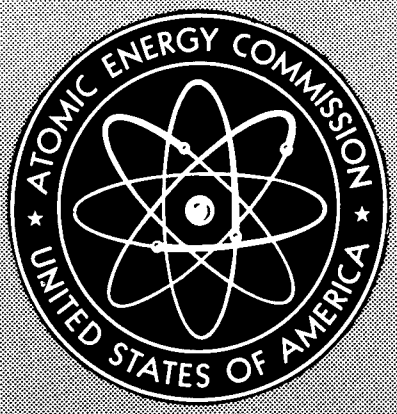

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A Statistical Tracer Theory for Analysis of Whole-System Retention Data

The application of tracer methods have, especially in medicine, opened a new class of physical problems, which have seemingly been given little consideration. Probably this is because the theoretical physicists today are so much occupied by quantum mechanics and theory of relativity that there is hardly any interest left over for what belongs to old fashioned statistical mechanics. Therefore, in the interpretation of tracer data, people have been forced to rely mainly on inductive methods, i.e., the construction of "models" that are not derived from basic principles but borrow their significance from how well they fit data. In an attempt to find a more deductive approach, I started some years ago a construction of what was intended to be a general physical tracer theory, a tracer dynamics (1). Although the emerging theory was general enough, and made possible a more precise formulation of some existing problems, it did not lead to anything of much practical result. One exception, however, can be noted: an ergodic-like relation (2) which in the following shall be explained and discussed.

Let us first consider the practical biologic problem in terms of a specific example. In a normal adult human there is a daily uptake and a balancing output of natural potassium ions. The result is a fairly constant amount of such ions in the body. This naturally present substance (in the present example, potassium) is in the following referred to as mother substance. A common experiment for the study of the potassium metabolism is to inject a small amount of potassium-42 ions (the chloride, for instance), the tracer, and then to measure how much $^{42}K^{+}$ remains in the body at different

times; frequently one also observes the specific activity in blood (i.e., the amount of tracer in a sample of blood divided by the amount of mother substance in that sample). The problem then is: on the basis of such data what can be said about the mother substance?

One answer is given by the following equation (3):

$$b_S^o = \frac{\int_o^\infty b_S(t)dt}{\int_o^\infty a_\upsilon(t)dt} \qquad \text{Eq. 1}$$

where b_S^o = the total steady-state amount of mother substance in the whole system (organism) S

$b_S(t)$ = the total amount of tracer in S at the time t

$a_\upsilon(t)$ = the specific activity at the time t in some arbitrary subsystem $S_\upsilon \subseteq S$

The term steady state means that the system is such that everywhere within S the amount of mother substance remains constant in time. The physical significance of this condition will be discussed in the following sections.

The total content of mother substance can thus be obtained from this type of experiment. However, there are several critical remarks that could be made about equation 1:

(i) In practical applications one would expect that there are subsystems in S with such a low turnover that hardly any significant amount of tracer ever appears there, although they might contain a significant amount of mother substance. These subsystems will not contribute to the data (the integrals) and, therefore, the right hand member of equation 1 does not determine b_S^o but rather a smaller amount, commonly referred to as the exchangeable mass (3). This quantity

lacks any precise physical definition of its own, and has to be introduced as an heuristic concept (4).

(ii) It is often difficult, if not even impossible (as seems to be the case for $^{42}K^{+}$), to follow the curves sufficiently long to obtain reasonably good estimates of the integrals.

(iii) Different functions $b_S(t)$ can give the same integral, which means that in equation 1 no attention is paid to the shape of the tracer curves. In other words, the tracer curves must contain more information than is revealed by equation 1; i.e., the exchangeable mass cannot be the only parameter characterizing mother substance that should be obtainable from tracer data.

In the following a new approach to tracer analysis will be outlined, which positively meets the objections (i - iii).

BASIC PRINCIPLES OF THE APPROACH

A rough formulation of the problem would be that what is needed is a more detailed analysis of tracer curves, giving a more detailed description of the mother substance, its distribution and "kinetic properties" within S. The most commonly used approach today is LFCA, linear first order compartment analysis (5). The metabolic system S is subdivided into a finite number of so-called compartments C_1, C_2,.....,C_n, where n usually is somewhere between 1 to 6. The underlying assumption is that all tracer flows from C_i ($i=1,2,...n$) are linear functions of the tracer content in C_i. In other words, one assumes first-order kinetics, which occasionally is interpreted such that each C_i constitutes a "homogeneous" or "well-stirred" subsystem of S; see further the section on the concepts of state and homogeneity. The compartment model, which thus results, is represented by a system of first-order linear differential equations with constant coefficients, having the tracer amounts in the compartments as t-functions. By fitting this equation system to the observed

functions $b_s(t)$ and $a_0(t)$ one obtains estimates of the coefficients, which then give the fluxes of mother substance (steady state assumed) between the compartments and to the environment of S, as well as the amounts of mother substance in the different compartments.

However, the concept of homogeneity applied to the compartment concept implies, as will be discussed later, some basic difficulties that obscure the physical significance of LFCA (5,6), and another approach has been suggested by Marshall (7). Instead of considering the metabolic pool as subdivided into compartments defined in morphological and chemical terms, Marshall considers compartmentalization in terms of "turnover times." But Marshall works with a specific system (calcium metabolism), and his method does not seem to be generally valid. In the following I shall outline a modification of Marshall's approach that seems to possess the desired generality. The emerging theory has a solid physical significance, based on earlier works, and the resulting representation is applicable to a broad class of systems, whether or not they are homogeneous (well-stirred). The applicability is not only in metabolic tracer work, but also in biochemistry (in vitro kinetics of complicated enzymatic systems) as well as in technologic tracer methodology.

THE ERGODIC PROPERTY

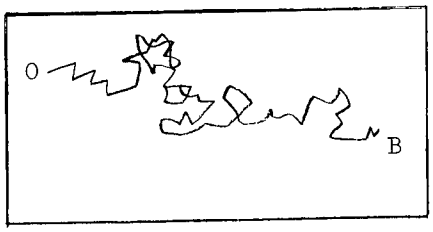
I shall try to limit the use of abstract arguments to a minimum. The essential parts of the mathematical formalism have already been worked out (8). I shall use the term <u>particle</u> meaning any kind of physical object that retains its identity throughout the system S; the "particle" may thus be an ion, a dye molecule, a labeled fish and so on.

The theory is based on the following postulate: While in S the particle is at any time in a definite <u>state</u>. By this I mean that at any time a complete description of the particle's physical condition is in principle possible;

this does not imply that such a laborious procedure has to be carried out. If the particle is in some state x_1 at the time t_1, there exists a definite probability for this particle to be in some other state x_2 at the time t_2. This probability depends only on x_1, x_2 and t_2-t_1, and is thus the same for all particles and all values of t_1; it has finite derivatives, of all orders, in the difference t_2-t_1.

In a more sophisticated language the postulate implies that the movement of a particle through S is a stationary, time-homogeneous Markovian process, but there is nothing important in that terminology and we can instead concentrate our attention on Figure 1. The rectangle denotes a closed S, and each possible position within it represents a definite state of a particle; it is assumed that no matter what state the particle is in it can sooner or later always reach any other state within S (the system is said to be irreducible).

Figure 1



The particle moves thus at random around in S. Assume that at the time $t = 0$ we observe a particle in position (state) 0, and then follow its movements until it finally reaches the state B at $t = T$. As illustrated in Figure 1, the particle has not passed through all states, some states only once, other states twice and so on. If now T increases, the particle will eventually have passed through all the states (9), but some states more often than others. Assume that T is very large, and that we at some arbitrary time $t > T$ observe the particle in the state x: the number of trajectories through x (i.e., the number of times the particle has passed through x) can then be

used to measure the probability of the particle to be found in that particular state.

This has the following important consequence. Consider the situation where, instead of one single particle, S contains a very large number of identical particles, strolling around independently of each other. It can then be shown (10) that if the system is left undisturbed for a sufficiently long time, it will eventually come to a stationary state, or equilibrium, which means that the density (or number) of particles in each state remains constant in time. Moreover, and this is the very crucial thing indeed, it can be shown that for any state x inside S, this equilibrium density of particles is directly proportional to the number of times our previous, lonely particle has passed through that state.

What makes this important is that by observing a time process (the movement of a particle) we can estimate the stationary (i.e., time independent) distribution of particles. Commonly this is referred to as an ergodic property, and the kinds of system here considered (closed and irreducible) are therefore frequently called ergodic systems.

Let us now consider an open system, i.e., a system which a particle will eventually leave no matter what states it passes through (Fig. 2). To simplify the discussion also this S is assumed to be irreducible (there is no serious loss of generality by this assumption). It is clear that a particle entering S will then spend only a finite time in S, which means that there is no justification in assuming T to be arbitrarily large. Consequently, the trajectory described by this particle (the solid one in Figure 2) can hardly be expected to pass through all possible states in S: observations of the particle will therefore not be representative of the whole system. However, if we repeat the experiment with another particle, the obtained trajectory will, with large likelihood, be different (the dotted trajectory in Figure 2). Thus, if we repeat the experiment over and over again, we shall eventually obtain a family

of trajectories that covers the whole S. It can then be shown that the number of trajectories passing through some state x in S has definite physical significance (11):

Figure 2

Let n(x) be the number of trajectories passing through x. Assume that there is a constant input q^o of particles to S (q^o = number of particles entering S per unit of time). It is then easily shown (12) that if this situation prevails for a sufficiently long time, the system will come to a steady state: the input of particles becomes equal to the output and, moreover, the number of particles in each state x remains constant in time. If $b^o(x)$ denotes the steady-state amount of particles in the state x, the following relation can be proved (13):

$$b^o(x) = q^o k\, n(x) \qquad \text{Eq. 2}$$

where k is a positive constant to be defined later. In other words, the steady-state density of particles in a state x in S is proportional to the number of tracer trajectories through that state.

The similarity between equation 2 and ergodic property that was formulated for the closed system is obvious: equation 2 may therefore be referred to as an ergodic relation for the open system, and is identical with the one previously mentioned as the origin of equation 1, which in the following will be the main topic of discussion.

Before proceeding, however, let us first notice and appreciate the fundamental differences between open and closed S. When dealing with a closed

S, we were considering a single particle, its trajectory within S during the time T, and we could carry out a limiting process (i.e., allow T to increase to arbitrarily large values); the number of trajectories through a state x will then measure the probability of finding that particular particle in that particular state. Nothing similar is possible for an open S, but we have to repeat the experiment over and over again, and interpret the number n(x) of trajectories through x not as the probability of a single particle but in terms of a large population of particles in steady state.

Moreover, when considering a closed S we made use of an equilibrium, stationary distribution. An open S, however, can never achieve such a state: the ordinary equilibrium of closed S is thus, for an open S, replaced by the steady state. That is, for a closed S, the stationary distribution is the natural reference system (time-independent) in terms of which we can interpret the observed time-process, and for an open S the steady-state distribution is the corresponding reference system. This is the reason why, when considering open metabolic systems, the steady-state assumption is absolutely necessary! (14).

THE APPLICATION TO TRACER THEORY

The application is straightforward. We can, in equation 2, identify q^o with the input (daily uptake) of mother substance, $b^o(x)$ with the amount of mother substance in the state x when S is in steady state, and n(x) with the number of times tracer particles (no matter which ones) have passed through x. The remaining problem is the factor k and it is solved as follows:

n(x) can be interpreted as being directly proportional to the <u>time of sojourn</u> $\theta(x)$ in state x, i.e., the total time a particle is "expected" to spend in x. In other words

$$n(x) = k' \, \theta(x)$$

where k' is another constant independent of x. If we consider an arbitrary subsystem (domain) S_v in S, the sum over all states x in S_v is

$$n_v = \sum_{S_v} k'\theta(x) = k'\,\theta_v$$

where θ_v is the time of sojourn in S_v, i.e., the total time a particle is expected to spend in S_v. Summing over all states in S,

$$n_S = \sum_{S} k'\theta(x) = k'\theta_S$$

which thus gives the total time a particle is expected to spend in S, i.e., between entrance and exit (15). Finally it can be shown (16) that k.k' = 1 and that

$$\theta(x) = \frac{1}{b_S(0)} \int_0^\infty b(x,t)\,dt$$

where b(x,t) is the amount of tracer in x at the time t, and $b_s(0)$ is the total amount tracer supplied to S at the start of experiment (t = 0). It is thus assumed that there is a single instantaneous tracer supply, but this assumption is not necessary and is introduced merely to simplify the formalism.

Using these results we obtain from equation 2 that

$$b_v^o = q^o\theta_v = \frac{q^o}{b_S(0)} \int_0^\infty b_v(t)\,dt$$

where b_v^o is the steady state amount of mother substance in S_v and $b_v(t)$ is the corresponding amount of tracer at the time t. Writing the subscript $v = S$ we get the same quantities for the whole system (i.e. $S_v = S$) and if we define

$$a_v(t) \equiv b_v(t)/b_v^o$$

equation 1 is immediately obtained.

My motivation for using this particular way of deriving equation 1 is that it provides us with a tool, by which we can easily visualize the physical significance of the basic equation

$$b_\upsilon^o = q^o \theta_\upsilon \qquad \text{Eq. 4}$$

namely that the steady-state amount of mother substance in some arbitrary subsystem S_υ is proportional to the density of tracer trajectories through that domain. This gives us an intuitive understanding of why and how tracer tells us something about mother substance. And it is on the basis of this interpretation of equation 4 we shall be able in the next section to make use of the previously mentioned idea induced by Marshall.

Before concluding this section there is another quantity that has to be introduced. Let us consider a particle in some state x. As long as the particle remains there it has, according to the postulate, a definite probability to leave the system S within some fixed time interval. Denote this probability by $\lambda_E(x)$ and consider the expression

$$\lambda_{E\upsilon}^o = \frac{1}{b_\upsilon^o} \sum_{S_\upsilon} b^o(x)\ \lambda_E(x)$$

Hence, $\lambda_{E\upsilon}^o$ is the <u>mean exit probability</u> in S_υ, calculated with respect to the steady-state distribution in that subsystem. By putting $\upsilon = S$ we obtain the mean exit probability in the whole system with respect to the steady-state distribution of mother substance in S. It holds that (17)

$$\lambda_{ES}^o = 1/\theta_S \qquad \text{Eq. 5}$$

Hence, as

$$\theta_S = \frac{1}{b_S(0)} \int_0^\infty b_S(t)\ dt \qquad \text{Eq. 6}$$

the area under the whole-body retention curve can be given a precise microphysical interpretation: it determines the mean exit probability λ_{ES}^o with respect to the steady-state distribution.

To me this is an important result, because it shows that data from systems of enormous microphysical complexity can, occasionally, be given a precise microphysical interpretation. In some way λ^{o}_{ES} is analogous to the temperature concept in physics: temperature can be interpreted as a parameter characterizing the mean energy over the population of molecules (i.e., temperature is given a microphysical significance). But, for this interpretation to be valid the physical system must be in thermal equilibrium (the Bolzman distribution, for instance), and similarly for the interpretation given here of retention curves, S must be in steady state. So here again we have an illustration of how the steady-state distribution plays a role for the open system similar to that of the equilibrium or stationary distribution in the theory of closed systems.

TURNOVER COMPARTMENTS

There is one major conclusion that can be made from the preceding analysis: it is the complete tracer process, and not separate parts of it that tells us something about mother substance; this is why integrals appear in the equations. Phrased differently, it can be said that one has to follow the trajectory of a tracer particle from its beginning to its end (i.e., when the particle leaves S). However, one single trajectory is a random event and gives a poor or, rather, uncertain information about the system. It is, therefore, necessary to carry out a large number of statistically independent "experiments", a condition that actually is rather well satisfied in a tracer experiment: each tracer particle represents one independent trial. A tracer experiment might be looked upon as generating a large population of trajectories through S, and the observed tracer variables may be considered as averages over this population; the magnitude of Avogadro's number implies that the statistical error is negligible.

It is an obvious statement that, among the population of trajectories generated by a tracer experiment, some are fairly short whereas others are of a considerable length. That is, some tracer particles leave S shortly after they entered, whereas other particles will stay in the system for quite a while. Hence, we can subdivide the trajectory population into subpopulations with respect to the length of the different trajectories.

Let us consider the total t-interval (0,T) during which the experiment is carried out, and divide it into intervals

$$0 < t_1 < t_2 \ldots . < t_{n-1} < t_n = T \ .$$

As before, $b_S(t)$ denotes the amount of tracer present in S at the time t; i.e., the whole-body retention curve. Then $b_S(0) - b_S(t_1)$ is the number of tracer particles leaving S in the first t-interval and, generally, $b_S(t_{i-1}) - b_S(t_i)$ is the number of particles leaving S in the ith interval. This corresponds to a subdivision of the total population of trajectories, as discussed in the preceding paragraph. But, as the density of trajectories in each such subpopulation corresponds to a steady-state density of mother substance, the procedure simply implies a subdivision of the total pool of mother substance with respect to when the particles leave S. Consequently, we have a division of the population of mother substance particles into n mutually exclusive <u>turnover compartments</u> K_1, K_2, ..., K_n, each of which has a definite steady-state amount of mother substance and a "kinetics" (i.e., a λ_E°-parameter) of its own. The following will show how these quantities can be estimated from $b_S(t)$-curves.

Since in the present context, the main purpose of the integral of $a_0(t)$ in equation 1, is to determine q° this integral will from now on be omitted: the amount of mother substance is always directly proportional to q°, and we shall thus here satisfy ourselves with the <u>relative sizes</u> of K_1, ... K_n. If the a(t)-integral can be obtained, the absolute sizes are easily calculated from the quantities obtained by the methods described in the following.

Let us start by defining the function

$$P_i(t) = \frac{b_S(t) - b_S(t_i)}{b_S(0) - b_S(t_i)}, \quad \begin{matrix} t \leq t_i \\ i = 1,2,\ldots,n \end{matrix} \qquad \text{Eq. 7}$$

Consequently, $P_i(0) = 1$, $P_i(t_i) = 0$, and the function itself may be interpreted as the probability that a particle will be in S at the time t, given that it belongs to any of the compartments K_1, K_2, ... K_i. That is, $P_i(t)$ is a so-called conditional probability, where the condition is that the particle is known to leave S somewhere during the time t_i (we start at t = 0).

$$\theta^*_{o,i} = \int_o^{t_i} P_i(t)\, dt \qquad \text{Eq. 8}$$

is the conditional time of sojourn. To get the "absolute" time of sojourn $\theta_{o,i}$, $\theta^*_{o,i}$ must be multiplied by the probability that a particle entering S will belong to the set of particles corresponding to the joint compartments K_1, K_2 ... K_i. This probability is given by $[b_S(0) - b_S(t_i)]/b_S(0)$ and, consequently

$$\theta_{o,i} = \int_o^{t_i} \frac{b_S(t) - b_S(t_i)}{b_S(0)}\, dt \qquad \text{Eq. 9}$$

Finally, if θ_i denotes the time of sojourn of K_i alone,

$$\theta_i = \theta_{o,i} - \theta_{o,i-1} . \qquad \text{Eq. 10}$$

Here θ_i has the same significance as θ_v in equation 4. That is

$$b_i^o = q^o \cdot \theta_i \qquad \text{Eq. 11}$$

is the steady-state amount of mother substance in compartment K_i; in other words b_i^o is the "size" of K_i, and θ_i determines what we then call the relative size of K_i.

Compartment K_i can be meaningfully subdivided into two mutually exclusive subcompartments:

$$K_{i1} = \text{the initial } K_i\text{-compartment}$$
$$K_{i2} = \text{the final } K_i\text{-compartment}$$

where K_{i1} corresponds to those particles in K_i that have spent a time less than t_{i-1} in S, and K_{i2} to those particles in K_i that have spent a time greater than t_{i-1}. Only K_{i2} contributes to the output of S; i.e., the exit probability of particles in K_{i1} is zero.

From equation 9 we understand that, because times of sojourn of mutually exclusive compartments are additive, the integral between t_{i-1} and t_i gives the time of sojourn in K_{i2}, that is

$$\theta_{i2} = \int_{t_{i-1}}^{t_i} \frac{b_s(t) - b_s(t_i)}{b_s(0)}\, dt \qquad \text{Eq. 12}$$

and obviously,

$$\theta_{i1} = \theta_i - \theta_{i2} \qquad \text{Eq. 13}$$

which is the time of sojourn in K_{i1}.

θ_{i1} and θ_{i2} measure the relative sizes of the corresponding compartments. What remains is to find a measure of the kinetics of K_i. However, as K_{i1} does not contribute anything to the output of S, we are only interested in K_{i2}. Consequently, we are interested in obtaining the mean exit probability with respect to the steady-state distribution in K_{i2}. Thus, given that the particle is in K_{i2}, its probability of still being in S at the time t ($t_{i-1} \leq t \leq t_i$) is given by

$$P_{i2}(t) = \frac{b_s(t) - b_s(t_i)}{b_s(t_{i-1}) - b_s(t_i)}, \quad t_{i-1} \leq t \leq t_i, \quad t_o = 0 \qquad \text{Eq. 14a}$$

and

$$\theta^{*}_{\cdot i2} = \int_{t_{i-1}}^{t_i} P_{\cdot i2}(t)\, dt \quad . \qquad \text{Eq. 14b}$$

The corresponding mean-exit probability is given, finally, by

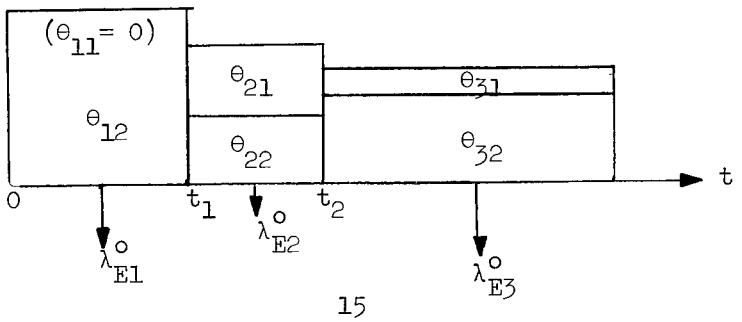
$$\lambda^{o}_{Ei} = 1/\theta^{*}_{\cdot i2} \quad . \qquad \text{Eq. 15}$$

It is important to recognize that $\theta^{*}_{\cdot i2}$ does not determine the size of K_{i2}; it gives only the time of sojourn in K_{i2} under the condition that the particle arrives at K_{i2} according to the distribution over the states in K_i at the time t_{i-1}; under the present conditions only a fraction of the total input q^o contributes to K_i. On the other hand, $\lambda^{o}_{Ei}(q^o \theta_{\cdot i2})$ gives the contribution of K_i to the total output from S.

THE TURNOVER DIAGRAM

It is essential that the results of the calculations (the data processing) can be exhibited in a neat form, so that the qualitative interpretation is simplified. Each turnover compartment K_i ($i = 1,2,\ldots,n$) is characterized by four parameters: θ_{i1}, θ_{i2}, λ^{o}_{Ei} and the time interval $[t_{i-1}, t_i]$. Figure 3 shows one possible type of diagram: the area of each "box" gives the corresponding θ-value (the relative size), together with the actual number printed inside the box. The length of the arrows is proportional to $\lambda^{o}_{Ei}\ \theta_{i2}$, i.e., proportional to the relative contribution of each compartment to the total output of mother substance from S.

Figure 3



An advantage of the suggested diagram is that it clearly reveals the fundamental character of the concept turnover compartment: There is no a priori assumed relation between the turnover compartments and the physico-chemical structure of the metabolic system. If any such relation exists it has to be shown by other means, independent of the tracer experiment. That is, the turnover compartment is defined independent of "location" and physico-chemical "functions", and from that viewpoint the concept becomes rather abstract. It is also clear that it makes no sense to speak of exchange of matter between turnover compartments.

THE CONCEPT OF "STATE"

A theory of the type considered here may be looked upon as language consisting of a set of basic concepts (words), and a postulate (grammar) ascribing some general properties to them. On this basis all other concepts and relations (equations) are derived; the basic concepts are thus not defined but considered "obvious". The basic concepts here used are, particle, state, and probability (18). Among these I feel that the concept of state needs some further considerations.

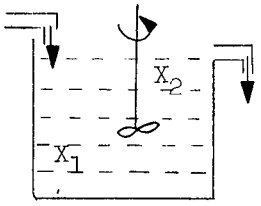
Let us start with an impossible type of system: S consists of one single state. According to the postulate all particles in S have the same constant probability λ_{ES} of leaving S. Hence, if we start at the time $t = 0$, the probability $P_S(t)$ that a particle will still be in S at some time $t \geq 0$ is given by (19)

$$P_S(t) = e^{-\lambda_{ES}t} \quad . \qquad \text{Eq. 14}$$

Hence, the expected number of particles remaining in S decreases according to a simple "exponential law".

Next, consider a system, which might be referred to as a well-stirred system (Figure 4): a small "open" vessel equipped with some stirring device. The system is in steady state: there is a constant inflow of plain water, an equal outflow, and the amount of water in the vessel remains constant. If now a small amount of tracer particles is added to the vessel at the time $t = 0$, and the tracer content of S is observed as a function of time, it is a well established experience that these data will satisfy equation 14. Hence, the well-stirred S behaves as if it consisted of one single state, which, however, is in bad agreement with the intuitive understanding of the state concept.

Figure 4



Thus, if at some instant we observe two particles simultaneously, one at x_1 and the other at x_2, we should hardly consider them as being in the same state. Moreover, it seems rather obvious that, at the moment of observation, the particle in x_2 has a higher probability of leaving S than has the particle in x_1. Consequently, on the basis of our intuitive understanding of the state concept, we cannot accept this S as consisting of one single state; the other possibility, namely that all states in S are equal with respect to the exit probability, must be rejected as well.

As a matter of fact we are here faced with a kind of a time-scale problem. Let $\lambda_E(x,\Delta t)$ stand for the probability of a particle in state x (a position x in the vessel of Figure 4) to leave the vessel within the time interval Δt; when $\lambda_E(x,\Delta t)$ is the same for all x then the process becomes a simple exponential $e^{-\lambda_E t}$, where $\lambda_E = \lambda_E(x)$ is such that $\lambda_E(x,\Delta t) = \lambda_E \Delta t + o(\Delta t)$. However, for $\lambda_E(x,\Delta t)$ to be independent of x depends on the scale in which Δt is measured. For instance, if Δt stands for a certain number of milliseconds,

$\lambda(x,\Delta t)$ will be different for different x, whereas it becomes almost independent of x if Δt is measured in hours. In other words, if we ask for the probability of a particle to leave S within 10 hours, say, we shall obtain the same answer for x_1 as for x_2, whereas this is not so when, in the question, hours is replaced by milliseconds (it is here assumed that the overall tracer process is so fast that after one hour almost all tracer particles have left S). That this has corresponding phenomenologic consequences has been shown elsewhere (20): when the time scale of observation is increased the system behaves as if the different states becomes lumped together, i.e., S behaves "simpler" in large time scale than in a small one. The phenomenon may be referred to as a time scale lumping.

The commonly employed way out of the dilemma is to state that the distribution of particles over the different states in S is time independent. Such a time independency of distribution leads to the desired system behavior (21). This is why in classic chemical kinetics the different chemical compounds in the reaction can be considered as "states": the necessary condition is that the system is in thermal equilibrium, which means that the energy distribution over the population of molecules is time-independent (the Bolzman distribution). In these concepts, then, one could consider the concept of "efficient stirring" as a generalization of the condition in chemical kinetics. However, the reason that the concept of time-invariant state distribution works in chemistry is that the condition of thermal equilibrium seems to be well satisfied also for rather fast chemical processes (22). In other words, within the range of time units in which chemical reactions are usually studied, the underlying concepts are invariably applicable: what is thermal equilibrium for a fast reaction is exactly the same thing for a slow one.

In the present investigation we are, however, faced with a different class of systems. First, the spectrum of time scales and experimental precision (absolute and relative) in which tracer studies are carried out has a

considerable width. Second, the discussion of Figure 4 shows that the well-stirred system is highly heterogeneous when considered in terms of the two basic concepts, state, and probability. Due to time scale lumping the phenomenologic behavior of such a system is likely to depend on the method of observation in terms of time scale and precision and, consequently, what in one tracer study appears to be a homogeneous S (all states are equal) may not necessarily be so in another tracer experiment. Or phrased differently, when we consider the concept of homogeneity in terms of time-invariance in the distribution of particles over the states in S, what under certain circumstances appears to be a time invariant distribution can under other conditions show significant variation.

The concept of homogeneity (or efficient stirring), which is further discussed in the next section, has played a fundamental role in current tracer theories (23), where it is usually defined in phenomenologic terms (the "exponential law"). Thus, the concept of the homogeneous compartment is the cornerstone in the LFCA-method previously mentioned, but as the preceding paragraph indicates, the approach is likely to lead to a rather uncontrolled situation: although different investigators might use the same terminology (compartment, efficient stirring, and so on) the microphysical significances of the words can be quite different. The situation is similar to describing a moving body in terms of a coordinate system that changes unpredictably with time. That a similar approach works in classic chemical kinetics is due to the particular circumstances there.

From this viewpoint it is therefore important to recognize the significance of the state concept introduced here: it is an invariant microphysical entity, which has the same meaning for all systems (experiments) to which the theory applies. Thus, whereas the number of exponential terms necessary to describe a tracer curve is a crucial point for many LFCA-methods the present theory puts no significance in this system property. The well-stirred system

is represented by the same kind of turnover diagram as is the highly "heterogeneous" system.

THE CONCEPT OF HOMOGENEITY

So far $b_S(t)$ has been considered both as the actual function observed by the experimenter and as the result of the observation. In the following it will be necessary to discriminate between those two quantities. Thus, from now on $b_S(t)$ denotes the physical function (or observed function), which is defined by S alone independently of experimental procedures, i.e., $b_S(t)$ is defined only by the set of states, corresponding to S, and a transition probability function on this set. The experimenter observes $b_S(t)$ and records the observations in a table or diagram and, by some fitting procedure, he ends up with a mathematical form $b_S^*(t)$, which conveniently describes the result of the complete experimental procedure (including the data processing). I shall call $b_S^*(t)$ the resulting function.

We may thus consider a procedure C operating on the physical function $b_S(t)$ giving the result (or image) $b_S^*(t)$:

$$C[b_S(t)] = b_S^*(t) \qquad \text{Eq. 15}$$

There is no reason to assume that the mathematical form of $b_S(t)$ is preserved by the mapping C. Thus, if we still assume that S contains a finite number of states $b_S(t)$ will consist of a sum of a finite number of exponentials, i.e., terms of the form $\beta_i e^{-\alpha_i t}$, where α_i is positive definite and t-independent, whereas β_i may contain sine and cosine functions of t. It can then be shown (22) that $b_S^*(t)$, written as a sum of $\beta_i^* e^{-\alpha_i^* t}$ (where β_i^* is t-independent), will in all likelihood contain a less number of exponentials than $b_S(t)$; so in these concepts there is no simple relation between $b_S(t)$ and $b_S^*(t)$. It is thus possible that the resulting function contains only one single exponential,

while the observed function contains a fairly large number of such terms. The only condition under which we can make a rather safe statement about $b_S(t)$, on the basis of the resulting function $b_S^*(t)$, is when $b_S(t)$ consists of one single exponential.

DEFINITION: S is said to be homogeneous if and only if $b_S(t)$ consists of one single exponential. S is said to be approximately homogeneous when it is non-homogeneous but $b_S^*(t)$ consists of one single exponential.

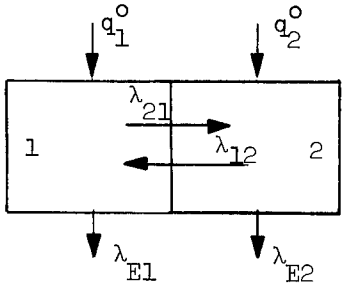
For illustrative purposes I shall discuss the simple two-state system shown in Figure 5. q_i^o is the number of mother-substance particles that enter S at state i (i = 1,2) per unit of time, and $\{\lambda_{ij}\}$ are the "rate constants" or "probability factors". The instantaneous tracer supply at t = 0 is throughout assumed to be equivalent (24): the amounts of tracer added to 1 and 2 are proportional to q_1^o and q_2^o respectively. It is only under this condition that tracer represents mother substance, and the ergodic relation holds.

It can easily be shown (25) that S becomes homogeneous in the present sense when $\lambda_{E1} = \lambda_{E2}$. Efficient stirring, on the other hand, gives rise to an approximately homogeneous S, as has been discussed in the preceding section. Commonly the concept of efficient stirring is interpreted as $\lambda_{12}, \lambda_{21} \gg \lambda_{E1}, \lambda_{E2}$. However, this condition does not approximate the homogeneous S defined by $\lambda_{E1} = \lambda_{E2}$, and the question becomes whether the behavior of the efficiently stirred S is due to its approximation to an homogeneity condition and, if so, what kind of condition is it?

First of all, the behavior of the efficiently stirred S cannot be a consequence of C alone, because we are here considering a property of S itself. Second, as I have pointed out in the previous section, attempts to consider homogeneity in terms of time independency of the tracer distribution in S lead to time-scale problems. We are thus looking for a quantitative condition (other than $\lambda_{E1} = \lambda_{E2}$), which will be insensitive to time-scale variations (or

the experimental procedure C in general) but will result in a homogeneous S that efficient stirring does approximate. The problem is solved by the following theorem.

Figure 5



THEOREM: S is homogeneous if

(i) S contains a finite number of states

(ii) $b(x_1,0) = b(x_2,0)$ for all $x_1,x_2 \in S$

(iii) $\dot{b}(x_1,0) = \dot{b}(x_2,0)$ for all $x_1,x_2 \in S$

That means, in terms of Figure 5, that $q_1^\circ = q_2^\circ$ and, if $b_i(t)$ is the amount of tracer in state i (i = 1,2) at the time t, it also is true that $\dot{b}_1(0) = \dot{b}_2(0)$. The proof of the theorem will show what restrictions on the matrix $\{\lambda_{ij}\}$ are implied by the conditions (ii) and (iii).

PROOF:

$\overline{b}(t) = \begin{pmatrix} b_1(t) \\ \vdots \\ b_n(t) \end{pmatrix}$ is the column vector of tracer contents in the states $x_1 \ldots\ldots x_n$ (S contains n states altogether.) For a unit instantaneous input of tracer at t = 0 (equivalent input assumed) one has (26)

$$\overline{b}(t) = \overline{\overline{\Lambda}}\, \overline{b}(t) \qquad [I]$$

where $\overline{\overline{\Lambda}} = \{\lambda_{ij}\}$, is the matrix of transition probability factors. Then

$$(ii) \Rightarrow \overline{b}(0) = \frac{1}{n} \begin{pmatrix} 1 \\ 1 \\ \vdots \\ 1 \end{pmatrix}, \quad \text{and} \quad (ii) \Rightarrow \dot{\overline{b}}(0) = \begin{pmatrix} -1 \\ -1 \\ \vdots \\ -1 \end{pmatrix}$$

Hence, according to [I]

$$\frac{1}{n}\begin{pmatrix}\lambda_{11} & \lambda_{12} & \cdots & \lambda_{1n}\\ \lambda_{21} & \lambda_{22} & \cdots & \lambda_{2n}\\ \cdot & \cdot & \cdots & \cdot\\ \lambda_{n1} & \lambda_{n2} & \cdots & \lambda_{nn}\end{pmatrix}\begin{pmatrix}1\\1\\\vdots\\1\end{pmatrix}=\frac{1}{n}\cdot\begin{pmatrix}\sum_i \lambda_{1i}\\ \cdot\\ \cdot\\ \cdot\\ \sum_i \lambda_{ni}\end{pmatrix}=\begin{pmatrix}-1\\-1\\\vdots\\-1\end{pmatrix}$$

$$\sum_{i=1}^{n}\lambda_{1i}=\sum_{i=1}^{n}\lambda_{2i}=\cdots\cdots=\sum_{i=1}^{n}\lambda_{ni}$$

Such a matrix $\bar{\bar{\Lambda}}$ has the eigenvector $\begin{pmatrix}1\\1\\\vdots\\1\end{pmatrix}$ and the corresponding eigenvalue $-n$. That is

$$\bar{\bar{\Lambda}}\begin{pmatrix}1\\1\\\vdots\\1\end{pmatrix}=-n\cdot\begin{pmatrix}1\\1\\\vdots\\1\end{pmatrix} \qquad [III]$$

The solution of [I] can be written as (27)

$$\bar{b}(t)=\sum_i C_i\,\bar{X}_i\,e^{a_i t} \qquad [IV]$$

where $\bar{X}_i$ is the eigenvector of $\bar{\bar{\Lambda}}$ corresponding to the eigenvalue a_i. It is assumed that $\bar{\bar{\Lambda}}$ of order $n \times n$, has n distinct eigenvalues (28). To obtain the coefficients $\{C_i\}_{i=1}^{i=n}$ we take the first derivative of $\bar{b}(t)$ and calculate its value at $t = 0$:

$$\dot{\bar{b}}(0)=\begin{pmatrix}-1\\-1\\\vdots\\-1\end{pmatrix}=\sum_i a_i C_i\begin{pmatrix}X_{1i}\\X_{2i}\\\vdots\\X_{ni}\end{pmatrix}=\begin{pmatrix}a_1C_1+a_2X_{12}C_2+\cdots+a_nX_{1n}C_n\\ a_1C_1+a_2X_{22}C_2+\cdots+a_nX_{2n}C_n\\ \cdots\cdots\cdots\cdots\\ a_1C_1+a_2X_{n2}C_2+\cdots+a_nx_{nn}C_n\end{pmatrix} \qquad [V]$$

where we have identified $\bar{X}_1$ with the known eigenvector $\begin{pmatrix}1\\1\\\vdots\\1\end{pmatrix}$.

The matrix

$$\begin{pmatrix} 1 & a_2X_{12} & \cdots & a_nX_{1n} \\ 1 & a_2X_{22} & \cdots & a_nX_{2n} \\ \vdots & \vdots & & \vdots \\ 1 & a_2X_{n2} & \cdots & a_nX_{nn} \end{pmatrix}$$

is nonsingular (29). Moreover, it is easily seen that $C_1 = \frac{-1}{a_1}$, $C_2 = C_3 = \ldots = C_n = 0$ is a solution to [V]. But as the matrix is nonsingular, this is the only solution (29). Hence, from [IV] we obtain that the solution of [I] has the form

$$\bar{b}(t) = \frac{-1}{a_1} \begin{pmatrix} 1 \\ 1 \\ \vdots \\ 1 \end{pmatrix} e^{a_1 t}, \quad a_1 = -n$$

which proves the theorem.

Consequently, the theorem states that if a finite tracer system has a transition matrix such that the sum of the elements in each row gives the same value for all rows, then the system will exhibit a single exponential tracer curve, provided the initial distribution of tracer is rectangular over the states in the system. In terms of Figure 5, this type of homogeneous S may be illustrated by $q_1^o = q_2^o = 1$, $\lambda_{E1} = 3$, $\lambda_{E2} = 1$, $\lambda_{21} = 1$, $\lambda_{12} = 2$. Certainly, this example presents us with a microphysically heterogeneous system: the two states are quite different in terms of λ_{Ei}, and the efficient stirring condition (i.e., $\lambda_{12}, \lambda_{21} \gg \lambda_{E1}, \lambda_{E2}$) is far from satisfied. I shall refer to this new class of homogeneous S as <u>weak homogeneity,</u> as distinguished from the <u>strong homogeneity</u> defined by the condition $\lambda_{Ei} = \lambda_{Ej} (i,j = 1,2,\ldots,n)$. In contrast to weak homogeneity, strong homogeneity implies that $b_S(t)$ is a single exponential no matter how the tracer is added to S; as a matter of fact, strong homogeneity implies that S behaves as if it contained one single state (the different states appear "lumped" together).

Now, which class of homogeneity does the well-stirred S illustrated by Figure 4 approximate? We have already seen that it does not approximate the strong homogeneity, and a moment's contemplation will show that it approximates the type of weak homogeneity defined by the preceding theorem. The fact that its behavior is independent of how the tracer is added is, in this particular case, irrelevant.

The existence of weak homogeneity is fundamental for our understanding of the behavior of many tracer systems, and of their striking simplicity. Characteristic of the weak homogeneity is that the behavior is partly due to how the tracer is added to S, a fact that again demonstrates how irrelevant it is to ascribe significance to the number of exponentials into which $b_S^*(t)$ can be resolved.

CONCLUDING REMARKS

Let us now return to equation 1 and the criticism of it. Objection (i) is obviously met by the approach I have outlined in the preceding sections. The term "exchangeable mass" thus appears somewhat inadequate, at least if it is not accompanied by a specification of the time interval under consideration. Hence, instead of speaking of exchangeable mass we now consider the sizes of certain turnover compartments, i.e., the amount of mother substance that under steady state conditions will stay in S a specified period of time.

A striking feature of the theory is the way in which it meets objection (ii). Thus, no matter how short the time period T of observation is we can always construct a turnover diagram. But, on the other hand, the diagram ends promptly at T, which means that the theory does not allow for any conclusions to be made beyond the time of observation. In other words, the turnover diagram represents actual data and nothing else. This is somewhat different from, say, LFCA, which is not a pure kinetic analysis of data in the present

sense: opposite to the present theory, LFCA takes into account also a priori knowledge or hypotheses about S, and makes possible a more extensive data analysis. In my own opinion this is a disadvantage of LFCA, in that this approach contains pitfalls and does not clearly discriminate between the observed kinetics of S and specific hypotheses; that is, there is in LFCA an inbuilt possibility to prove what one wants to prove.

Concerning objection (iii) it is clear that a turnover diagram can be made as detailed as one wishes: there is no theoretical limit as regards the fineness of the subdivision of the interval 0 to T. On the other hand, there is no sense in making the representation more detailed than is motivated by data. Here also the a priori knowledge about S becomes significant: there is no reason for making the turnover compartmentalization more detailed than the number of functions and properties of S one wants to study.

In this context I should like to emphasize that there is a considerable redundancy in the type of turnover diagram I have suggested; i.e., it contains less information than is apparent at a first glance. This is, of course, almost necessary because otherwise, from what I just said in the previous paragraph, there would be no limit in the amount of "information" one could extract from $b_S(t)$-data. Part of this redundancy is revealed by the fact that the different parameters are not entirely independent of each other. For instance, it is easily shown, and intuitively obvious that

$$\theta_{i1} = t_{i-1} \left[b_S(t_{i-1}) - b_S(t_i) \right] / b_S(0)$$

i.e., the size of the initial part of K_i is strongly correlated to t_{i-1}. By the same token, θ_{i2} is positively correlated to the difference $|t_i - t_{i-1}|$.

NOTES AND REFERENCES

1. Bergner, P.-E. E. J. Theor. Biol. 1, 120 (1961).
2. Bergner, P.-E. E. J. Theor. Biol. 6, 137 (1964).

   Bergner, P.-E. E. "Kinetic Theory: Description of metabolic systems." In: Dynamic Clinical Studies with Radioisotopes (Eds. Andrews, Kniseley, Tauxe). AEC Symposium Series TID-7678 (1965, second edition).
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4. Bergner, P.-E. E. "The concepts of mass volume and concentration." In: Compartments, Pools and Spaces in Medical Physiology (Ed. Bergner and Lushbaugh). AEC Symposium Series CONF-661010. In press.
5. Bergner, P.-E. E. "Tracer theory: A review" In: Isotopes and Radiation Technology, 245-262, (1966).
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7. Marshall, Y. In, Compartments, Pools and Spaces in Medical Physiology (Eds. Bergner and Lushbaugh). AEC Symposium Series CONF-661010. In press.
8. Bergner, P.-E. E. Ergodic properties of the tracer system. USAEC Report ORAU-103. In press.
9. This statement is, as it stands, not absolutely correct except, seemingly so, for a "finite" S, that is when S contains a finite number of states only (cf. Kintchin, A. T.: "The mathematical foundation of statistical mechanics." Dover, 1949). Therefore, to avoid in the present context uninteresting mathematical difficulties, I shall assume that S is finite, although the number of states it contains might be "arbitrarily" large.
10. This is a well-known property of finite Markovian processes (cf. (9)), as is the following ergodic property. An excellent presentation of this part of the theory is found in Khintchine, A. T. "Mathematical methods in the theory of queueing." Griffin's 1960.

11. The reasoning presented here is "backwards" in the sense that chronologically it is an interpretation of the following equation 4, which was first proved in the first reference in (2). The interpretation in terms of "trajectories" was first given in the second reference in (2), and further developed in (8).

12. This is an elementary property of finite (cf. (9)) linear differential equations with constant coefficients; see, for instance, Theorem 6 in Bergner, P.-E. E. J. Theor. Biol. 1: 359, (1961).

13. The fact explained in (11) makes this part of the presentation somewhat clumsy: equation 2 might thus be considered as an interpretation of equation 4.

14. It is important to recognize that the concept of steady state appears automatically in the closed logic, which the physical tracer dynamics defines (8).

15. The time of sojourn is here used instead of the more appropriate but clumsier mean-time of sojourn. It should not be confused with the concept of mean-transit time used by Zierler, K., In: "Compartments, Pools and Spaces in Medical Physiology" (Eds. Bergner and Lushbaugh). AEC Symposium Series CONF-661010. In press. The transit time is identical with what occasionally in stochastic theory is called first passage time; only in the special case $\upsilon = S$ is the time of sojourn equal to, incidentally, the mean transit time. When applied to turnover compartments the time of sojourn becomes rather abstract and may, conceptually, be more conveniently thought of as a size measure.

16. $k \cdot k' = 1$ is not formally proved anywhere, but is a necessary consequence of the fact that we must eventually end up with equation 4 (see (11)).

17. First reference in (2). Equation 5 holds only in the form it stands; it is not allowed, e. g., to replace "S" by "υ".

18. Of course, this is partly a matter of personal taste, e.g., some people would not consider the given list of basic concepts as being complete without entities like "time" and "system."

19. This is a basic and well-known property of what might be called a stationary Markovian state (cf. Marsaglia, G. Report D1-82-0280, Boeing Scientific Laboratory 1963).

20. Chapter 6 in reference (6).

21. See for instance, Theorem 2 in reference (1).

22. Denbigh, K. G. "The thermodynamics of the steady state." Methuen and Company, Ltd., London, 1951.

23. See for instance reference (5) and, especially, Sheppard, C. W. "Basic principles of tracer method." John Wiley and Sons, Inc., New York, 1962.

24. See the first reference in (2) and (3).

25. Theorem 2 in reference (1).

26. A detailed derivation of this differential equation is given in reference (1), and further discussed in reference (12).

27. This type of solution was first pointed out to me by Dr. Lawson, Waterloo, Canada, and it has been used in kinetical contexts by Montroll, E.W., and Shuler, K. E. in "Advances in chemical physics" (Ed. Prigogine, J.) p. 361, Interscience Publishers, Inc., New York, 1958.

28. It has been pointed out to me by Professor Uhlhorn, Lund, Sweden, that from a physical viewpoint the likelihood of multiple eigenvalues is negligible, though mathematically possible.

29. Aitken, A. C.: "Determinants and Matrices." Oliver and Boyd, Edinburgh and London, 1954.