2. INTRODUCTION

This investigation, of which the present paper accordingly is a third report, was originally started with the aim of analysing the "significance" of the modern tracer kinetic methods in biology, especially as regards the so-called compartment analysis. To describe the dynamic properties of metabolic systems in terms of homogeneous compartments is conceptually a rather natural method from a biological point of view, and when the tracer techniques had begun to develop after the Second World War the biologists were quite familiar with these ideas. Hence, when it was found that after a single injection of tracer into animals the amount of tracer in the blood could be written as a function of time of the form

\[ t = \sum_{i=1}^{n \leq 5} \beta_i e^{-\alpha_i t} \quad (0 \leq \alpha_1 < \alpha_2 < \ldots < \alpha_n), \]

(2.1)

the interpretation in terms of homogeneous compartments appeared to be a natural procedure. Functions of this form were at that time already well known, e.g. from the work by Teorell (1937) on the kinetics of drugs, and from the experimental and theoretical work on the decay of radioactivity (e.g. Bateman, 1910). By a formal analogy with the curves of radioactive decay the coefficients \{\alpha_i\} were sometimes interpreted as "rate constants" of individual processes (e.g. Thomas et al., 1952), but, as is apparent, for instance from the paper by Teorell (loc. cit.), such an interpretation cannot be expected to hold in general. Only if the system consists of homogeneous compartments irreversibly connected to each other and without recycling, e.g.

\[ \rightarrow 1 \rightarrow 2 \rightarrow 3 \rightarrow 4 \rightarrow \]

this interpretation seems correct, and attempts to perform a more general analysis were published in which no general restrictions were put on the system as regards the connection between the different compartments (see, for instance, Berman and Schoenfeld, 1956; Rescigno, 1954, 1956, 1960).

This was the situation when the present investigation was started, and it soon became apparent that before any theoretical analysis of the methods could be performed, it was necessary to build up a conceptual background, relevant to the biologist as well as to the physicist, and it is this part of the work that has been reported in I. In terms of two postulates a new representation of the atoms in metabolic systems is introduced, making it possible to give a "scalar" chemical reaction
of any order) the same representation as is given to a "vectorial" diffusion flow; concerning the importance of the distinction between "scalar" and "vectorial" flows, see Denbigh (1951). This may, for instance, prove useful during in vivo experiments on complicated membrane transports, as the fluxes can be given a tracer dynamic description without making necessary any assumptions on the mechanism of flow (cf. section 4 of I). In any case, the simplifications thus achieved are necessary if a general over-all picture of complicated metabolic systems is to be at all possible.

It sounds perhaps trivial to point out that the biological systems are complicated, but it is indeed a fact that has certain important consequences. One of them is that, in order to be general, the theoretical formalism has often to be set forth in new and somewhat abstract concepts, a phenomenon that is common in modern physics and which unfortunately may cause difficulties in the communication between the experimentalists and the theoreticians. To illustrate the necessity of new concepts we may consider the biological concept of "compartment". From a linguistic point of view, a compartment is just a part or a section of the system, e.g. the creatine bound phosphorous in the right biceps may be considered as a compartment of the phosphorous system constituted by all the phosphorous in the animal, but this is a mere example and not a definition of the concept. Further, it is not the phosphorous in the creatine phosphate that is the compartment but what we could call the state of phosphorous corresponding to this compound and this geometrical position (i.e. the right biceps). A moment's consideration will make apparent that the common elementary concepts are hardly sufficient when it comes to the general definition, thus illustrating the necessity of new concepts. For this purpose the set theoretical concepts introduced in I have proved rather satisfactory and have given rise to the formalism which in the following is referred to as the tracer dynamics.

Usually, however, we are not concerned with just compartments but with "homogeneous compartments", which complicates the conceptual part of the analysis even further. In the literature the homogeneity of a compartment is often referred to as "complete mixing" or "instantaneous mixing", but this is obviously only a merely intuitive description of the situation (the word "compartment" is sometimes used for the concept which here has been denoted by "homogeneous compartment"). In the tracer dynamics, on the other hand, the compartment, by virtue of its definition, is given properties which indicate the significance of a concept like homogeneity, but it is not until the "experimental precision" has been introduced in the analysis (cf. chapter 6) that it has been possible to give this concept a more quantitative definition (see page 37), which, however, is still rather tentative.

To make the above reasoning more substantial, let \( C_u \) stand for a compartment of the kind previously exemplified by the creatine phosphate and consider the total amount of the considered species \( u \) in \( C_u \). If \( u \) is a definite kind of atom, i.e. with a definite atomic number, we may consider the naturally present isotopes of \( u \) in
the metabolic system as constituting the *mother substance*; the *tracer* is the population of non-naturally present isotopes of \( u \) which have been supplied to the system in such a way that the amount of tracer is everywhere much less than that of the mother substance. Let us assume that the total amount of mother substance in \( C_v \) is constant and that there is a constant flux, \( \zeta_v^0 \), of mother substance from \( C_v \) (counted in, for instance, gram atoms per second). The term "constant" is here used in the common statistical sense: the number of atoms leaving \( C_v \) in unit time is, when plotted versus time, randomly scattered around a mean value without showing any trend with respect to time. We are thus concerned with what could be called a "stationary stream", and the corresponding mean value is \( \zeta_v^0 \). Then, the following interpretation becomes natural: if \( b_v^0 \) is the total amount of mother substance in \( C_v \) (e.g. counted in units of gram atoms),

\[
\lambda_v^0 \cdot \delta t = \left( \frac{\zeta_v^0}{b_v^0} \right) \delta t
\]  

(2.2)

is the probability that an atom of the mother substance in \( C_v \) will leave \( C_v \) during the very short time interval \( \delta t \).

In the following \( \lambda_v^0 \) is called the *turnover factor* of the population of \( u \)-atoms in \( C_v \) and it exists by virtue of (2.2) as long as \( \zeta_v^0 \) exists and \( b_v^0 \neq 0 \). However, the efflux \( \zeta_v^0 \) is often unobservable and we have then simply to assume its existence. But to assume the existence of \( \zeta_v^0 \) is certainly equivalent to assuming that a quantity like \( \lambda_v^0 \) exists, and it is this assumption which has been made in the tracer dynamics. This approach would, however, not mean much of an improvement if the existence of \( \lambda_v^0 \) was just stated: we want to have at least some idea of what kind of restrictions must be put on the system for the turnover factor to exist. This is a motivation for the form given to the tracer dynamics, which is ultimately based on the two postulates formulated in I. The postulates ascribe to the individual atoms certain fundamental properties in terms of the so called transition probabilities, which are introduced as physical entities. This then enables us to define \( \lambda_v^0 \) as a mean value of the corresponding probabilities, possessed by the individual \( u \)'s in \( C_v \), to leave \( C_v \) within the unit of time, the mean being taken over the atoms of the mother substance in \( C_v \). This motivates the term "expected probability" for \( \lambda_v^0 \), often used in I and II.

Assume that in \( C_v \) there is some tracer present, the total amount of which is equal to \( b_v(t) \). The tracer dynamics now states that there exists a tracer turnover function \( q_v(t) \) such that

\[
\zeta_v(t) = q_v(t) b_v(t),
\]  

(2.3)

where \( \zeta_v(t) \) is the efflux of tracer from \( C_v \) at time \( t \) and \( q_v(t) \) is dependent on the distribution of tracer in \( C_v \) but not on the total amount present there. From the analysis in II we know that if the tracer is supplied to the system in exactly the same way as is the mother substance, then

\[
\lim_{t \to \infty} q_v(t) = \lambda_v^0
\]  

(2.4)
when the system is *perfect*; the system is perfect when, for any \( C_v \) in the system, both \( \lambda^0_v \) and \( b_v^0 \) are time independent.

This gives the desired connection between the tracer and its mother substance, though in a somewhat too restricted form (cf. page 20), but it still remains to show how \( \lambda^0_v \) can be experimentally estimated. Usually \( b_v \) is the variable that is observed and, as shown in chapter 7, it is in the general case not possible to estimate \( \lambda^0_v \) from such observations. We have accordingly to restrict ourselves to more special systems, essentially those to which the tracer can be supplied in a single dose at time zero into the considered compartment \( C_v \) and where \( C_v \) is homogeneous (cf. chapters 7 and 8). The concept "homogeneous compartment" has already been mentioned. For a more precise definition of this concept the reader is referred to chapters 6 and 7, and here we merely state in a somewhat loose manner that \( C_v \) is homogeneous if and only if \( q_v(t) \) can be considered as time independent, a condition which certainly depends on the "precision" of the applied experimental procedure (cf. chapter 6).

An important feature of the tracer dynamics is that it provides us with tools which simplify the physical considerations whether or not a compartment is homogeneous. This is important as, according to the results of chapter 6, the tracer curves cannot usually give much information about the homogeneity of a compartment; in fact, the tracer dynamics gives no justification for writing tracer data in the form of (2.1), except of course when the system is known in its details, as, for example, sometimes may be the case in biochemical experiments *in vitro*.

At the very beginning of this chapter it was stated that the aim of the present investigation has been to test the "significance" of some tracer kinetic methods, where now the term significance is to be taken with respect to the physical concepts introduced by the tracer dynamics. In medical work it is frequently assumed that, after a single injection of tracer, the ratio of the specific activities in different parts of the system will approach unity as time increases (the system being assumed perfect). By the specific activity in a volume-element of the system we understand the quotient between the total amount of tracer and the total amount of mother substance contained in that volume-element, and the method has been often used to determine the "size" or "volume" of what is sometimes called "metabolic pools". However, the present investigation has shown that this assumption is reasonable only if the system is perfect and the tracer is supplied in exactly the same way as is its mother substance; when the system is open, this is a condition which is not very attractive to the experimentalist (cf. chapter 5).

A method deserving special attention is the compartment analysis, mentioned above. The tracer dynamics gives no motivation for this method: a compartment can be chosen at will and lacks therefore any physical significance of its own (cf. section 6 of II), and it is therefore obvious that the models derived by the compartment analysis, using such compartments as basic entities, are not likely to have much physical significance. However, the inefficiency of this method can be under-
stood from another and somewhat more rational point of view, but before we can enter into a discussion of that it is necessary to introduce the concept of commensurability of data, which will be of importance for the analysis in the following chapters.

We shall not enter too deeply into this question but merely indicate what we are aiming at. Let us start by considering a first order chemical reaction, in which a substance $a$ is irreversibly transformed into another modification $a'$, i.e.

$$a \rightarrow a'.$$

It is well known that, in this case, the concentration $[a]$ of $a$ will be a function of time of the form

$$[a] = [a]_0 e^{-kt},$$

where $[a]_0$ is the concentration of $a$ at time zero. Hence, on observing $[a]$ as a function of time we may, with the help of convenient graphical or numerical methods, estimate the value of the "rate constant" $k$. A variable like $k$ may be defined in statistical mechanical terms, independent of any special experimental procedure, and we shall therefore refer to it as a physical variable. In the present case the experiment leads to a numerical value $k^*$ which thus may be considered as an estimate of the value possessed in the experiment by a definite physical variable. It accordingly makes sense to make a quantitative comparison between $k^*$ of different experiments or of different first order reactions, and, for this reason, the type of data represented by $k^*$ will here be referred to as commensurable data.

According to this point of view, to say that experimental data are commensurable means that they are considered as estimates of definite physical variables, and throughout the present communication the following statement will be accepted: experimental data can be commensurable only if the corresponding physical variables can be given, at least theoretically, in advance of the experiment. For instance, if there is a function containing certain parameters of interest, we try to find out, by suitable curve-fitting methods, what values of the parameters are most compatible with given experimental data. We are thus not concerned with the form of this function, nor with the composition of the set of parameters; in general, other functions can be found that can be fitted to the experimental data with a much better accuracy than can the function given by the theory. It is also from this point of view that the compartment analysis cannot be accepted: as will be shown in chapter 4 the compartment analysis does not in general lead to a commensurable description.

The turnover factor may be considered as a physical variable, but, as indicated above, only under rather special circumstances can it be experimentally estimated. In order to give a simple illustration of a case when a commensurable description in terms of turnover factors is possible, let a perfect system be given subdivided into two mutually exclusive compartments $C_1$ and $C_2$. It is desired to determine
the turnover factors $\lambda_1^0$ and $\lambda_2^0$ of the respective compartments. From the discussions in chapter 7 it will be clear that, in general, such a determination is possible only when one of the compartments is homogeneous and when the tracer can be supplied to that compartment in a single dose. The turnover factor of the homogeneous compartment is determined from the initial slope of the corresponding tracer curve, after which the other turnover factor is estimated by the expression

$$\lambda_1^0 \delta_1^0 - \zeta_1^0 = \lambda_2^0 \delta_2^0 - \zeta_2^0,$$

where $\delta_i^0$ is the total amount of mother substance in $C_i$ and where $\zeta_{1i}^0$ and $\zeta_{2i}^0$ are the fluxes of mother substance from $C_i$ out to the environments of the system and from the environments into $C_i$ respectively; these four variables are usually observable.

It is of importance that only the compartment to which the tracer is added has to be homogeneous, and in this respect the tracer dynamic point of view means a considerable simplification. This is illustrated by the in vitro system of plasma and red blood cells discussed in chapter 8, where it is also shown that the somewhat broader aspect of "homogeneity" means a reduction in the requirements with respect to the mixing mechanism of the tracer in the plasma compartment. In the same chapter it is also indicated that even if the circulatory systems of mammalian animals are in general not homogeneous, published papers seem to point to possible methods for the determination of turnover factors also for the fluxes from the blood; on the basis of this it is shown that a rather detailed and commensurable description of such systems seems in fact possible without making too many restrictions as regards the homogeneity of the different compartments.

In chapter 9 it is stated that we often have to distinguish between clinical and physical significance, i.e. clinical findings cannot always be translated into common physical terms. The types of experiments now discussed are in general not applicable in clinical work: the observations often make necessary the destruction of the system. Also, in medicine, the systems are usually open and only a single injection of tracer is performed, and, as shown in II, such systems are not very attractive as their tracer variables usually cannot be expected to represent the mother substance (compare what was stated above about the specific activities). To this we may add the results of chapter 7: the fact that observations are performed with finite "precision" (cf. the definition in chapter 6) implies that what in chapter 7 is called the macroscopic complexity of the metabolic systems seems to give rise to a fundamental difficulty as regards the physical significance (with respect to the turnover) of the tracer variables. Hence, in the general case, a commensurable description of metabolic systems does not seem possible in terms of the turnover.

In fact, the mere complexity of the biological systems seems to give rise to problems of a rather fundamental and general character. What has been on an intuitive
basis commonly held among the biologists (e.g. Commoner, 1961) seems thus here to be given a motivation. It is true that during recent years similar ideas have been reported by physicists as well (e.g. Elsasser, 1961), but the discussions have hitherto been of a more abstract and principle nature. The tracer dynamics provides us with a somewhat more concrete illustration of some difficulties that may be caused by the complexity of the biological systems, by considering these difficulties as a consequence of the fact that the experimental precision is always finite.

An immediate practical result of this is that the turnover generally cannot be estimated, hence, any hypothesis as regards the turnover must be stated in advance of the experiment, and all that can be done is to see whether or not this hypothesis is consistent with experimental data. This is, among other things, in agreement with what has been found in an other field of “biological kinetics”, namely at the stochastic interpretation of cell survival curves (Bergner, 1961c), and it suggests that the most profitable thing we can do is not to try to determine the turnover but to find other physical variables that can be observed and which are in some way correlated to the turnover. What we want is to find rigorous methods by which it would be possible to see, from experimental data, whether or not a stated hypothesis is a probable one.

In the last chapter an example is given of a variable that seems to make possible a commensurable description of metabolic systems, also in the quite general case (the system is certainly always assumed stationary). However, the considered variable, called the “time of sojourn” of tracer particles, is not so immediately connected to the properties of the mother substance in the corresponding compartment as is the turnover. The strong “physiological location” is certainly a factor that makes the turnover such an important concept as actually it is in the biological discussions, and this importance is underlined by the fact that the turnover factor has here been given a significance that will appear relevant to the physiologist and the pharmacologist as well as to the chemist and the physicist. Hence, it would mean a great achievement if it were possible to find variables that are easily observed, like the time of sojourn, and that are as strongly as possible correlated to the turnover factor.

This problem, however, still remains. Hence, to sum up, what in the present investigation has been done is, first of all, that there has been given a rigorous formulation of conditions that appear necessary—or at least sufficient—for certain forms of interpretation of tracer data to be valid. When these conditions are satisfied, the theory provides a general tool for a quantitative and significant description of fluxes; tracer methods that have often been applied to certain types of metabolic processes (e.g. transports through membranes) have thus been given a general physical motivation. On the other hand, when these conditions are not satisfied, the tracer dynamics shows why the ordinary methods are not applicable, and makes it accordingly possible to state what kind of problems there are to be solved.