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Tracer Methods in Hormone Research

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It also includes discussions on "turn-over times" published with Dr. ALDO RESCIGNO, Department of Physiology, University of Minnesota.

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Discussions with Drs. Paul C. MacDonald, Raymond L. Vande Wiele and Seymour Lieberman at Columbia University, New York, held during the development of tracer methods to determine rates of secretion of dehydroisoandrosterone sulfate, are pleasantly recalled. That project motivated theoretical treatments presented in this monograph.

Contents

Introduction

The purpose of this monograph is to describe theoretical aspects of the interpretation of data obtained from experiments performed with labeled hormones.

Quantitative endocrinologic studies involving the use of tracers include the determination of rates at which hormones are secreted by endocrine glands and are produced outside these glands by conversion of other secreted hormones. Tracer experiments are also performed with the purpose of measuring rates of metabolic reactions. These measurements reveal the contribution of secreted hormones to the formation of circulating compounds and urinary metabolites. The estimation of rates of fetal and placental production and exchange of hormones characterizes a class of *in vivo* quantitative studies performed with isotopically labeled hormones (radioactive or not). In addition, tracers are used to measure permeability and rates of reaction in *in vitro* systems, and to study the uptake of hormones by tissues, both *in vivo* and *in vitro*.

The stability of the steroid nucleus carrying the isotopic label and the large number of reversible metabolic reactions in which steroids are involved, both facilitated and motivated the development of a sophisticated theoretical treatment of tracer experiments in the field of endocrinology. Although the practical examples used to illustrate the concepts and calculations presented in this monograph involve labeled hormones, the theory is presented in a general symbolic manner and is applicable to other fields of investigation.

Different problems require the application of special experimental designs and lead to calculations that may not be found in a collection of formulas. Therefore, the intention of this monograph is to emphasize the description of procedures followed to derive presently available formulas and the assumptions on which these derivations are based, both to facilitate their proper application and to stimulate further advances. Formulas to calculate kinetic parameters from isotopic data represent logical consequences of *a priori* assumptions concerning the biologic system under study. The current assumptions may eventually become unsatisfactory and may be superseded by more realistic and sophisticated schemes.

Most of the analyses presented in this monograph refer to open systems in the steady state. Under these conditions, rates can be assigned constant values and clear relationships can be established between rates and isotope concen-

trations obtained in tracer experiments. In reality, however, many hormones are produced at a variable rate and steady state conditions are not commonly fulfilled. It is then important to note that the metabolic fate of a labeled hormone is usually (but not always) unaffected by changes in hormone production. Therefore, parameters with values estimated entirely from isotope measurements may be validly discussed in terms of steady state conditions, although the calculation of rates requires consideration of the variable rate of production of the hormones. These considerations are discussed throughout the monograph and particularly in Chapter 5.

The reader should not confuse mathematical difficulty, which is minimal in this monograph, with annoyances arising from the symbolism employed. Regardless of efforts to simplify the notation, the large number of parameters and experimental data to be handled makes the symbolism quite cumbersome. This is the most irritating feature of all publications on analysis of tracer experiments. In fact, sometimes it is easier to derive a formula than to decipher someone else's notation. This seems to be a common problem, as revealed by the high incidence of rediscovery of theorems in compartmental analysis.

The monograph is organized as follows:

Chapter 1 describes open systems in the steady state and provides definitions for rates of transfer between different pools or compartments is such systems. Various other parameters describing the dynamics of production and movement of material in the system are also defined, and the relationships among them are derived. No reference to tracers is made in this chapter. However, rates and parameters are defined with the advanced knowledge that they can be estimated from tracer experiments. In fact, the proper definition of rates of transfer was found to be a key point in the interpretation of isotopic data.

Chapter 2 provides the formulas necessary for the estimation of the parameters described in Chapter 1 using data obtained from experiments in which the tracers are infused at a constant rate until a steady state with respect to the isotopes is achieved. The derivation of these formulas is simply based on equations describing the conservation of labeled and unlabeled material in any space, at the steady state. The solution of the resulting systems of linear equations is quite elementary. This chapter emphasizes the advantages and possibilities offered by the simultaneous use of two tracers labeled with different isotopes.

Chapter 3 presents an analysis of experiments in which tracers are administered as a single dose rather than infused at a constant rate. It shows a theoretical relationship that exists between the isotopic data obtained in the same system by these two methods of administration of tracers. This simple relationship justifies the application of the formulas derived in Chapter 2 to data obtained after rapid injection of a tracer. Therefore, no differential equations are needed to derive the formulas used in the calculation of rates. This chapter also covers the procedures used in the determination of specific

activity functions from isotopic data and describes parameters that are calculable when these functions are known.

In Chapter 4, a more conventional compartmental analysis is presented. Models consisting of several compartments are used to derive expressions describing the isotope content in each compartment in terms of the parameters of the system and the time following the administration of tracers. This is a formal mathematical analysis based on abstract models.

Chapter 5 deals with the interpretation of tracer concentration curves in biologic systems, with a specific reference to blood data. In contrast to the analysis in Chapter 4, the physiologic meaning of pools or compartments is questioned and the assumptions necessary to justify the calculations of rates are critically reviewed. This chapter includes a discussion of several "turnover" terms defined in Chapter 3 and describes applications of 2 isotope methods to the study of peripheral interconversion and fetomaternal transfer of hormones.

Chapter 6 examines the information made available by experiments in which the isotope content of urinary metabolites is determined after the administration of tracers. The interpretation of urinary data is complex because of the multiplicity of precursors of the metabolites, but deserves special attention inasmuch as urine is a convenient and widely used source of experimental data.

Chapter 7 describes an *in vitro* experimental design based on superfusion of tissue slices or cell suspensions with a mixture of metabolically related tracers. It shows how isotopic steady state data can be used to estimate rates of entry, exit, synthesis, and metabolism, as well as intracellular compartmentalization of compounds.

Although some of the formulas presented in this monograph can be derived without involving the concept of compartments, most of the theory is based on the consideration of multicompartmental models. In order to achieve physiologic and biochemical relevance through these models, a form of analysis was developed that is based on data obtained from a few accessible compartments among the many that exchange the isotope introduced into the system. The calculation of rates of movement of material into, out of, and between these selected pools can be validated even when the number and identity of the other pools in the network is unknown. Consequently, the complexity of the system need not be neglected by limitations imposed by the models used to interpret the isotopic data.

A fundamental characteristic of the systems considered in this monograph is that the movement of the labeled molecules out of a compartment can be described by a first-order expression, viz. that the rate of exit of the isotope towards any destination is always proportional to the amount of isotope in the compartment. A simple manner to assure this property is to specify that the system is at the steady state with respect to the unlabeled molecules, as is discussed in Note 3.2, Chapter 3. Because hormonal steady state may be the

exception rather than the rule, it is important to realize that the movement of isotope may follow first-order kinetics even in systems that are not at the steady state, as is apparent in situations in which the kinetic behavior of the labeled molecules is not influenced by changes in the levels of the endogenous hormones. These systems can be studied with the tracer methodology described in this monograph. However, the proposed methods are not applicable to systems in which the values of the rate constants of processes involving the tracer vary during the experiment. These situations are apparently rare in the field of endocrinology, at least at the present level of experimental precision. Therefore, it can be expected that the concepts based on "first-order" tracer kinetics or even on steady state will continue to yield useful information on the production, distribution, and metabolism of hormones.

Chapter 1

Rates in Open System in the Steady State: Definitions and Relations

Labeled compounds introduced into living systems are distributed in various spaces and undergo chemical reactions. In order to give a structure to the system, it is usually assumed that there are regions in which a compound is uniformly distributed or, at least, in which internal dispersion occurs much more rapidly than does outward flow; a compound distributed in one such space constitutes a pool. The term "pool" is used in preference to "compartment" to avoid the purely spatial implication that might be assigned to the latter term.

In a biologic system the total number and the anatomic boundaries of most of the pools defined in this manner cannot be specified. Therefore, the definitions of rates and related parameters described in this monograph are chosen for their applicability to multicompartmental systems in which the total number of pools is unknown. When a rate of transfer between two pools is defined and a formula for its measurement from isotopic data is described, it is understood that the two pools are well defined and accessible to the investigator, even though they may be embedded in a complex network of many other pools, some also accessible, some hopelessly inaccessible. The definition of rates, as given here, allows for the existence of unknown intermediate pools in the path of movement of material between two pools. In contrast, typical compartmental analysis is based on models consisting of a determined and explicit number of pools.

The manner in which rates are defined in this chapter is a key to the development of simple tracer equations applicable to systems of an unknown number of pools (HART, 1966; MANN and GURPIDE, 1966).

Formulas for the calculation of each of these rates from experimental data are given in subsequent chapters.

One, two, three, or, in general, m accessible pools embedded in a network of an unknown number of compartments may be considered in any particular study, depending upon the number of pools that are sampled or directly labeled. These cases are discussed following the order of increasing complexity. Definitions of average rates in non-steady-state systems are discussed in other chapters.

A. One Pool Embedded in a Multicompartmental System

Rates of Entry

Figure 1.1 represents one of the pools in the system, viz. pool 1. This pool may represent a hormone in systemic circulation. The molecules of the compound entering the pool can be divided into two categories: molecules entering the pool for the first time (at a rate v_{01}) and those which are reentering the pool (at a rate w_1). The sum of these two rates represents the total amount of material coming into the pool per unit of time.

Production Rate

The symbol PR_1 denotes the "production rate" of the compound in the pool, i.e., the rate at which *new* material is fed into the pool. It contrasts with the "unproductive" recycle of material denoted by w_1. When only one pool embedded in a multicompartmental system is considered, PR_1 equals v_{01}. Originally the term production rate was used to distinguish the total rate of *de novo* formation of a hormone from the "secretion rate" of the hormone by endocrine glands (VANDE WIELE *et al.*, 1963), because some hormones are produced by more than one gland as well as by metabolic conversion of other secreted hormones. This parameter is easily determined when a tracer is administered into the pool (Eq. 2.1, Chapter 2).

Rates of Removal

The compound leaves the pool in one of two ways: irreversibly, at a rate v_{10}, or temporarily, bound to return to the pool. The latter rate is, by definition, equal to w_1. At the steady state, PR_1 equals v_{10}, since the rate at which the compound enters *de novo* into the pool must equal the rate at which it irreversibly leaves the pool.

The processes represented by the rates v_{10} and w_1 do not necessarily refer to different metabolic paths. Rather, each path of removal of the compound from the pool may contribute to both of these two rates. Formulas to calculate w_1 from isotopic data are given in Chapter 3.

B. Two Related Pools Embedded in a Multicompartmental System

Rates of Entry, Exit, and Transfer

Figure 1.2 represents two pools between which there is a transfer of material in at least one direction. Pools 1 and 2 may represent two blood-borne hormones such as estrone and estradiol between which interconversion is noted,

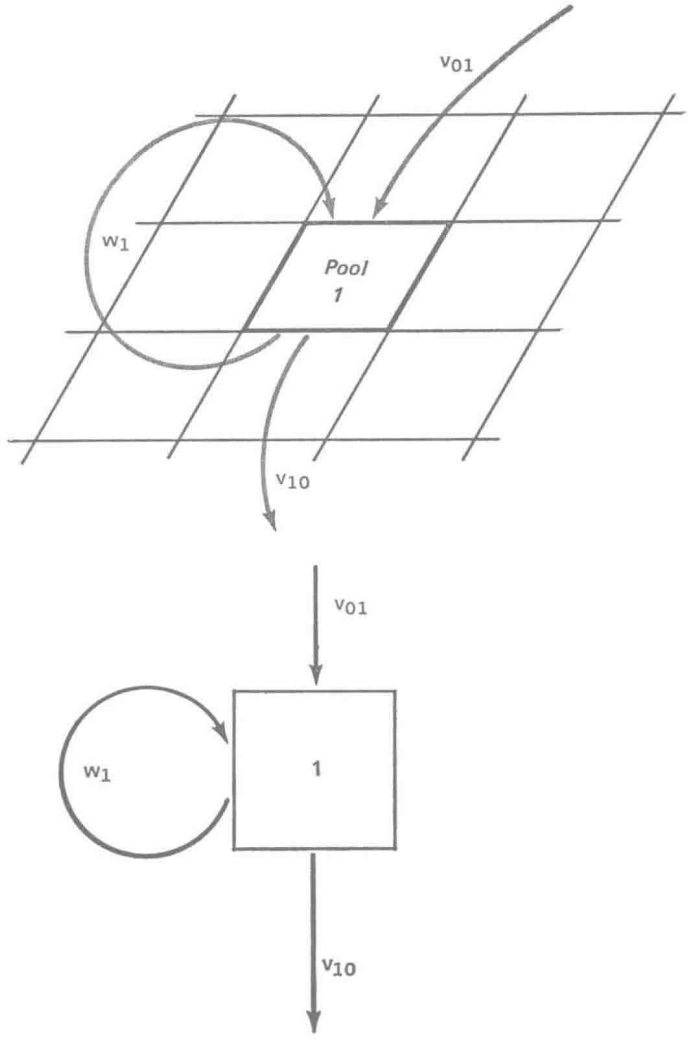

Fig. 1.1. Representation of a pool embedded in a multicompartmental system. Pool 1 represents a compound uniformly distributed in a space in which mixing occurs instantaneously or, in experimental terms, in which internal dispersion occurs much more rapidly than outward flow. In a biologic system, a pool exchanges material with an undetermined number of other pools represented by the network on the upper figure. Material appears *de novo* in pool 1 at a rate v_{01} and leaves the pool irreversibly at a rate v_{10}. Some material leaving the pool returns to it a rate w_1. The lower figure is the simplest representation of the system; the cycle indicated by the arrow implies the existence of other pools exchanging material with pool 1

or they may designate one hormone distributed in two spaces, such as cortisol in fetal and maternal circulations.

The rate at which material is transferred from pool 1 to pool 2 is denoted by v_{12}. It describes the total transfer, by all paths, of material from one pool

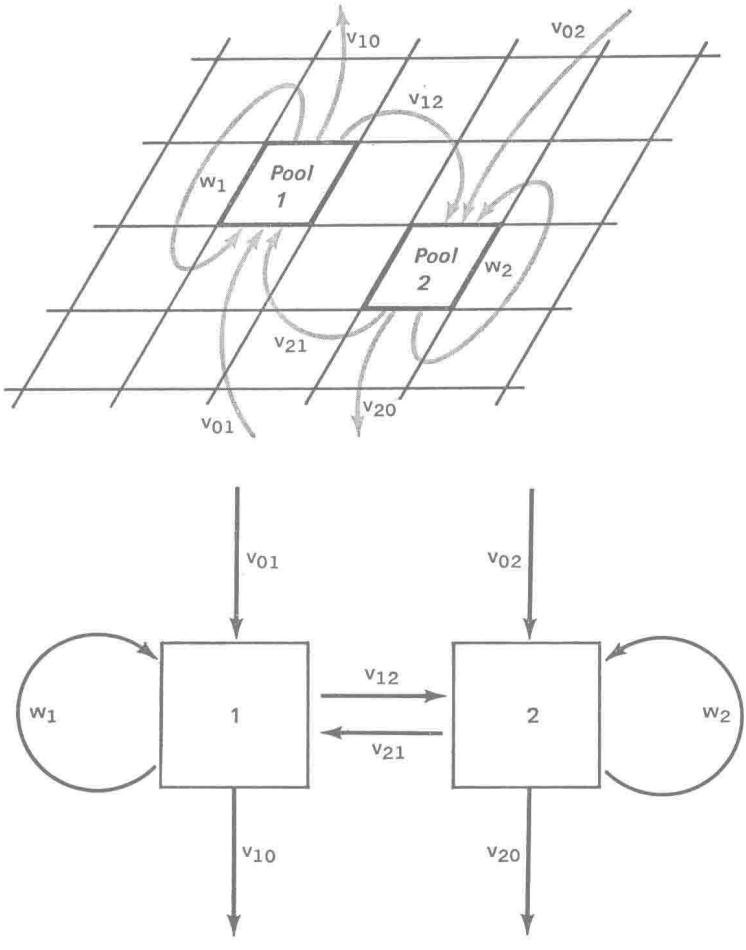

Fig. 1.2. Representation of two pools embedded in a multicompartmental system. Another pool of the network (pool 2) has been singled out for examination of its relation to pool 1 (see Fig. 1.1). Note that the transfer of material between pools 1 and 2 (rates v_{12} and v_{21}) may occur through other intermediate pools. Note also that v_{01} and w_1 in Fig. 1.2 may be smaller than v_{01} and w_1 in Fig. 1.1. Superscripts for these symbols (e.g., $v_{01}^{(2)}$) are used in the text only when the possibility of confusion arises. The lower figure is the simplest representation of this system; the cycle arrows imply the existence of an undetermined number of exchanging pools

to the other. The rate of transfer from pool 2 to pool 1 is v_{21}; the net exchange between the two pools is expressed by the difference between these two rates.

Each rate of transfer, e.g., v_{12}, can be considered to be the sum of two other rates:

$$v_{12} = v'_{12} + v''_{12} \tag{1.1}$$

where v'_{12} refers to material that has never been in pool 2, and v''_{12} refers to material that is returning to pool 2. Such a distinction is justified because both