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THE APPLICATION OF COMPARTMENTAL ANALYSIS TO RESEARCH IN NUTRITION

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"The greatest contribution which a compartmental model may make is to stimulate the investigator to think about his system in a different way, to ask new questions, and to run new experiments."

R. Moore (53)

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INTRODUCTION

Many nutritionists have used stable or radioactive isotopes *(tracers)* to obtain information about a particular substance of interest [the *tracee* (14)]. In fact, according to Robertson (66), the first use of radioisotopes in biology was to study the mineral element phosphorus, which is a nutritionally important macronutrient. Most of us who have performed isotopic studies are familiar with *tracer kinetics,* the term used to denote "the temporal and spatial interrelations of the tracer and the system" (14). In the course of carrying out kinetic experiments, it becomes evident that the data contain more information than can be extracted by conventional methods of analysis. For example, data interpretation is limited to very qualitative statements if the results of an in vivo kinetic study are analyzed as tracer distribution in tissues or as percent of dose versus time in a particular organ. As an alternative, a properly designed and executed tracer kinetic study can be analyzed using mathematical modeling to obtain quantitative and predictive information about the dynamic behavior of the system under study and to generate hypotheses that can be tested experimentally.

Our purpose here is to discuss compartmental analysis, a mathematical modeling approach that is particularly useful for nutritionists. Although we do not review papers in which the approach has been applied to nutritionally interesting systems, we refer to selected applications as examples of various points. We discuss compartmental analysis more from a biologist's perspective than from the mathematical point of view. We expect that potential users, once convinced of the power of these techniques, will be motivated to tackle the underlying mathematical and statistical methods. Our primary goals are to sharpen the ability of nutritionists who are not modelers to read and more readily understand literature that uses this approach and to encourage nonmodelers to add these methodologies to their experimental repertoire. We hope to convince readers that, although it is a serious undertaking, compartmental analysis can significantly enhance our appreciation and understanding (or misunderstanding, if inappropriately used) of biological systems.

MODELING APPROACHES

Several types of mathematical modeling have been used to investigate the dynamic behavior of biological systems. These include deterministic models that are compartmental in structure (1, 15, 43, 52, 70), models of distributed systems in which the "compartments" are nonhomogeneous and contain (for example) concentration gradients (15), stochastic (probabilistic) models (52), and models based on difference equations (46, 52). Some kineticists also

include a category called *noncompartmental analysis* (15, 26), which may make use of convolution/deconvolution techniques from linear systems theory. Although some forms of noncompartmental analysis have been called *model-independent,* that designation may be misleading, since physiological interpretation of the kinetic parameters is based on knowledge of the dynamic structure of the system (i.e. a conceptual model).

If modeling is appropriate, choosing the best modeling strategy depends on the system under investigation, one's ability to satisfy the underlying assumptions of the method, the availability of computer hardware and software, and previous experience. Ideally, the choice is made before the data are collected, just as for statistical tests, although it is possible to choose and apply modeling techniques after the experiment has been performed (12, 30, 38). Here we limit the discussion to two tracer kinetic approaches, namely empirical modeling and model-based compartmental analysis, which are usually classified as subcategories of compartmental analysis. These two techniques are discussed in detail later. Briefly, by empirical modeling, we mean fitting the data (typically the plasma tracer response after a bolus tracer input) to a one- to 5-component exponential equation and then using the exponential constants and coefficients to calculate certain kinetic parameters related to the dynamics of the system. The system dynamics may or may not be conceptualized compartmentally. By model-based compartmental analysis, we mean fitting similarly obtained plasma data, plus any other appropriate tracer and tracee data from the same or other experiments or from the literature, to an hypothesized compartmental model, and then adjusting the model parameters and structure, or performing further experiments, until a compartmental model is identified that is compatible both with the available data on the system dynamics and with known characteristics of the system. The latter approach can also be used to simulate (predict) the system responses in different situations and to plan future experiments.

Compartmental modeling is currently the best method for many nutritionally relevant systems because the dynamics of such systems can be approximated by a finite number of homogeneous states and lumped processes that exhibit deterministic behavior (6, 43, 70). *Deterministic behavior* means simply that the future state of the system is predictable based on its current state and future input; it contrasts with *stochastic behavior,* in which there is an intrinsic (probabilistic) uncertainty about the future state of the system even if the current state and future inputs are completely known (15). Since sophisticated computer programs (e.g. the Simulation, Analysis and Modeling programs discussed subsequently) can now handle large models, some distributed behavior can be approximated by deterministic compartmental models.

Compartmental analysis can be applied to many systems of interest to

nutritionists. As evidenced by the following examples, compartmental analysis can be used to model (i.e. describe and quantitate) digestion and absorption of nutrients (10, 56, 59, 68, 72, 75) and the whole-body metabolism of nutrients (18, 29, 39, 47, 76). It can be used to estimate rates of tissue turnover or metabolic interconversion and utilization of nutrients. It can also be used to estimate masses of nutrients or their metabolites that are not directly measurable. In addition, several interesting time parameters can be computed: for example, how long, on average, a particular nutrient spends in the body or in the blood before reversible or irreversible utilization, and how many times it cycles through the blood before irreversible utilization. The models developed by applying model-based compartmental analysis to data from experiments in vivo or in vitro can also provide unique insights into underlying processes and control mechanisms (15; 43, chap. 13); most important, modeling can generate ideas for experiments to validate hypotheses developed during the modeling process.

NOMENCLATURE AND THEORETIC BACKGROUND

Anyone who begins to study tracer kinetics immediately confronts the problem of nomenclature. Many different names are used for the same kinetic parameter, different orders are used to name vector spaces of parameters, and different kinetic behavior is referred to by the same name. These inconsistencies hamper one's reading and analysis of tracer kinetic papers, because the reader must often establish which conventions are being used.

In addition, to become a competent kineticist, one must make a decision and commitment to delve into the relevant mathematics. Many excellent texts and reviews can be recommended as general references (1, 2, 15, 26, 40, 43, 45, 51, 52, 70). An alternative and frequently preferable approach is to collaborate with a competent biological modeler who has expertise in compartmental analysis and who is willing to become acquainted with the nutritionist's chosen system. Even the latter route requires one to learn as much as possible about kinetic theory in general and about experimental design as it applies to tracer kinetic experiments in particular.

Steady State

A system is in a steady state over a defined period of time if, for every compartment, the total rate of appearance of the substance(s) of interest is equal to its total rate of disappearance. The input and output rates may be very different in the various compartments. One could say that such a system and its individual compartments are "in balance." In such a system, the mass of material is time invariant (i.e. constant over time). True steady states rarely occur in biological systems; however, minor fluctuations around a steady state

are not uncommon and these can often be obtained by careful experimental design (15). The steady state is usually assumed in empirical analysis and it facilitates model-based compartmental analysis. In the past, the use of the steady state greatly simplified the solution and physiological interpretation of the ordinary (versus partial), first-order differential equations used to approximate the deterministic behavior of multicompartmental systems; with modern digital computers and advanced algorithms, the steady state is not required for model-based compartmental analysis.

Linear and Nonlinear Behavior; Tracers

If a system shows linear behavior, then, for a change in input, one sees a proportional change in output (i.e. response). Berman (6, p. 5) used the superposition principle to explain this phenomenon: "Let the response of the system to any arbitrary time dependent input $u_1(t)$ be $r_1(t)$, and the response to another arbitrary input $u_2(t)$ be $r_2(t)$. The system is said to be linear if the response to any linear combination of the two inputs, $k_1u_1(t) + k_2u_2(t)$, is $k_1r_1(t) + k_2r_2(t)$." In contrast, most biologic systems exhibit nonlinear behavior. A classic example of nonlinear behavior is Michaelis-Menten kinetics. Although kinetic behavior is nonlinear throughout the range of substrate concentrations when enzyme concentration is held constant, at very low substrate concentrations, linear behavior is approximated (i.e. doubling the substrate concentration doubles the reaction velocity). At higher substrate concentrations, the system displays nonlinear kinetic behavior.

To study the behavior of nonlinear systems and thus learn more about homeostatic control mechanisms, biological systems are often studied in a number of different physiological states (10, 37, 60, 75). Nonlinearities often can be studied directly by perturbing the system away from steady state and then following the transients as the system returns to the original or a new steady state (15, chap. 5).

An *ideal tracer* introduced into a biological system of interest to nutritionists nearly always displays linear kinetic behavior. By *ideal tracer,* we mean one that is easily detectable, follows the same kinetics as the tracee, and does not perturb the mass or underlying kinetics of the system. For example, a rat is administered 1 μCi of tracer and plasma tracer concentration at time t is x dpm/ml. If 2 μCi had been administered, tracer concentration at time t would be $2x$ dpm/ml. Thus the tracer displays linear kinetics. If we can verify that the tracer follows the same kinetics as the tracee, we may be able to model tracee behavior. However, the fact that the tracer follows linear kinetic behavior does not necessarily mean that the tracee does so.

If the system is in a steady state, then the tracer kinetics can be described by specified initial conditions (i.e. the amount and location of the tracer immediately after injection or at the start of an infusion) and by time-invariant

(i.e. constant) fractional transfer coefficients (see next section). If the underlying system is in a non–steady state but is being studied in a dynamic region that displays linear kinetic behavior, then the tracer kinetics can still be described by time-invariant fractional transfer coefficients; if the system displays nonlinear kinetic behavior, then the tracer kinetics will be described by time-variant fractional transfer coefficients.

Although most of the nutrition-related studies that have used compartmental analysis employ stable or radioactive isotopes as tracers, tracers are not a prerequisite in compartmental analysis. One can also perturb the state variables of the system (i.e. change the mass and concentration of the tracee) and model the resulting transients using compartmental analysis (35).

Compartment, Transfer Rate, Fractional Transfer Coefficient

A compartment is a mathematical construct that may or may not define a discrete physiological or biochemical space. Each compartment contains an amount of tracer and tracee that is well mixed (i.e. homogeneous) and that turns over more slowly than the time required for mixing within the compartment. Fortunately, many of the compartments that nutritionists are interested in do consist of a physiologically definable space. For example, the plasma pool of some entities acts as a kinetically distinct compartment if mixing of the constituent in blood is rapid compared with its rate of turnover from the blood. Several organs or parts of organs may act kinetically as one compartment or, within a given organ, there may be several kinetically distinguishable compartments. In empirical modeling, each compartment is, by definition, kinetically distinct, whereas in model-based compartmental analysis, several sampled organs might show similar kinetic behavior but be modeled as separate (measured) sets of compartments.

In a steady state a certain fraction of the material in a compartment leaves per unit time. Thus we can calculate the rate of transfer of tracee (mass/time) from compartment J to compartment I as $R(\mathrm{I}, \mathrm{J}) = L(\mathrm{I},\mathrm{J}) * M(\mathrm{J})$, where $L(\mathrm{I},\mathrm{J})$ is the fractional transfer coefficient (time^{-1}; i.e. the fraction of the material in compartment J transferred to compartment I per unit time), and $M(\mathrm{J})$ is the mass of tracee in compartment J. Figure 1 depicts these parameters for a specific two-compartment model. In a steady state, an amount of material equal to the amount leaving per unit time enters the compartment for the first time from inside or outside the system [$U(1)$, the production rate] or by recycling from other compartments [$R(1,2)$]. $R(0,2)$ is the rate at which material leaves compartment 2 and the system irreversibly. The mass balance equation that describes the change in amount of material in compartment 1 with time [$\mathrm{d}M(1)/\mathrm{d}t$] can be stated as $\mathrm{d}M(1)/\mathrm{d}t = U(1) + R(1,2) - R(2,1)$. For models with multiple sites of input and output from compartment 1, $R(1,2)$ would be replaced by the sum of $R(1,\mathrm{J})$, and $R(2,1)$ by $R(1,1)$, where

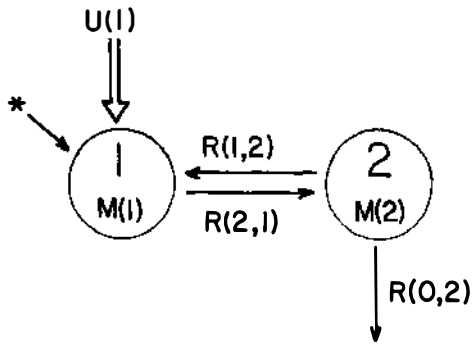

Figure 1 Two-compartment model. Compartments 1 and 2 are shown as circles; connectivities *(arrows)* are labeled with the appropriate transfer rate [e.g. R(I,J) is the rate of transfer of material to compartment I from compartment J (mass/time)]. The rate of tracee input into the system (production rate) is shown as $U(1)$, output from the system is from compartment 2 [$R(0,2)$], M(I) represents tracee mass in compartment I, and the asterisk denotes the site of introduction of tracer.

$R(1,1) = L(1,1) * M(1)$, and $L(1,1)$ is the sum of the fractional transfer coefficients describing output of tracee from compartment 1. In a steady state, the $U(1)$, $L(1,2)$, $L(2,1)$, $M(1)$, and $M(2)$ are all constant, and $dM(1)/dt = dM(2)/dt = 0$.

The transfer of material between compartments is equivalent to movement to a kinetically distinct pool in the same or different space [e.g. it could be a chemical transformation in the plasma space or a transfer from the plasma to an organ (i.e. a different space)]. Such movement can occur by flow, diffusion, transport across membranes, transfer between organelles, chemical modification, or transfer to different blood constituents. The fractional transfer coefficients described here should not be confused with classical chemical rate constants, since the processes approximated by the L(I,J) may show first-, higher-, mixed-, or zero-order kinetics, and the coefficients often reflect a number of complex processes (6, 15).

The fractional transfer coefficient (also referred to as the rate constant, transfer rate constant, and even turnover rate) is one of the kinetic parameters that is defined differently by various kineticists. In contrast to the convention given above [i.e. that L(I,J) represents the fraction of tracee in compartment J transferred to compartment I per unit time], some authors define L(I,J) as the fraction transferred from I to J.

Conceptual Versus Mathematical Models; Large Models; Pooled Data

Two types of compartmental models are discussed here: conceptual models and mathematical models. A conceptual compartmental model is a diagram

that summarizes current knowledge or hypotheses about the compartmental structure and interconnectivities of a system. Figure 1 is an example of a conceptual compartmental model. In contrast, a mathematical model is a mathematically formalized compartmental representation of the system. The mass balance equation stated above is an example; integration of the differential equations representing that model results in an equation that can be used to simulate the kinetic behavior of compartment 1. In the case of the differential equations describing the two compartment model in Figure 1, integration would yield a pair of algebraic equations, each of which is the sum of exponential functions with real coefficients. Given a set of initial values and a description of $U(1)$, we can calculate all the parameters of the system.

Ideally, modeling is done on the data for individual subjects; this procedure is feasible in empirical modeling because the data are usually derived from sampling blood after introduction of the tracer into the vascular system. However, when developing large models of complex systems, it may be necessary to kill groups of animals at different times to collect data for various organs and then to pool the data into one large data set for modeling. This technique has been called *the superrat approach* (45). Some very useful insights into system dynamics can be obtained in such experiments; these insights can then be validated or abandoned after subsequent studies. One needs to keep in mind, however, that a pooled model is not the mean of the dynamic behavior for individual animals. See Jacquez (43, chap. 16) and Cocchetto et al (20) for discussions of this topic.

Model Structure and Parameter Identifiability; Model Validation

Identifiability is used in theoretical (a priori) and practical (a posteriori) contexts (15, 17, 19, 26, 43–45). Theoretically, *identifiability* means determining whether, with ideal (i.e. error-free) data, the model parameters could be estimated with a specified level of precision. It relates to when and where the system is sampled (i.e. whether the parameters of interest are sensitive to the data collected). As examples of failed identifiability, consider trying to understand traffic dynamics in San Francisco by monitoring the flow of traffic on US Interstate 80 (which bypasses the city), or trying to quantify the kinetic processes occurring in liver when only plasma was sampled and when changes in kinetics in the liver had little influence on plasma kinetics. In practice, *parameter identifiability* means evaluating the accuracy or uniqueness of parameter estimates based on statistical analysis during the modeling process. *Structural identifiability* means finding a conceptual model that is statistically compatible and consistent with the data collected and with knowledge of the system (i.e. determining the minimum number of compartments and connectivities necessary to explain the data). As in the use of regression

analysis for other applications, both internal and external validations of the model should be performed (15, 16).

Kinetic Parameters

The *mean transit time* in compartment I [$\bar{t}$(I)] is the mean of the distribution of times that molecules entering compartment I spend there during a single transit before leaving reversibly or irreversibly. This parameter has also been called the *turnover time* (79). Note that mean transit time is calculated for molecules that actually enter compartment I. It is the mean of the distribution of times because not all molecules of the substance of interest spend the same amount of time in the compartment. That is, even though molecules leave the compartment at a known rate, we do not know which particular molecules will leave during any specific finite time interval.

The *mean residence time* in compartment I [$\bar{T}$(I,J)] is the mean of the distribution of times that the substance of interest spends in compartment I from the time it enters the system via compartment J until it leaves compartment I irreversibly. Residence time in compartment I is also the mean time of exposure of compartment I to the tracer dose that entered the system via compartment J. For any molecules that never reach compartment I, their residence time in that compartment is zero. Thus, the residence time in compartment I can be less than the transit time in I. Also, the residence time in I can be much longer than the transit time in I for systems in which there is a substantial amount of recycling through compartment I. A 1984 review by Covell, Berman, & DeLisi (21) gives excellent information on residence time.

The residence time for a tracer may not equal that for its tracee in a system with a single site of tracee input unless the tracer enters the system by the same route as does the tracee. For example, newly absorbed vitamin A enters the lymphatics as a component of chylomicrons. Thus, if the tracer is injected intravenously as retinol associated with retinol-binding protein, the residence time calculated for the tracer in plasma is not the same as that for newly absorbed vitamin A, if any is lost from the liver before secretion into plasma bound to retinol-binding protein. For a system with multiple sites of tracer input, the calculated residence time depends on the site of entry (i.e. it is not necessarily the same for each site of entry). Also note that residence or transit times that are potentially physiologically meaningless can be calculated if tracers are administered via a nonphysiological route (e.g. retinol given intraperitoneally or intravenously but suspended in ethanol).

In empirical modeling of plasma tracer response data (see subsequent discussion) and noncompartmental analysis, residence time can be obtained by integrating a plasma response curve extrapolated from the finite experimental period to infinity. When plasma data are expressed as a fraction of the tracer dose, the area under the curve [AUC(P)] so computed is the mean

exposure time of the plasma to the entire dose, or the plasma mean residence time [$\bar{T}$(P)]. This fact can be verified by considering that if every tracer molecule spent the same amount of time in plasma, then, numerically, AUC(P) = 1.0 (i.e. fraction of dose) * $\bar{T}$(P). If the dose is administered orally, then AUC(P) reflects the extent of digestion and absorption as well as later dynamics. Thus, unless the extent and time course of absorption is known or included in the modeling process, physiological interpretation of the computed AUC(P) may be complicated.

The inverse of the mean residence time is the *fractional catabolic rate* for compartment I [FCR(I,J); time^{-1}]. FCR(I,J) is thus the fraction of the mass in compartment I that irreversibly leaves compartment I per unit time after entering the system via compartment J. The rate of irreversible utilization *(disposal rate, DR)* for the substance that enters the system via compartment J is equal to FCR(I,J) * M(I). Its value may or may not be the same as the system disposal rate, depending on the site of entry of tracer and tracee. By definition, in a steady state, the system disposal rate is equal to the production rate (i.e. the rate of entry of the substance into the system for the first time).

The *system mean residence time* [$\bar{T}$(SYS)] is the total amount of time the tracer or tracee spends in the system from the time of input until irreversible loss. Like the mean residence time for a compartment, $\bar{T}$(SYS) is dependent on the point of entry. A related parameter, *mean sojourn time (MST),* can be calculated in empirical modeling. For blood-borne substances, it is defined as the mean of the distribution of times that tracer molecules spend in the system from the time of first entry (e.g. into plasma) until the time of "irreversible" exit from plasma. The MST will be less than $\bar{T}$(SYS) because it does not include the mean time that tracer molecules spend in the system after leaving the plasma irreversibly. The *total traced mass* in the system (57) can be computed as the product of $\bar{T}$(SYS), or of MST and DR, or as the sum of the model-derived masses of the substance of interest in individual compartments.

Two other kinetic parameters are sometimes calculated in compartmental analysis. One is the *recycle number* [$\bar{\nu}$(I)], or the average number of times a tracer or tracee molecule recycles through compartment I before it irreversibly exits from compartment I (40). $\bar{\nu}$(I) is calculated as [$\bar{T}$(I)/$\bar{t}$(I)] − 1. Another is the *recycle time* [$\bar{\pi}$(I)], or the time it takes for the average tracer molecule leaving compartment I to cycle back. After the tracer is introduced into compartment I, $\bar{\pi}$(I) equals [MST − $\bar{T}$(I)]/$\bar{\nu}$(I).

EMPIRICAL MODELING

When the research goal is to determine specific kinetic characteristics and/or parameters describing a biologic system (e.g. the number of kinetically

distinct compartments, the total traced mass, the disposal or utilization rate, or the plasma transit time, residence time, and transfer rate for a substance of interest), the form of compartmental analysis called empirical modeling is the method of choice. Empirical modeling has been applied to many nutritionally interesting entities including cholesterol (34, 71), amino acids (63), iron (73), calcium (50), sodium (74), thyroid hormone (25), retinol (37), and transthyretin (48). Its use is well documented (26, 40, 41, 45, 70).

Empirical modeling is usually applied to kinetic data on plasma tracer response versus time after introduction of a tracer into the vascular system. An adequate number of samples must be obtained so that the response profile is well enough defined for subsequent mathematical analysis. The number of samples to be collected can be minimized by applying optimization procedures (3, 24) to predicted or preliminary data. The experiment must also be carried out long enough so that the tracer can mix with the exchangeable tracee; that is, the temporal change in plasma tracer level must have reached a final slope when the data are plotted semilogarithmically. It may be possible to determine an optimal duration in a pilot study or by using data from the literature.

Typically, the plasma data are expressed as tracer concentration (dpm/ml), fraction of dose in plasma, or specific activity (dpm/mass) versus time-after-dose administration. Data are plotted semilogarithmically and then fit to an exponential equation of the form

$$y(t) = \sum_{i=1}^{n} I_i \exp(-g_i t)$$

where n is unknown, the I_i are the exponential constants, and the g_i are exponential coefficients. This procedure is called *curve fitting* and often inappropriately called "modeling the data" (64). Plasma data from an in vivo turnover study will fit a one- to, at most, four-component exponential equation. See Landaw & DiStefano (45) for a discussion of why there is such a low upper limit on the number of components determined by this method. The data are fit to an exponential equation because such equations are solutions to the ordinary, first-order differential equations that describe the assumed deterministic behavior of tracers in biologic systems. Simply obtaining a satisfactory fit of the data to a multiexponential equation does not guarantee that the system follows deterministic behavior---only that it is compatible with such a hypothesis.

Fitting data to the exponential equation given above requires weighted nonlinear least squares regression methods and is thus facilitated by an appropriate computer program [For reviews of weighted least squares methods of nonlinear regression, including appropriate weighting factors, see (45, 54,

61)]. Most frequently, we use the conversational (interactive) version of the Simulation, Analysis and Modeling (SAAM) computer program [CONSAM (7)] to fit plasma tracer data to an exponential equation. The mechanics of this process are described in the SAAM tutorials.[1] Statistical tests [e.g. the runs test, the F-test, the Akaike Information Criterion, or the Schwarz Criterion (see 45, 54)] are applied to determine the minimum number of components that best fit the data; this number is equivalent to the minimum number of lumped compartments in the system. Typically, one or more of these compartments is plasma, which is often the site of tracer introduction. Connectivities between the central plasma compartment and the other compartments, as well as sites of input and output, can then be added to the conceptual compartmental model in view of prior knowledge of the system, hypotheses being tested, and appropriate or inappropriate simplifying assumptions. Other statistical considerations (e.g. multicolinearity and autocorrelation) are reviewed in texts on regression analysis such as that by Neter et al (55).

The exponential constants and coefficients that define the equation are sometimes referred to as *macroparameters* (45). These, along with the initial conditions, are used to calculate the identifiable fractional transfer coefficients (the model's microparameters) and other kinetic parameters such as those defined earlier (e.g. transit time, residence time, fractional catabolic rate, recycling time and number). Equations for calculating the microparameters and other kinetic parameters can be found in several sources (36, 37, 40, 65, 70).

Before concluding our discussion of empirical modeling, several other points should be mentioned. Assume that an experiment is planned in which data will be collected only from the system's central compartment (which includes the blood) for analysis by empirical modeling and that the investigator knows enough about the system to draw an initial model. Ideally, identifiability procedures can be applied to determine whether one will be able to obtain reasonable estimates for the fractional transfer coefficients defining the model (15, 44). Sometimes the coefficients cannot be uniquely determined, but an interval for the parameter can be estimated (25, 26, 45, 71); or the experimenter can make assumptions that allow calculation of all the model's fractional transfer coefficients (48).

We refer to empirical modeling as the "back-door" approach to compartmental analysis because one first estimates the macroparameters (and usually their statistical uncertainties) and then, through combinations of the macroparameters, calculates the system microparameters [the $L(I,J)$]. In so

[1]Foster, D. M., Boston, R. C., Jacquez, J. A., Zech, L. A. 1988. The SAAM tutorials: An introduction to using conversational SAAM version 29. Resource Facility for Kinetic Analysis (RFKA), Seattle, Wash.

doing, the statistical uncertainty for the $L(I,J)$ is usually not computed, and one thus cannot say how unique are the values for the microparameters. In contrast, the microparameters are estimated directly in developing a model using model-based compartmental analysis (hence nicknamed the "front-door" approach to compartmental analysis; see next section) and one obtains an estimate of their uncertainty.

MODEL-BASED COMPARTMENTAL ANALYSIS

The goal of model-based compartmental analysis is to develop and quantify a compartmental description that characterizes the dynamics of a particular system. Model-based compartmental analysis using the SAAM computer programs has been employed to develop models describing the in vivo metabolism of many nutritionally important entities. These entities include minerals (10, 27, 29, 56, 67, 75, 77), vitamins (39), blood-borne fuels (33, 49, 69, 76), lipids and lipoproteins (4, 8, 38, 62, 78), hormones (22), and water (28). The approach has also been applied to in vitro systems (11, 12, 30).

The approach taken in model-based compartmental analysis is different from that used in empirical compartmental analysis. For example, because the questions under study and the resultant compartmental model are often more complex, the design of these studies generally involves more than serial sampling of blood. Note, however, that a number of useful models [e.g. for plasma lipoproteins (8)] have been developed using only plasma data. In addition to sampling plasma, the red blood cells, organs, and/or excreta might be sampled versus time. Obviously, organ or tissue sampling is not always feasible in humans, although, for some tracers such as zinc, whole-body counting over a specific region provides organ data noninvasively (29, 42). The inability to sample organs in the case of most isotopes emphasizes the usefulness of experimental animals in developing whole-body models.

Ideally, model-based compartmental analysis begins at the desk rather than at the laboratory bench. The investigator first formalizes what he or she knows or hypothesizes about the system into a conceptual compartmental model. In fact, postulation of the starting model frequently does not take place until the researcher has collected his data and is ready to begin the modeling process. The initial model should be complex enough to describe both the data and accepted ideas about the system, but it should also respect the principle of parsimony and Occam's razor: "Entities ought not to be multiplied except out of necessity" (William of Occam, ca 1285). The initial model includes not only the postulated compartmental structure and connectivities among compartments and the environment but also estimates of the fractional transfer coefficients. If the initial model is developed after the experiment has been

done, it should include at least one compartment for each component of the system that was measured. If a compartmental model for the system of interest, or a closely related one, already exists, it can be used as an initial model; if not, information in the literature or results from empirical analysis of the data can be used to estimate initial model parameters. Only the most gifted and experienced modelers acquire the ability of Mones Berman, the original architect of the SAAM program and the "father" of model-based compartmental analysis in biology. He sometimes obtained his initial estimates for these coefficients by looking at a plot of the response data!

After the experiment has been done, tracer data (generally calculated as fraction of dose versus time) and other relevant information [e.g. initial estimates for $L(I,J)$, initial conditions, tracee masses, sites of input and output] can be entered into a SAAM input file. See Foster & Boston (31) for an excellent discussion of this process and for details on the format of a SAAM input file. The input file is solved using CONSAM (7), and the observed data are compared to the response predicted for the starting model. If one considers the computational requirements for solving even a small model, generation of a model solution by SAAM is incredibly rapid (e.g. a few seconds for a small model to several minutes for a large one). Solution results are evaluated by comparing the observed and calculated data graphically and/or numerically. Then the modeler systematically adjusts the model parameter values and, if necessary, the structure of his starting model until a close fit between observed and predicted values is obtained. Additional experiments to expand the existing data set may also be required to identify a model. Finding a satisfactory match between the experimental data and the model predictions can require any imaginable amount of effort and time, from hours to years.

The SAAM programs provide statistical information about model solutions so that closeness of fit can be evaluated objectively and subjectively. During parameter estimation, the modeler not only seeks a close fit between the data and the model simulation but also must keep in mind the known or suspected physiology and biochemistry of his system, so that a physiologically reasonable model is presented as a working hypothesis for the system dynamics. The final model should not be too complex; otherwise one may obtain a close fit to the data but at the cost of very little confidence in the model parameters. In SAAM, this possibility can be evaluated by examining the statistical uncertainties for the parameters and the correlation matrix that tabulates the statistical relationship among parameters. In regression analysis, the occurrence of large correlation coefficients (>0.85) for certain combinations of parameters is called *multicolinearity*. This occurrence indicates that various combinations of parameter values will fit the data equally well. Note that when using SAAM and CONSAM for model-based compartmental analysis, the investigator is the one who controls model development. The

software sets up and solves the differential equations numerically, aids in simulation and evaluation of the proposed models, and, in the end, is used to do weighted least squares nonlinear regression to provide convergence with a local [versus global (54)] best-fit model. It is important to keep this point in mind, as some researchers may entertain the misconception that one simply enters data in a deck, runs SAAM, and is rewarded with the best model. In fact, SAAM and CONSAM will block nonlinear regression analysis if the model simulation does not fit the observed data adequately.

To conclude this section, we reemphasize some of the distinctions between model-based compartmental analysis and empirical analysis. For the former, the experimental design and the resulting model are usually more complex. In model-based compartmental analysis, the number of compartments and parameters in a model is constrained by the type of data collected, statistical criteria, limitations of the software, and the investigator's imagination and good sense. In comparison, in many applications of empirical modeling, compartment number is determined by the shape of the tracer response curves (which seldom can be resolved into more than four exponential components) or by an interest in investigating the scientific implications of a minimal model. Model-based compartmental analysis is also powerful in terms of generating hypotheses, bringing conventional wisdom into question, using past knowledge, incorporating tracee data in model development, and dealing with non–steady state situations. For both approaches, more than one study may be needed to optimize the experimental design (24) and thus obtain the data needed to identify (15, 44) the kinetic parameters of interest. As discussed by several authors (15; 23; 26; 52, chap. 4; 58), compartmental analysis is ideally an iterative process. Both empirical modeling and model-based compartmental analysis have made important contributions to our body of knowledge.

THE SAAM AND CONSAM COMPUTER PROGRAMS

Although a number of computer programs are available for analyzing data by compartmental analysis (53), here we concentrate on the powerful and unique SAAM programs mentioned above. The batch SAAM program (9) was introduced by Mones Berman and colleagues in 1962. According to Robertson (66), Berman and Robert Schonfeld began to develop the program in the 1950s, when they recognized the need for computational devices to provide numerical solutions for the equations required to describe compartmental systems with more than four compartments. Since then SAAM has been extensively improved, expanded, revised, and documented by Berman (until his death in 1982) and many associates. An interactive version, conversational SAAM (CONSAM; 7, 13), was introduced in 1980.

SAAM and CONSAM were specifically developed for model-based com-

partmental analysis of biological systems, although they can be used for other modeling applications (e.g. empirical modeling, Gaussian distribution analysis, Michaelis-Menten kinetics, integration, and deconvolution). Version 30 of the programs was recently released. In addition to a number of new features, this version can handle much larger models than its predecessors. Specifically, SAAM 30 can work with 75 compartments, 75 adjustable parameters, and 1000 observed or simulated data points. The programs have been written to run on the VAX and SUN computers and are being modified to run on the AT&T UNIX PC. The Resource Facility for Kinetic Analysis (RFKA) currently administers the programs, provides copies of current versions of the programs and tutorials, and publishes an informative newsletter.

Learning to use SAAM and CONSAM is well worth the effort required because the programs have powerful and unique features. Although Reference 31 and Footnote 1 are excellent resources for these programs, the best way to learn SAAM is undoubtedly by working with an experienced user. Workshops sponsored by RFKA are an ideal introduction to using the programs.[2]

One feature of SAAM, termed the forcing function capability, is particularly useful for large databases that include information on tracer and tracee over time in plasma, organs, and perhaps urine and feces. Plasma data are first fit to a multiexponential equation, called the plasma forcing function. The fraction of the dose in plasma can then be simulated over time using this equation. Since all organs are linked via the blood, the forcing function can be used to "uncouple" the system, organ by organ. Thus, to model the liver subsystem, for example, one sets up a SAAM input file with the liver data, the plasma forcing function, and relevant fractional transfer coefficients. A model for the liver is then developed without the modeler's need to worry about the influence of other organs on the simulated plasma response. Once a model has been developed for each organ subsystem, then all data, including that for the plasma, are put together in a single input file and modeled. At first the plasma forcing function may be retained while adjustments are made in individual organs. Ultimately, the plasma data are also freed so that the fit of the final working hypothesis model to all of the data can be evaluated. This approach was used in part by Foster et al (29) to develop a whole-body model for zinc metabolism in humans; its technical aspects are described by Foster & Boston (31).

FINAL COMMENTS

We have found that most researchers who apply compartmental analysis to nutritionally interesting systems are staunch advocates of the approach. Mod-

[2]For information, contact D. M. Foster, RFKA, Center for Bioengineering, FL-20, University of Washington, Seattle, WA 98195 (800-421-SAAM).

eling no doubt helps us think about a system, the experimental methods, and relevant (and seemingly irrelevant) literature in a different way. Berman commented (5, p. 102) that "a detailed model extracts all the information in the data," and it frequently generates novel ideas to test in subsequent studies. Besides, modeling is challenging and, more often than not, fun!

Unfortunately, one sometimes encounters strong-willed resistance (e.g. from peer reviewers) to compartmental analysis, especially to the application of model-based compartmental analysis. Some nonmodelers seem to feel that kinetic methods are akin to black magic and should thus be viewed with a healthy dose of skepticism. Such a viewpoint is sometimes attributable to a less-than-thorough acquaintance with the theoretical (i.e. mathematical) underpinnings of this form of kinetic analysis. Just as often, skepticism may arise in the reader because the modeler has not properly documented the approach or is misusing it. Attitudes are clearly changing, however, and will continue to do so as more biologists are exposed to the power of quantitative kinetic analyses. Continued acceptance of these methods assumes that they will be rigorously and correctly applied and that they are thoroughly justified to readers.

At a recent lipoprotein kinetics workshop held after the 1988 scientific sessions of the American Heart Association in Washington, DC, several modelers made valuable suggestions about important information to include in a manuscript presenting a multicompartmental model. Since some of their ideas may also be useful in formulating a modeling project, we paraphrase them here from that point of view. First, clearly state the purpose of the project, and verify that the experimental methods are appropriate. Decide whether empirical modeling or model-based compartmental analysis (or some other approach) is most appropriate to achieve the experimental goals; then formally or informally optimize the experimental design. Make every effort to ensure that the dose is prepared in such a way that it will be administered and metabolized physiologically. For model-based compartmental analysis, formulate a starting model, making as much use as possible of information in the literature. During model development, keep a record of alternate models tested and any assumptions made; the new "history" and "library" subroutines in CONSAM 30 facilitate such records. Be sure the "final" model is statistically sound (i.e. state why it was preferred over other models tested). In presenting the results, make every effort to include the primary data and the final model so that others can evaluate them, and be sure that interpretation of the modeling results is intelligible to a nonkineticist. Emphasize the main biological conclusions and state how the kinetic data support these ideas. Mention any new questions that modeling has generated. Also, as Berman has remarked (6), do not be surprised if the model is not totally consistent with conventional wisdom; rather, consider such inconsistencies as hypotheses to

be tested in the next experiment. And, finally, view the model as a working hypothesis subject to validation and evolution as a result of subsequent experiments.

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