Editorial

Do Tracers Trace?

By PETER REIZENSTEIN

ONLY A FEW YEARS after the discovery of artificial radioisotopes by the Joliot-Curies in 1934, de Hevesy, Chievitz and Ottesen used tracers in hematology. Even today, there are few areas in clinical medicine where as many radioisotopes are used both for investigative and diagnostic purposes as in hematology. Consequently, the methods of tracer studies are often described in the hematologic journals. The methods of interpreting tracer studies are more rarely mentioned, although these methods were described (for nonradioactive drugs) as early as in 1937.[1] The reason for reviewing them here is the experience from some recent symposia, where it proved difficult to analyze tracer kinetics in a short discussion. At a time when clinical experiments have become so sophisticated that the interpretation of results has become as time-consuming and laborious as the obtaining of them, equal attention should be given to both.

The purpose of a clinical tracer study is often to obtain information on the behavior of an unlabeled substance. Whether it is the uptake, the loss, the distribution or the movement from one part of the body to another which is sought, the information needed can usually be expressed as the rate of flow of a substance between pools of different sizes. One studies the behavior of the tracer, hoping to get information on the flow rates of the unlabeled substance. Under certain conditions (chemical identity, undisturbed steady state, etc.) this is possible. Tracers do trace in the sense that there is a mathematically definable relation between the behavior of tracers and that of its unlabeled equivalent. This does not imply, however, that the exponents in the equation describing the tracer curve equal the flow rates.[2-11]

Intuitively, it appears probable that some time after its administration, a minute amount of labeled material will "equilibrate" with the large amount of preexistent, unlabeled material in the body, and such equilibration has often been assumed.[12-24] It is almost customary to assume that "equilibration" has taken place when the curve of radioactivity—studied plasma, tissue, or whole body concentration—has "reached its final level" or "final slope." At that time, it appears natural to calculate the total or "exchangeable" amount of the material under study by "dilution analysis," or to apply the rate of change (i.e., the exponent) last observed for the radioactive material to the flow of the unlabeled equivalent. In this manner some figures for human losses of vitamin B_{12} or of iron or for body stores of these and other materials have been ob-

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https://doi.org/10.1182/blood.V27.5.744.744

tained,[12-24] and it is not surprising that the results "remain controversial" or that other methods were thought to "provide more meaningful data."[25]

To biophysicists it has long been clear that this simplified view is justifiable only in a very limited number of strictly defined metabolic situations. For most substances, metabolism includes several pools. A pool may be defined here, tentatively, as a population of molecules of the material under study, the average rate of outflow of which differs sufficiently from other molecule populations, so that with the methods of study available they can be distinguished from each other. These pools may be anatomical, biochemical or biophysical in nature.

Only for closed systems (no excretion) or single-pool systems is the assumption of isotopic equilibrium—i.e., a uniform specific activity in all pools—justified. This is important to remember, since it means that the tracer excretion rate is never equal to the corresponding rate for the unlabeled material in multiple pool systems. Unfortunately, systems which reach isotopic equilibrium are physiologically rare.

For virtually all other systems the relation between tracer behavior and the behavior of the unlabeled material under study is more complex. Mathematically, this is easy to show, but even intuitively it is not difficult to realize that the initial specific activity will be high in the pool into which the tracer is first introduced, and in those pools which have a rapid uptake (or turnover) of tracer. Later the specific activity will, on the other hand, be high in the pools with a slow turnover and a slow release of the tracer. Thus, there will be no equilibrium.

Mathematically the change in specific activity registered in accessible pools or, with the help of a whole body counter, in all together, can be expressed in a number of different ways. Of these, it is most meaningful to choose the sum of several exponentials for the following reason.

Empirically the movement of tracers in multipool models of biological systems can be described by a system of first order differential equations, the solution of which will have the form of sums of exponentials of the type Ae^{-bt}. These differential equations contain the symbols for the information sought—i.e., the sizes of and flow rates between the different pools. The sizes are expressed in grams, the flow rates usually as the fraction of the tracer in a pool per time unit—for example, per cent per minute. Once these sizes and flow rates are known, the size of the sum of the pools ("body stores") and the sum of outflow ("total losses") can be calculated.

The equations describing the curves obtained experimentally have the form of a sum of exponentials of the same type, where t = time. The coefficients (A) and exponents (b) of the different terms, however, are devoid of any physiologic meaning. It is not generally true that exponents indicate a rate of flow between different pools, or coefficients their sizes. In order to translate the experimental data (in themselves meaningless) into physiologically meaningful terms, a kinetic analysis is needed. To perform such an analysis of the experimental curves, a theoretical model must be selected. The simplest possible model has as many pools as the number of exponential terms.

The purpose of the kinetic analysis is to express the constants obtained

experimentally (A and b) in terms of the total pool sizes and flow rates of the model selected. This is done by integrating the differential equations describing the theoretical model to obtain equations for the activities in each pool at the time *t*. The solutions thus found of the differential equations have the form of exponential polynomials, like the experimental equations, and thus the constants in the experimental curve can be expressed in terms of pool sizes and flow rates. In this way it becomes possible to obtain information regarding not only the body stores, the physiologic distribution between different pools or the uptake and the losses of a substance under different clinical conditions, but also of the normal flow rates between the different pools and of the pathologic changes in these rates caused by diseases or drugs.

However, the figures thus obtained are dependent on the model chosen, and the particular model found is usually not unique; other models could fit the experimental results. Methods have been described and used to map these other models.[2,26] Sometimes the pool sizes themselves indicate the nature of pools, but most often no information is obtained from the kinetic analysis on the nature and localization of the pools; concomitant physiologic studies and independent considerations must furnish this information.

Some hematologic examples—iron, vitamin B_{12}—have been mentioned, where correct kinetic analyses of tracer experiments can yield valuable information. Examples from other areas of medicine are calcium, potassium and other electrolytes, trace metals, etc. It is possible that some drug effects or diseases can be defined one day as changes in a specific flow rate.

A kinetic analysis of the type mentioned is less complex than it may perhaps sound, and solutions have been developed[2,3] which can be applied to many problems. In other problems, analog computers[9] may be of help, and work is in progress to develop digital computer programs applicable to a number of clinical problems.

However, the present purpose is not to provide a "do it yourself" manual. Just as he seeks the advice of a statistician or other experts, a clinician active in tracer research might want to collaborate with "kineticists" to plan and interpret his experiments. In this manner, a maximum of physiologically really meaningful information[7,9,10] can be obtained from the data collected.

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