast, the most convenient way to express stable isotope measurements as discussed in §2.5 is the tracer mass per unit tracee mass:

$$z(t) = \frac{m(t)}{M} \tag{2.2.6}$$

Since the volume V is the same for both the tracee and tracer, $z(t)$ also represents the ratio between tracer and tracee concentrations:

$$z(t) = \frac{m(t)}{M} = \frac{c(t)}{C} \tag{2.2.7}$$

2.2.4 The Tracer-Tracee System

The link between the tracer and tracee system comes from the tracer-tracee indistinguishability assumption. This assumption implies that the probability that the tracer leaves the pool is equal to the probability that a particle in the pool is a tracer. This can be written as

$$\frac{f(t)}{F + f(t)} = \frac{m(t)}{M + m(t)} \tag{2.2.8}$$

This equation can be reorganized:

$$\frac{\frac{f(t)}{F}}{1 + \frac{f(t)}{F}} = \frac{\frac{m(t)}{M}}{1 + \frac{m(t)}{M}} \tag{2.2.9}$$

from which one obtains

$$f(t) = \frac{F}{M} m(t) \tag{2.2.10}$$

which, when this expression for $f(t)$ is substituted into (2.2.4), gives

$$\frac{dm(t)}{dt} = u(t) - f(t) = u(t) - \frac{F}{M} m(t) = u(t) - km(t) \tag{2.2.11}$$

where $k = \frac{F}{M}$. This equation is a linear, constant coefficient differential equation which provides the link between the tracer and tracee systems since the tracer parameter k reflects tracee events, $k = \frac{F}{M}$.

2.2.5 System Parameters from Tracer and Tracee Measurements

In the single pool system under consideration, the unknown parameters of interest are F and M. It is the purpose of the tracer experiment to generate the tracer and tracee data from which these parameters can be estimated. One possible method is based on the solution of the tracer model given by (2.2.11). Here $m(t)$ is expressed as a function of the unknown tracer parameter, k, (and thus of the tracee parameters since $k = F/M$) and the known tracer input $u(t)$. For instance, if the tracer

experiment consists of injecting the tracer as a bolus of dose d at time zero, then the solution of (2.2.11) is

$$m(t) = de^{-kt} \tag{2.2.12}$$

Hence the tracer measurement can be related to the model parameters. In particular, if a radioactive tracer is used and its concentration $c(t)$ is measured, then

$$c(t) = \frac{m(t)}{V} = \frac{d}{V}e^{-kt} \tag{2.2.13}$$

where the unknown parameters are the volume V and the exponential k. Both parameters can be estimated from the tracer data: the ratio $\frac{d}{V}$ equals the tracer concentration at time zero whence

$$V = \frac{d}{c(0)} \tag{2.2.14}$$

while k can be estimated from the rate of decay of the tracer. From the estimates of k and V, and knowing the tracee concentration C, the system tracee mass and fluxes can be quantified since, from the definition of C and k,

$$\begin{aligned} M &= C \cdot V \\ U &= F = kM \end{aligned} \tag{2.2.15}$$

The same procedure applies if a stable isotope is used. In this case, the tracer measurement is the tracer to tracee ratio $z(t)$. The counterpart of (2.2.13) become

$$z(t) = \frac{m(t)}{M} = \frac{d}{M}e^{-kt} \tag{2.2.16}$$

Here M plays the role that V played in (2.2.13). The parameters k and M can be estimated from the tracer data as before, whence $U = F = kM$.

The rationale applied above serves as the basis for the compartmental modeling analysis which will be expanded in Chapters 4–6. Alternatively, the flux F can be quantified from the tracer and tracee data by using the noncompartmental analysis approach discussed in Chapter 3. Briefly, the conservation of mass principal applied to the tracer (i.e., the amount of tracer introduced into the system equals the amount leaving the system), can be written

$$d = \int_0^\infty u(t)dt = \int_0^\infty f(t)dt \tag{2.2.17}$$

racer as a bolus of dose d at time

$$e^{-kt} \tag{2.2.12}$$

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since d, the total amount of tracer introduced into the system, is equal to $\int_0^\infty u(t)dt$. Substituting the expression for $f(t)$ given in (2.2.10) into this equation, one obtains

$$d = \int_0^\infty \frac{F}{M}m(t)dt \tag{2.2.18}$$

which, when solved for F, gives

$$F = \frac{d}{\int_0^\infty \frac{m(t)}{M}dt} = U \tag{2.2.19}$$

From (2.2.19), F can be expressed as a function of tracer and tracee measurements. If the tracer is quantitated in terms of the tracer to tracee ratio $z(t)$, it follows immediately from the definition that

$$F = \frac{d}{\int_0^\infty z(t)dt} = U \tag{2.2.20}$$

If the tracer measurement is concentration $c(t)$, then the expression for F as a function of $c(t)$ can be derived from the equality $\frac{m(t)}{M} = \frac{c(t)}{C}$, hence

$$F = \frac{d}{\int_0^\infty \frac{c(t)}{C}dt} = \frac{d \cdot C}{\int_0^\infty c(t)dt} = U \tag{2.2.21}$$