INTEGRAL EQUATION DESCRIPTION OF TRANSPORT PHENOMENA IN BIOLOGICAL SYSTEMS

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1. Introduction

In considering transport phenomena in biological systems, usually the purpose is to gain some information about the structure of the system from the analysis of flux and concentration data. In a typical experiment radioactively labeled molecules of some kind are introduced into an animal; subsequently radioactivity of various components of the system is measured, and from this information an attempt is made to characterize the transfer of molecules from one part of the system to another and the chemical conversion of one species to another. Most commonly this characterization has been in terms of a number of compartments or "pools" and "turnover" coefficients between the different pools. A recent review article by Robertson [1] and a forthcoming book by Sheppard [2] survey the literature in this area. Mathematically [3] this approach can be shown to represent an approximation to the basic transport equation

$$-\operatorname{div} \mathbf{J}_k(\mathbf{r}, t) + s_k(\mathbf{r}, t) = \frac{\partial c_k(\mathbf{r}, t)}{\partial t}, \tag{1.1}$$

where $\mathbf{J}_k(\mathbf{r}, t)$ is the total vector flux of particles of the kth species at position $\mathbf{r}$ and time t, $s_k(\mathbf{r}, t)$ is the net production per unit volume from chemical reactions, and $c_k(\mathbf{r}, t)$ is the concentration. Here and subsequently we always refer to the labeled particles unless specifically stated otherwise. Physically this approach can be justified by the existence of various more or less discrete anatomical and physiological compartments and pools in biological systems. It also has the practical justification that frequently flux data take the form of the sum of several exponential terms, which is the form of solution obtained for the compartmental model.

The main difficulty with this approach is that it quite clearly is not a good approximation for certain problems, for example problems in which transport via the circulatory system is important. Such systems can of course be described by the partial differential equation (1.1), but this is essentially vacuous because the equation can rarely be solved for biological geometries. An attempt has been made therefore to find mathematical descriptions more general than the compart-

mental approximation and yet not completely useless from a manipulative point of view. One such is to describe the system by an integral equation or system of integral equations in which the kernel is regarded as characteristic of the system. This approach has been applied to a variety of problems. (See [3] for references.) Historically the method was applied by Volterra to a variety of hereditary phenomena including biological problems [4], [5]. In earlier papers [3], [6] we have discussed in detail the formulation of these equations for certain types of biological systems. In this paper we will consider the formulation from a more general point of view and will discuss the problem of determining the kernel from experimental data. To illustrate the theory we will briefly discuss a particular problem in lipid metabolism.

2. Formulation of equations

We will assume that the primary experimental variables in a system are a set of fluxes, finite in number, denoted by $\gamma_1(t), \gamma_2(t), \cdots, \gamma_n(t)$. For the moment we will consider these to be isotopically labeled molecular fluxes, although the theory we develop is obviously not so restricted. We make the fundamental assumption that each flux in this set at time t is given by a linear functional on all previous flux in the set plus an additive term accounting for material being newly introduced into the system. Specifically, we assume

$$\gamma_i(t) = \sum_j \int_{-\infty}^{t} w_{ij}(t, \omega)\gamma_j(\omega)\, d\omega + m_i(t). \tag{2.1}$$

If we give the fluxes the vector representation

$$\boldsymbol{\Gamma}^T = [\gamma_1(t), \gamma_2(t), \cdots, \gamma_n(t)], \tag{2.2}$$

and the additive term the representation,

$$\mathbf{M}^T = [m_1(t), m_2(t), \cdots, m_n(t)], \tag{2.3}$$

the system (2.1) can be written as the operator equation

$$\boldsymbol{\Gamma} = \mathbf{W}\boldsymbol{\Gamma} + \mathbf{M}. \tag{2.4}$$

In (2.4) we define $\mathbf{W}\boldsymbol{\Gamma}$ to be a column vector with ith entry

$$\mathbf{W}\boldsymbol{\Gamma}_i(t) = \sum_j \int_{-\infty}^{t} w_{ij}(t, \omega)\gamma_j(\omega)\, d\omega. \tag{2.5}$$

Equation (2.4) has the formal solution

$$\boldsymbol{\Gamma} = (\mathbf{I} - \mathbf{W})^{-1}\mathbf{M} \tag{2.6}$$

and

$$\boldsymbol{\Gamma} = (\mathbf{I} + \mathbf{W} + \mathbf{W}^2 + \cdots + \mathbf{W}^n + \cdots)\mathbf{M}. \tag{2.7}$$

Either (2.6) or (2.7) gives a formal solution as can be seen by direct substitution in (2.4). However, the series in (2.7) must be computable and convergent to have any real meaning. This problem will be discussed below. One can regard

equations (2.6) and (2.7) from two points of view, depending on the situation. One is that the operator **W** is known or assumed and that the problem is to compute the response of the system to an arbitrary input **M**. This, for example, is the problem the engineer faces in computing the response of an electrical network composed of known circuit elements. This is primarily the point of view taken in earlier papers [3], [6]. The other point of view is to regard **W** as unknown, and from measurement of the fluxes after known input to determine **W**. As can be seen from (2.6), what can actually be determined experimentally is $(\mathbf{I} - \mathbf{W})^{-1}$, from which $(\mathbf{I} - \mathbf{W})$ and hence **W** can be found, at least in principle, by some appropriate inversion process. It is this problem we will now consider. Before going on to it, however, some additional remarks can be made about the above equations. The first is that in equation (2.1) the summation can be replaced by integration over one or more parameters, say over space or momentum. In actuality fluxes usually are more or less continuously distributed in space and momentum, but in biological systems this problem is too difficult to solve; hence it must be replaced by some approximation in a finite number of fluxes. Also, fluxes integrated over some range of space and momentum usually constitute the actual experimental data. The second remark is that if the continuous distributions in time are replaced by discrete terms, the vector notation and the operator formalism remain unchanged, although the operator instead of being of the integral type is an ordinary matrix. This fact is highly useful in numerical computations, particularly machine computations, and also frequently is of considerable heuristic value in more general considerations. A final comment is that the integrations in (2.1) are ordinarily taken from some initial zero time, marking the original perturbation of the system by the introduction of labeled material.

3. Determination of the operator W from experimental data

3.1. *Finite matric representations.* We will first consider the case in which the continuous time distributuion is approximated by a finite number of discrete terms. Specifically we will suppose that in (2.1) each $\gamma_i(t)$ is replaced by a finite number of discrete numbers, each number representing the total flux during a time interval Δt_i, the time interval of integration being divided into m such periods, it being assumed there is some finite lower limit, say zero, to the integration. Each $\gamma_i(t)$ is then represented by a column vector with m entries, whose transpose is

$$\boldsymbol{\gamma}_i^T = [\gamma_{i1}, \gamma_{i2}, \cdots, \gamma_{im}]. \tag{3.1.1}$$

Equation (2.1) then becomes the linear system

$$\gamma_{il} = \sum_j \sum_k w_{ijlk}\gamma_{jk} + m_{il}, \tag{3.1.2}$$

where w_{ijlk} is the probability that a particle which contributes to the jth flux

in the kth time interval contributes to the ith flux in the lth time interval. Equation (3.1.2) can be written

$$\gamma_i = \sum_j \mathbf{w}_{ij}\gamma_j + \mathbf{m}_i, \tag{3.1.3}$$

where each $\mathbf{w}_{ij}$ is an m by m matrix. If as in (2.2) we define

$$\boldsymbol{\Gamma}^T = [\gamma_1, \gamma_2, \cdots, \gamma_n] = [\gamma_{11}, \cdots, \gamma_{1m}, \gamma_{21}, \cdots, \gamma_{nm}], \tag{3.1.4}$$

then $\boldsymbol{\Gamma}$ is a column matrix with nm entries, and the operator equation (2.4) retains its form

$$\boldsymbol{\Gamma} = \mathbf{W}\boldsymbol{\Gamma} + \mathbf{M}, \tag{3.1.5}$$

but the operator $\mathbf{W}$ is now a matrix built up from the $\mathbf{w}_{ij}$ matrices, thus with $(nm)^2$ scalar entries. As above we can write the solution of (3.1.5)

$$\boldsymbol{\Gamma} = (\mathbf{I} - \mathbf{W})^{-1}\mathbf{M}. \tag{3.1.6}$$

Let us now consider the problem of determining $(\mathbf{I} - \mathbf{W})^{-1}$ from experimental data. Mathematically it is obvious that $(\mathbf{I} - \mathbf{W})^{-1}$ is completely determined by its transformation of any linearly independent set of inputs nm in number, that is, any basis of the $\boldsymbol{\Gamma}$ space. Clearly for experimental simplicity the orthonormal basis $(1, 0, \cdots, 0)$ $(0, 1, 0, \cdots, 0), \cdots, (0, \cdots, 0, 1)$ is the logical choice. Each $\boldsymbol{\Gamma}$ obtained by operating on this basis is then the corresponding column of the matrix $(\mathbf{I} - \mathbf{W})^{-1}$. That is, if we have

$$\mathbf{M}_1^T = (1, 0, \cdots, 0), \tag{3.1.7}$$

$$\boldsymbol{\Gamma}_1 = (\mathbf{I} - \mathbf{W})^{-1}\mathbf{M}_1, \tag{3.1.8}$$

then the entries in $\boldsymbol{\Gamma}_1$ are those in the first column of $(\mathbf{I} - \mathbf{W})^{-1}$ and so on. Once $(\mathbf{I} - \mathbf{W})^{-1}$ is known it can be inverted to give $(\mathbf{I} - \mathbf{W})$ and hence $\mathbf{W}$. We assume it is nonsingular. This is virtually assured by the physical situation, because if not, then two different inputs would give the same output, or to put it differently some nonzero input would give zero output. Experimentally the vector $(1, 0, \cdots, 0)$ corresponds to the introduction of unit quantity of material into the system with momentum and spatial distribution corresponding to $\gamma_1(t)$ during the first time period; and the input with the sth entry unity, others zero, and $s = (j - 1)m + k$, corresponds to the introduction of unit material into the jth flux during the kth time period.

3.2. *Volterra systems.* Instead of the above finite approximation let us return to the system of integral equations (2.1). The operator $\mathbf{W}$ now becomes a matrix composed of the integral operators of (2.1). Thus, by definition, we have

$$(\mathbf{IM})^T = [m_1(t), m_2(t), \cdots, m_n(t)], \tag{3.2.1}$$

$$(\mathbf{WM})^T = [\mathbf{WM}_1(t), \mathbf{WM}_2(t), \cdots, \mathbf{WM}_n(t)], \tag{3.2.2}$$

where

$$\mathbf{WM}_i(t) = \sum_j \int_0^t w_{ij}(t, \omega)m_j(\omega)\, d\omega. \tag{3.2.3}$$

As indicated by (3.2.3) $\mathbf{WM}$ is a single column matrix with n entries, each a function of time. Successive iterates, which appear in the expansion of $(\mathbf{I} - \mathbf{W})^{-1}\mathbf{M}$, are defined in the usual way, that is,

$$\mathbf{W}^2\mathbf{M} = \mathbf{W}(\mathbf{WM}), \tag{3.2.4}$$

$$\mathbf{W}^s\mathbf{M} = \mathbf{W}(\mathbf{W}^{s-1}\mathbf{M}). \tag{3.2.5}$$

Denoting the transpose of $\mathbf{W}^{s-1}\mathbf{M}$ by

$$(\mathbf{W}^{s-1}\mathbf{M})^T = [\mathbf{W}^{s-1}\mathbf{M}_1(t), \cdots, \mathbf{W}^{s-1}\mathbf{M}_n(t)], \tag{3.2.6}$$

we obtain for the ith entry in $\mathbf{W}^s\mathbf{M}$ from the operator definitions

$$\mathbf{W}^s\mathbf{M}_i = \sum_j \int_0^t w_{ij}(t, \omega)\mathbf{W}^{s-1}\mathbf{M}_j(\omega)\, d\omega. \tag{3.2.7}$$

The resolvent transformation which we will denote by $\mathbf{K}$ is then defined by

$$(\mathbf{I} + \mathbf{K})(\mathbf{I} - \mathbf{W}) = \mathbf{I}. \tag{3.2.8}$$

Formally we clearly have for $\mathbf{K}$ the expansion

$$\mathbf{K} = \mathbf{W} + \mathbf{W}^2 + \cdots + \mathbf{W}^n + \cdots, \tag{3.2.9}$$

with the corresponding kernel matrix

$$\mathbf{K}(t, \omega) = \mathbf{W}(t, \omega) + \mathbf{W}^2(t, \omega) + \cdots + \mathbf{W}^n(t, \omega) + \cdots. \tag{3.2.10}$$

The entries in $\mathbf{W}(t, \omega)$ are the $w_{ij}(t, \omega)$ and the entries in $\mathbf{W}^n(t, \omega)$ are given by

$$w_{ij}^n(t, \omega) = \sum_k \int_\omega^t w_{ik}(t, \tau)w_{kj}^{n-1}(\tau, \omega)\, d\tau. \tag{3.2.11}$$

The convergence of the series

$$\mathbf{KM} = [\mathbf{W} + \mathbf{W}^2 + \cdots + \mathbf{W}^n + \cdots]\mathbf{M} \tag{3.2.12}$$

is assured by the fact that the entries in $\mathbf{W}(t, \omega)$ are Volterra kernels, that is, $\mathbf{W}(t, \omega) = 0$ if $\omega > t$. The argument remains essentially the same for the matrix as for a single function (see [7], p. 147). Briefly, if $W_{\max}$ is the upper bound of the entries in $\mathbf{W}(t, \omega)$ and A is the upper bound of the integrals $\int_0^t m_j(\omega)\, d\omega$, it is easily shown that the entries in $\mathbf{WM}$ are maximized by $nW_{\max}A$ and the entries in $\mathbf{W}^s\mathbf{M}$ by $(nW_{\max})^sA\, t^{s-1}/(s-1)!$. It follows that the series for $\mathbf{KM}$ is uniformly convergent for any finite upper bound of t.

Let us now consider the problem of determining the operator $\mathbf{K}$ from experimental data. We have the general operator equation

$$\boldsymbol{\Gamma} - \mathbf{M} = \mathbf{KM}. \tag{3.2.13}$$

We will suppose that at some time ω unit quantity of trace material is suddenly (that is, as rapidly as experimentally possible) introduced into the jth flux, all other inputs remaining zero throughout the experiment. Mathematically this corresponds approximately to the condition

$$m_j(t) = \delta(t - \omega). \tag{3.2.14}$$

If subsequently the fluxes are all measured, we will determine the entries in

$\boldsymbol{\Gamma} - \mathbf{M}$ for this injection function. The ith entry in the column matrix $\boldsymbol{\Gamma} - \mathbf{M}$ will then determine an entry $K_{ij}(t, \omega)$ in the $\mathbf{K}(t, \omega)$ matrix. In other words, with this isolated experiment we can determine one column of the $\mathbf{K}(t, \omega)$ matrix for one value of ω and all subsequent values of t. This is essentially *all* the information which can be obtained from a single experiment. In order to gain additional information further separate experiments must be performed. This obviously requires the assumption that the characteristic matrix for the system $\mathbf{W}(t, \omega)$ remains the same in the separate experiments. In other words, it must be possible to repeat the experiment either with the same system or with another nearly identical system. Let us assume that this can be done. If the above experiment is repeated, with the injection function for each of the n fluxes being $\delta(t - \omega_1)$ sequentially, each experiment will determine a column of $\mathbf{K}(t, \omega_1)$, and the set will determine the matrix $\mathbf{K}(t, \omega_1)$. If these experiments are repeated for a set of values of ω, say $0, \omega_1, \cdots, \omega_n$, the corresponding set of matrices $\mathbf{K}(t, 0)$, $\mathbf{K}(t, \omega_1), \cdots, \mathbf{K}(t, \omega_n)$ will be obtained. From this sequence a continuous approximation of $\mathbf{K}(t, \omega)$ which will be designated by $\mathbf{K}'(t, \omega)$ can be constructed by some suitable interpolation scheme. Then from $\mathbf{K}'(t, \omega)$ the characteristic matrix of the system $\mathbf{W}(t, \omega)$ can be computed approximately. For some purposes this may be superfluous, the entries in $\mathbf{K}'(t, \omega)$ giving the desired information, but usually the inversion will give additional information. Basically, the inversion utilizes the general operator equation,

$$(\mathbf{I} + \mathbf{K})(\mathbf{I} - \mathbf{W}) = \mathbf{I}, \tag{3.2.15}$$

from which we obtain the reciprocal expansion

$$\mathbf{W} = \mathbf{K} - \mathbf{K}^2 + \mathbf{K}^3 - \cdots - (-1)^n\mathbf{K}^n - \cdots, \tag{3.2.16}$$

whose convergence is assured by the fact that $\mathbf{K}(t, \omega)$ is again a Volterra kernel. If we now denote $\mathbf{K} - \mathbf{K}'$ by $\boldsymbol{\epsilon}$, substitution gives

$$\mathbf{W} = (\mathbf{K}' + \boldsymbol{\epsilon}) - (\mathbf{K}' + \boldsymbol{\epsilon})^2 + \cdots. \tag{3.2.17}$$

Since the series expansion of $\mathbf{W}$ given by (3.2.16) yields a uniformly and absolutely convergent series when operating on any vector for which the integrals, $\int_0^t \gamma_i(\omega)\, d\omega$, are bounded, equation (3.2.17) can be rearranged to give

$$\mathbf{W} = \mathbf{K}' - \mathbf{K}'^2 + \cdots - (-1)^n\mathbf{K}'^n - \cdots + (\text{terms in } \boldsymbol{\epsilon}). \tag{3.2.18}$$

Here, we will pass over a discussion of the terms in $\boldsymbol{\epsilon}$, that is, the error terms, and simply assume they are small. We then have

$$\mathbf{W}(t, \omega) \cong \mathbf{K}'(t, \omega) - \mathbf{K}'^2(t, \omega) + \cdots - (-1)^n\mathbf{K}'^n(t, \omega) - \cdots, \tag{3.2.19}$$

where the iterations have the same definition as above.

3.3. *Convolution kernels.* It can be seen that an enormous amount of experimental data is necessary to determine a general Volterra kernel of the type $\mathbf{W}(t, \omega)$. Suppose for example, that one has a modest 4 by 4 matrix, and takes six time values $\omega_0, \omega_1, \omega_2, \omega_3, \omega_4, \omega_5$. This requires 24 separate experiments for one complete set of values. To test for significant statistical variation in the entries, sev-

eral complete sets would be required. In systems in which the kernel is of the convolution type, $\mathbf{K}(t - \omega)$, the amount of experimental data necessary for an analysis is enormously reduced, because each column need be determined for only one value of ω. Thus, for a 4 by 4 matrix, four separate experiments would give one complete set of data. The particular time ω, chosen for injection clearly is not important. Experimentally systems with convolution kernels are so-called "steady-state" systems. Once $\mathbf{K}(t - \omega)$ is determined it can be inverted as above If $\mathbf{K}(t - \omega)$ can be given an analytical representation the mathematical machinery of the Laplace transform is available for carrying out the manipulations. A detailed analysis of systems of the convolution type has been given in earlier papers [3], [6] and will not be repeated here. It is worth noting, however, that formally the analysis of these systems carries over to systems of the more general type. Hence, the operator equations developed previously for these systems remain valid, although any numerical or analytical representation becomes more involved.

3.4. *Systems with incomplete information.* So far in our analysis we have assumed that all fluxes are available for injection and subsequent measurement. This may not be the case. Let us suppose that s are available for injection, t for measurement. This will permit the experimental determination of st entries in the $\mathbf{K}(t, \omega)$ matrix. Possibly from other information additional entries of $\mathbf{W}(t, \omega)$ or of $\mathbf{K}(t, \omega)$ are known. In addition some of the unknown entries may have other restraints on them. The problem is to utilize all the available information to restrict the range of the $\mathbf{W}(t, \omega)$ matrix. The prototype of this problem has been considered by Berman and Schoenfeld for compartmental systems. In our discussion here, we will do little more than state the general problem.

The problem appears in its most elementary form in a system in which the operator is represented by a finite n by n matrix. As before we have the general operator equation

$$(\mathbf{I} + \mathbf{K})(\mathbf{I} - \mathbf{W}) = \mathbf{I}, \tag{3.4.1}$$

where the $\mathbf{K}$ and $\mathbf{W}$ are matrices. If the multiplications indicated by (3.4.1) are carried out, we will obtain n^2 scalar equations in the $2n^2$ entries in $(\mathbf{I} + \mathbf{K})$ and $(\mathbf{I} - \mathbf{W})$. Thus, in general, n^2 of the entries can be expressed as functions of the other n^2. If these are all known, a determinate solution is obtained; if only r of them are known, a solution with $n^2 - r$ arbitrary parameters is obtained. Restrictions on the values which can be taken by these parameters and the solutions may yield useful information.

Another way of looking at the problem is to consider all transformations $\mathbf{P}(\mathbf{I} + \mathbf{K})$ with corresponding transformation of the inverse $(\mathbf{I} - \mathbf{W})\mathbf{P}^{-1}$, which will preserve the known restraints on $\mathbf{K}$ and $\mathbf{W}$. All such mappings will then generate the space within which admissible solutions must lie. This is the approach taken by Berman and Schoenfeld in their paper on compartmental systems [8].

The problem can quite clearly be extended to integral operators of the Volterra

type, or for that matter to linear operators in general. As yet the only case we have investigated at all systematically is that in which the integral operators are of the convolution type. Here, instead of (3.4.1) we have

$$[\mathbf{I} + \mathbf{K}(p)][\mathbf{I} - \mathbf{W}(p)] = \mathbf{I}, \tag{3.4.2}$$

where $\mathbf{K}(p)$ and $\mathbf{W}(p)$ are matrices composed of the Laplace transforms of the entries in $\mathbf{K}(t)$ and $\mathbf{W}(t)$. The remarks made above apply except that the numerical entries in the $\mathbf{K}$ and $\mathbf{W}$ matrices are replaced by the corresponding functions in the transform parameter p. Hence, we will have n^2 relations, involving $2n^2$ functions. From these equations n^2 of the functions can be expressed (at least theoretically) in terms of the remaining n^2. If r of these are known, we will obtain a solution in terms of $n^2 - r$ arbitrary functions of p. As before rather general restraints may restrict the range of possible solutions and lead to useful information.

4. Application to a kinetic problem

4.1. *Analysis of fatty acid transport.* For obvious reasons, steady-state systems, that is, systems with a kernel of the convolution type, have received more practical attention, and the above theory will be illustrated with a rather simple application of this type. Nevertheless, conceptually the analysis of a much more complicated system would be much the same.

Our illustrative application arose in the experimental studies of Dr. D. S. Fredrickson and colleagues on lipid transport and metabolism. Basically the question is whether unesterified fatty acid (denoted by UFA) and fatty acid derived from the splitting of triglyceride (TGFA) follow the same metabolic pathway—more exactly whether the fatty acid derived from splitting triglyceride all or nearly all passes through the plasma pool of unesterified fatty acid before it is metabolized. The experimental details of the work have been described previously [9] and here we give only an outline of the mathematical analysis, the details of which will be published elsewhere.

Two sets of experiments were performed. In one set, C^{14} labeled UFA was injected directly into the blood plasma of dogs. Subsequently the specific activity of UFA in the plasma, and the specific activity of C^{14} labeled carbon dioxide in the respiratory output were measured. In the other set C^{14} labeled TGFA was injected into the plasma as chylomicra. Subsequently the specific activities of plasma UFA and of respired CO_2 were measured. In terms of these experiments the problem was to determine whether in the second set, the flux of labeled UFA out of the plasma was sufficient to account for the total output of labeled CO_2 or whether a significant fraction of TGFA was metabolized without passing through the plasma UFA pool. To decide this question it is clearly necessary to compute the output of labeled CO_2 from the labeled UFA which passes through the plasma pool. This can be done utilizing the above mathematical theory and information from the first set of experiments.

To set up our model we will suppose that we have two compartments, plasma and tissue. The plasma is considered to be uniformly mixed, but the tissue is not. A labeled UFA molecule leaving the plasma has three possible fates: (1) after a certain interim it returns to the plasma as an UFA molecule, (2) it is metabolized and its C^{14} appears in the respired CO_2, (3) it is deposited in some tissue depot where for practical purposes it remains indefinitely (of the order of months or years). It must be recognized, of course, that en route to these fates the UFA molecules may follow complicated and devious pathways through other lipid compartments located in both tissues and plasma. Let us designate the flux of UFA molecules leaving the plasma by $\gamma_1(t)$, the returning flux by $\gamma_2(t)$, and the flux of labeled CO_2 by $\gamma_3(t)$. We assume that these fluxes are related by integral operators of the Volterra type, and the experiments were performed so that the dogs were in as near a physiological steady state as possible. Hence, it is reasonable to assume that these operators are of the convolution type. Thus, we obtain the equations for the system

$$\gamma_2(t) = \int_0^t w_{21}(t - \omega)\gamma_1(\omega)\, d\omega, \tag{4.1.1}$$

$$\gamma_3(t) = \int_0^t w_{31}(t - \omega)\gamma_1(\omega)\, d\omega. \tag{4.1.2}$$

In the first set of experiments the fluxes, $\gamma_1(t)$, $\gamma_2(t)$, and $\gamma_3(t)$, are known from the experimental data: $\gamma_3(t)$ is measured directly and $\gamma_1(t)$ and $\gamma_2(t)$ are determined from measurements of plasma UFA activity, denoted by $c_1(t)$, in a way we will now briefly outline. If V is the plasma volume, we have for the total quantity of labeled UFA in the plasma

$$q_1(t) = c_1(t)V. \tag{4.1.3}$$

The rate of change of the total quantity of labeled UFA in the plasma pool obviously equals the difference between the ingoing and outgoing fluxes, or

$$\frac{dq_1}{dt} = \gamma_2(t) - \gamma_1(t). \tag{4.1.4}$$

During the initial phase of the experiment, UFA radioactivity in the plasma falls very rapidly and nearly exponentially. It is reasonable to assume that during this initial period $\gamma_2(t) = 0$. With this assumption and the assumption that the plasma is well mixed, we have

$$\gamma_1(0) = kc_1(0)V = kq_1(0) = \frac{dq_1(0)}{dt}, \tag{4.1.5}$$

where k is a turnover coefficient, which can be computed from (4.1.5). Subsequently we assume that k remains constant, that is, that a fraction k of labeled UFA molecules present in the plasma UFA pool leave it per unit time, so that

$$\gamma_1(t) = kq_1(t) = kc_1(t)V, \tag{4.1.6}$$

in which k, $c_1(t)$ and V are known directly from the experimental data [$V = m/q_1(0)$, where m is the total quantity of labeled UFA introduced initially]. The

flux of UFA entering the plasma, $\gamma_2(t)$, can then be computed from (4.1.4). Knowing the fluxes, $\gamma_1(t)$, $\gamma_2(t)$ and $\gamma_3(t)$, we can determine $w_{21}(t - \omega)$ and $w_{31}(t - \omega)$ by solving equations (4.1.1) and (4.1.2). We have done this analytically by approximating $\gamma_1(t)$ and $\gamma_2(t)$ and $\gamma_3(t)$ by sums of exponentials and using Laplace transforms. We have also carried out numerical solutions on an IBM 650. Both $w_{21}(t - \omega)$ and $w_{31}(t - \omega)$ have physiological significance, but it is the latter we want for our computation of the labeled CO_2 due to plasma UFA in the second set of experiments.

In the second set of experiments, the chylomicra are removed from the plasma and enter the tissue. Here, they are at least partially split into fatty acids and glycerol; at least a fraction of the fatty acid returns to the plasma and enters the plasma UFA pool. Subsequently this labeled UFA leaves the plasma with the possible fates given above. If we assume that the turnover constant k is the same as in the first set of experiments, a condition which is reasonably assured by the equivalent total UFA concentration in the two sets of experiments [10], $\gamma_1(t)$ can be computed from the plasma level of labeled UFA as in the earlier experiments, that is, by means of (4.1.6); and if the transport function $w_{31}(t - \omega)$ is assumed to remain the same, the production of labeled CO_2 from $\gamma_1(t)$ can be computed by equation (4.1.2). The total production of labeled CO_2 is measured directly. The excess of the total production of CO_2 over that computed to arise from $\gamma_1(t)$, gives the production from TGFA whose fatty acids *do not* cycle through the plasma pool of UFA. We have not yet completed our analysis of these experiments, but it turns out that a considerable fraction of TGFA is oxidized without passing through the plasma UFA pool.

5. Concluding remarks

In essence in the theory presented in this paper we represent the state of a biological system by a vector in an abstract space and consider the basic characterization of the system to be a linear operator in the space. We have outlined how the structure of the operator can be obtained from experimental data. It is clear that this representation is primarily a data reduction scheme. Until the method has been applied to a larger number of biological problems, it is difficult to assess its ultimate usefulness. One of the greatest assets of this approach is that it provides a precise mathematical language in which a large number of systems can be described and in terms of which experiments can be formulated. This we have tried to illustrate with the example from fatty acid transport and metabolism. Another great advantage of the method is that both stationary and nonstationary problems can be handled by it, utilizing the same formal language. The method does not yield any direct information about the microscopic structure of a system, but any microscopic theory must yield an operator which agrees with that derived directly from the experimental data, in much the same way that thermodynamic coefficients computed by statistical theory must agree with those experimentally measured.

It is apparent that we have raised a number of unanswered problems in this paper. There are statistical sampling problems relative to the construction of the operator from experimental data. We have just touched on the question of the analysis of systems with incomplete information. We hope that these and other problems will be solved by further investigation along the directions we have outlined.

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