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Herman Branson Howard University

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HERMAN BRANSON
From the Graduate School, Howard University, Washington, District of Columbia

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The Kinetics of Reactions in Biological Systems

Herman Branson [1]

From the Graduate School, Howard University, Washington, District of Columbia
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INTRODUCTION

An integral equation description of metabolizing systems has been discussed by the author in a series of articles (1). It is the purpose of this paper to show how the rate function and metabolizing function of the description can be specified to treat the kinetics of many biological systems of great experimental interest which are now being actively studied by means of isotopic tracers. The results permit the determination of rates of entrance of the normal substance, the turnover time, and the free energies of activation of the rate-determining steps in the metabolic chain of the normal substance for systems where the behavior of the isotopic tracer may be described by a linear combination of exponentials.

The essential idea in the integral equation description is that the amount of material present in the system at time t is made up of two contributions: the amount remaining from the initial amount present and the amount remaining from that which has entered the system during the interval from $t = 0$ to t. The resulting equation is

$$M(t) = M_0F(M_0,t) + \int_0^t R(\theta)\, F(M_\theta, t - \theta)\, d\theta, \qquad (1)$$

where $M(t)$ is the amount present at time, t. $F(M_0,t)$ is the metabolizing function, defined as that function which when multiplied by the amount present at time, $t = 0$, gives the amount remaining at time, t. F will be a function of the amount present, as we shall see below.

One of the advantages claimed for this treatment is greater generality, since we have a single equation for all systems with the R and F to be determined by each specific case. In addition, the approach enables

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one to specify concisely such a property as the fact that the system does not discriminate between isotopic molecules in that the F functions are the same for both, excepting for slight differences which are dependent upon the small mass differences. Additional advantages will evolve from this discussion.

I

The concept of metabolizing function may be introduced most satisfactorily perhaps by considering reactions of a definite order. If the order of a reaction is known, the metabolizing function is easily found by factoring out M_0. A zero-order reaction yields

$$M(t) = M_0\left(1 - \frac{Kt}{M_0}\right); \tag{2}$$

a first-order reaction,

$$M(t) = M_0 e^{-Kt}; \tag{3}$$

a second-order reaction,

$$M(t) = M_0\left(\frac{1}{1 + M_0Kt}\right); \tag{4}$$

and a third-order reaction,

$$M(t) = M_0\left(\frac{1}{\sqrt{1 + M_0^2Kt}}\right). \tag{5}$$

If there is no material entering the system during the course of the reaction, $R(t) = 0$ and Eq. (1) becomes

$$M(t) = M_0F(t)$$

so that for each reaction of definite order, F is the factor multiplying each M_0 in Eqs. (1) through (5). F is a function of M_0 for all reactions except first order.

A condition which the metabolizing function must satisfy is that the fate of a certain number of molecules present in a system at time t should be the same as that for the same number irrespective of the past history of the molecules. For example, suppose that there are N_0 present at time $t = 0$, at time θ there will be $N(\theta) = N_0F(N_0, \theta)$. The number present at time t from the number originally present would be $N_t = N_0F(N_0,t)$. The number present at time t from the number present at time θ is $N_t = N_\theta F(N_\theta, t - \theta)$. Substituting shows that one

must have then to meet this condition,

$$F(N_0,t) = F(N_0,\theta)\, F(N_\theta, t - \theta). \tag{6}$$

Using the zero order as an example, we see

$$\left(1 - \frac{Kt}{M_0}\right) = \left(1 - \frac{K\theta}{M_0}\right)\left[1 - \frac{K(t-\theta)}{M_0\left(1 - \frac{K\theta}{M_0}\right)}\right] = \left(1 - \frac{Kt}{M_0}\right),$$

as required. The other reactions of definite order may easily be shown to satisfy this condition.

The condition expressed in Eq. (6) applies to a single metabolic pathway. This may be seen from the following discussion. A popular type of function used in describing natural phenomena is a linear combination of exponentials, which can be written in the form

$$M(t) = M_0 \sum_\kappa \beta_\kappa e^{-\alpha_\kappa t} \text{ with } \sum_\kappa \beta_\kappa = 1 \tag{7}$$

or

$$M(t) = \sum_\kappa A_K e^{-\alpha_\kappa t} \text{ with } \sum_\kappa A_K = 0 \text{ if } M = 0 \text{ at } t = 0. \tag{8}$$

Gellhorn *et al.* (2) found that such a form expressed satisfactorily their experimental results on the transcapillary exchange of sodium in the dog. They gave

$$C_p - 1000 = 2117e^{-1.040t} + 933e^{-0.95t}$$

which in our notation becomes

$$M(t) = 3050\left(\frac{2117}{3050}\, e^{-1.040t} + \frac{933}{3050}\, e^{-0.095t}\right)$$

or

$$M(t) = M_0(\beta_1 e^{-\alpha_1 t} + \beta_2 e^{-\alpha_2 t})$$

with

$$F(t) = \beta_1 e^{-\alpha_1 t} + \beta_2 e^{-\alpha_2 t}. \tag{9}$$

The $F(t)$ in Eq. (9) does not satisfy Eq. (6). The interpretation of Eq. (9) shows that it describes a system schematized in Fig. 1. The material present in the system at $t = 0$ is in the pool. At $t = \theta$, we have

$$N_\theta = N_0(\beta_1 e^{-\alpha_1 \theta} + \beta_2 e^{-\alpha_2 \theta}). \tag{10}$$

If we consider the initial time now as θ, the number of molecules

present at time t will be

$$N_t = \bar{N}_\theta[\beta_1 e^{-\alpha_1(t-\theta)} + \beta_2 e^{-\alpha_2(t-\theta)}]. \tag{11}$$

If N_θ is numerically equal to $\bar{N}_\theta$, are they interchangeable? The answer is clearly that they cannot be since in Eq. (10) the N_θ are distributed between the two metabolic pathways through the system, while in Eq. (11) the $\bar{N}_\theta$ are in the pool. If there is only a single metabolic pathway, the two would interchange, for there would be no distinction between the pool and the single path.

We may conclude, therefore, that it is legitimate to describe reactions in chemical and biological systems through equations of the type

$$M(t) = M_0 F(t) = M_0 \sum_{i=1}^{\kappa} \beta_i F_i(t). \tag{12}$$

In order for this description to represent possible processes, it is necessary that each value of $F_i(t)$ satisfy Eq. (6).

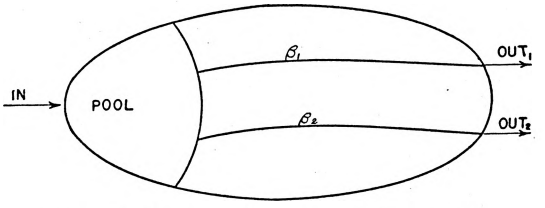

FIG. 1. The system represented by Eq. (9).

The description through Eq. (12) requires the interpretation that all of the material, M_0, is initially present in a pool, but after the experiment is underway ($t > 0$), the material is distributed among the various pathways in the proportions given by the β's, that is, 100 β_1% along pathway 1, 100 β_2% along pathway 2, etc.

If the basic reactions occurring in biological systems are of a definite order, this discussion indicates that the most common allowable metabolizing functions, $F_i(t)$, would be those given by Eqs. (2), (3), (4), and (5). The only allowed $F_i(t)$ of a definite order which does not depend upon the amount present is the exponential function for a first-order reaction. Although we shall limit our discussion in the final sections to systems described by a linear combination of exponentials, the discussion is applicable to linear combinations and linear mixed sums of all of the other types of allowed F_i's.

A linear combination of exponentials is one of the most popular means of describing the behavior of a substance in a chemical or biological system (3). From Eq. (1) we see that what we need are two independent determinations, each yielding a linear combination of exponentials,

$$M(t) = M_0F(t)$$

$$M(t) = \int_0^t R(\theta)\, F(M_\theta, t - \theta)\, d\theta + M_0F(t)$$

in order to determine both R and F for the material $M(t)$.

The most promising experimental procedure is to use double-tagged molecules (4). This procedure is very involved and difficult. With the experimental conditions permitting a mathematical description of the

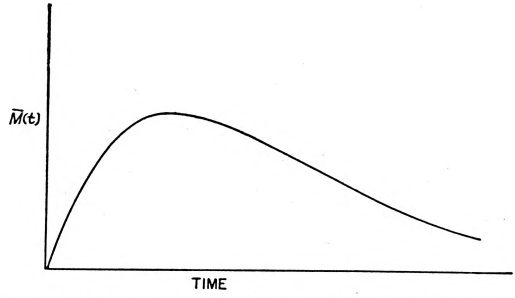

FIG. 2. A type of curve encountered in experiments with radioactive tracers which is approximated by Eq. (13).

form of Eq. (12), we can deduce extremely useful conclusions from a single determination with a tagged molecule. This application rests upon the assumption that the metabolizing function for the normal and the tagged metabolite are the same.

The usual experimental procedure is that a substance tagged with a radioactive or rare isotope is introduced into a system as a precursor of M:

$$P \longrightarrow \mathrm{M} \longrightarrow .$$

The arrows here merely indicate that P goes to M. The actual chain of events may be extremely complicated. The behavior of $\bar{M}(t)$, the tagged M, is shown in Fig. 2. Equation (1) becomes in this example

$$M(t) = \int_0^t R(\theta)\, F(M, t - \theta)d\theta.$$

We fit by some convenient method a series of exponentials to the experimental curve for $\bar{M}(t)$,

$$\bar{M}(t) = \sum_{\kappa=1}^{n} A_K e^{-\alpha_\kappa t}, \tag{13}$$

where the α's are numbered so that $\alpha_1 > \alpha_2 \cdots \alpha_n$. The initial condition is that $\bar{M}(0) = 0$; then $\sum_{\kappa=1}^{n} A_K = 0$. Equation (1) becomes for this example

$$\sum_{\kappa=1}^{n} A_K e^{-\alpha_\kappa t} = \int_0^t R(\theta)\, F(t - \theta) d\theta. \tag{14}$$

We seek solutions for R and F in the form

$$F(t) = \sum_{i=l}^{n} \beta_i e^{-\alpha_i t} \qquad n \geqq l > 1. \tag{15}$$

Since

$$F(0) = 1, \sum_{i=l}^{n} \beta_i = 1$$

and

$$\bar{R}(t) = \sum_{j=1}^{l-1} \beta_j e^{-\alpha_j t}. \tag{16}$$

The justification for these forms for R and F are deduced from Fig. 3. After a long time the tagged material should be fairly well concentrated

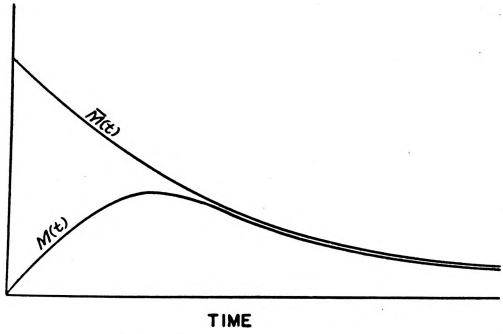

FIG. 3. The tracer was introduced as a precursor of $M(t)$ and directly as $\bar{M}(t)$. The curves would have the same slope after a sufficiently long time.

in $\bar{M}(t)$ from P. We could expect, therefore, the tail end of the curve to approach the curve for a sample of $\bar{M}(t)$ introduced directly into the system. The only way this can be so is for $F(t)$ to include the exponential terms with the smallest α's or α. If we substitute Eqs. (15) and (16) into Eq. (14) we have

$$\sum_{j=1}^{l-1} \beta_j \left[\frac{\beta_l}{\alpha_l - \alpha_j} + \cdots \frac{\beta_n}{\alpha_n - \alpha_j} \right] e^{-\alpha_j t} + \sum_{\kappa=l}^{n} \beta_\kappa \left[\frac{B_1}{\alpha_1 - \alpha_\kappa} + \cdots \frac{B_m}{\alpha_{l-1} - \alpha_\kappa} \right] e^{-\alpha_\kappa t} = \sum_{\kappa=1}^{n} A_\kappa e^{-\alpha_\kappa t}.$$

Equating coefficients gives

$$A_1 = B_1 \sum_{\kappa=l}^{n} \frac{\beta_\kappa}{\alpha_\kappa - \alpha_1}; \quad \cdots A_{l-1} = \beta_{l-1} \sum_{\kappa=l}^{n} \frac{\beta_\kappa}{\alpha_\kappa - \alpha_{l-1}} \tag{17}$$

$$A_l = B_l \sum_{\kappa=1}^{B\kappa} \frac{B_\kappa}{\alpha_\kappa - \alpha_l}; \quad \cdots A_n = \beta_n \sum_{\kappa=1}^{l-1} \frac{B_\kappa}{\alpha_\kappa - \alpha_l}.$$

Equation (17) is to be solved for the B's and β's.

The solutions are straightforward but tedious in the general case. The experimental results so far seem to require no more than three terms in Eq. (13). For specificity we shall consider such a three-term expression. Equation (17) gives two choices.

Case 1

$$\bar{R}(t) = B_1 e^{-\alpha_1 t}$$

$$F(t) = \beta_2 e^{-\alpha_2 t} + \beta_3 e^{-\alpha_3 t}.$$

Here

$$A_1 = B_1 \left(\frac{\beta_2}{\alpha_2 - \alpha_1} + \frac{\beta_3}{\alpha_3 - \alpha_1} \right)$$

$$A_2 = \beta_2 \left(\frac{B_1}{\alpha_1 - \alpha_2} \right)$$

$$A_3 = \beta_3 \left(\frac{B_1}{\alpha_1 - \alpha_3} \right).$$

From which we get

$$\beta_2 = \frac{A_2(\alpha_1 - \alpha_2)}{A_2(\alpha_1 - \alpha_2) + A_3(\alpha_1 - \alpha_3)}$$

$$\beta_3 = \frac{A_3(\alpha_1 - \alpha_2)}{A_2(\alpha_1 - \alpha_2) + A_3(\alpha_1 - \alpha_3)}$$

$$B_1 = A_2(\alpha_1 - \alpha_3) + A_3(\alpha_1 - \alpha_3).$$

Case 2

$$\bar{R}(t) = B_2 e^{-\alpha_1 t} + B_2 e^{-\alpha_2}$$

$$F(t) = \beta_3 e^{-\alpha_3 t}$$

with

$$A_3 = \frac{B_1\beta_3}{\alpha_1 - \alpha_3} + \frac{B_2\beta_3}{\alpha_2 - \alpha_3}$$

$$B_1 = A_1(\alpha_3 - \alpha_1); \; B_2 = A_2(\alpha_3 - \alpha_2)$$

$$\beta_3 = 1.$$

In order to decide between the two cases, we make use of the additional condition that $R(t) \geqq 0$ for all values of t. We shall illustrate this condition in the examples below.

The $F(t)$ in Eq. (15) is clearly of the form discussed in Eq. (12). Hence Eq. (15) implies that there are $(n - l + 1)$ metabolic pathways for the substance to follow through the system. These pathways are conveniently renumbered from 1 to K. We shall discuss each of those pathways with the appropriate $F_i(t)$.

The metabolic pathways derived from Eq. (15) are not necessarily the specific routes along which material is undergoing transformation or reaction. Rather the α's represent in general complicated functions of all the many phenomena occurring in the system, such as active transport, diffusion, and chemical reaction. This point will be further emphasized in the following paper.

The data available are from experiments with mature animals in nutritional equilibrium (4) in which the normal amount of the metabolite may be expected to remain constant during the experiment—or the rates encountered are such that the amount of the normal metabolite changes only slightly. Equation (1) becomes for the normal metabolite in such a system,

$$M(0) = M(0)\, F(t) + \int_0^t R(\theta)\, F(M_0, t - \theta)\, d\theta.$$

From the interpretation of Eq. (12), a percentage $100\, \beta_1$ of M_0 follows pathway 1, $\cdots$ $100\, \beta_\kappa$ follows pathway K. So that we have for each pathway

$$\beta_i M_0 = \beta_i M_0 F_i(t) + \int_0^t R_i(\theta)\, F_i(t - \theta) d\theta. \tag{18}$$

In our example $F_i(t) = e^{-\alpha_i t}$, so that Eq. (1) becomes a simple integral

equation of the first kind with the solution

$$R_i(t) = \alpha_i\beta_i M_0. \tag{19}$$

Since the β's are dimensionless, Eq. (19) is dimensionally correct and gives for the total rate of entry

$$R(t) = \sum_{i=1}^{K} R_i(t) = M_0 \sum_{i=1}^{K} \alpha_i\beta_i.$$

Equations (15) and (19) describe a system schematized in Fig. (4). Here the material is entering along K different metabolic routes at

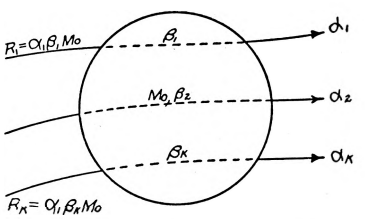

FIG. 4. The system described by Eqs. (15) and (19).

constant individual rates, $R_i = \alpha_i\beta_i M_0$. It leaves the system along K different routes as a first-order reaction along each. The rate of entry is such that the total quantity of material in the system,

$$M_0\beta_1 + M_0\beta_2 + \cdots + M_0\beta_K = M_0 \sum \beta_i = M_0$$

is constant, M_0.

The turnover time of the substance in the system may be defined

$$M_0 = \int_0^{\tau} R(t)\, dt.$$

Here the upper limit, τ, would be the turnover time. This equation yields

$$\tau = \frac{1}{\sum_{i=1}^{K} \beta_i\alpha_i}. \tag{20}$$

The conditions for the applicability of these results are as follows: The experimenter is considering an animal in nutritional equilibrium or a system in which the amount of the normal metabolite remains

essentially constant. His data on the behavior of the tagged metabolite can be described through Eq. (12). When he finds the β's in Eq. (15), he will have the rate of entry of the normal metabolite into the system through Eq. (19). In addition, he can determine immediately the turnover time of the normal metabolite from Eq. (20).

II

In this section, we shall consider some data which meet the conditions for the application of our equations. The first set of data are those of Hamilton and Soley (5) who studied the uptake of radioactive iodine (I^{131}) by a normal subject. Their data could be fitted by a function

$$\bar{M}(t) = \bar{M}_0(1 - e^{-4.5t})\, e^{-0.006t},$$

when t is in days. Here the only possibility for $F(t)$ according to Eq. (15) is

$$F(t) = e^{-0.006t}.$$

From Eq. (19), we have that the rate of entry of iodine into the normal thyroid is $R(t) = 0.006M_0$ per day. If the amount of iodine in the normal thyroid, M_0, is taken as 20 mg., then 0.12 mg. of iodine enters the normal thyroid per day. According to Bodansky (6), the minimum amount of iodine required per day (0.05 mg.) is approximately half this amount.

The turnover time of iodine in the normal thyroid is

$$\tau = \frac{1}{0.006} \text{ days} = 167 \text{ days}.$$

Sato and Tyler report that the radioactive phosphorus (P^{32})/g. of wet tissue in the rat liver after the P^{32} had been administered in a single intraperitoneal injection follows the curve

$$M(t) = -4.03e^{-0.045t} + 2.83e^{-0.0041t} + 1.36e^{-0.00017t},$$

where t is in minutes. In this example

$$\begin{aligned} A_1 &= -4.03 & \alpha_1 &= 0.045 \\ A_2 &= 2.83 & \alpha_2 &= 0.0041 \\ A_3 &= 1.36 & \alpha_3 &= 0.00017 \end{aligned}$$

With three terms we must consider the two possible cases. With the condition $R(t) \geqq 0$, we find that

$$F(t) = 0.655e^{-0.0041t} + 0.345e^{-0.00017t}.$$

Thus

$$\beta_2 = 0.655 \text{ and } \beta_3 = 0.345.$$

The rate of entry of phosphorus into the normal rat liver is then

$$R(t) = (0.655 \times 0.0041 + 0.345 \times 0.00017)M_0 = 0.00275M_0/\text{min.}$$

The turnover time is $\tau = 1/0.00275$ min. $= 363$ min. $= 6.0$ hr.

The final example is also from the work of Sato and Tyler. They find that the P^{32}/g. of wet tissue of ribonucleic acid from rat liver in the same experiment follows a curve

$$M(t) = -0.055e^{-0.025t} - 0.265e^{-0.0039t} + 0.32e^{-0.000059t}.$$

In this example

$$\begin{aligned} A_1 &= -0.055 & \alpha_1 &= 0.025 \\ A_2 &= -0.265 & \alpha_2 &= 0.0039 \\ A_3 &= 0.32 & \alpha_3 &= 0.000059 \end{aligned}$$

With the condition $R(t) \geqq 0$, we find that

$$F(t) = e^{-0.000059},$$

and for the normal metabolite, Eq. (19) $R(t) = 0.000059M_0$. The turnover time of phosphorus in the ribonucleic acid of rat liver is then $\tau = 1/0.000059$ min. $= 16{,}900$ min. $= 281$ hr.

These examples show that an alternative manner of assigning the terms in Eq. (13) to $F(t)$ or $\bar{R}(t)$, at least in these simple examples, is to assign the term with positive A's to $F(t)$ and those with negative A's to $\bar{R}(t)$. This assignment may be justified in that the $\bar{R}(t)$ terms in $\frac{d\bar{M}}{dt}$ would then be positive and the $F(t)$ terms negative.

III

The calculation of the α's permits a determination of the free energy of activation for each of the first-order reactions occurring in the description through Eq. (15). According to Eyring's theory (7)

$$\alpha_i = \frac{KTe^{-\frac{\Delta F_i^{\ddagger}}{RT}}}{h}. \tag{21}$$

At 37C°., Eq. (21) becomes

$$\Delta F_i\ddagger = \left(19{,}260 + 1510 \log_{10} \frac{1}{\alpha_i}\right) \text{cal.} \qquad (22)$$

This equation gives for iodine in the thyroid, $\Delta F\ddagger = 30{,}060$ cal.; for phosphorus in ribonucleic acid of rat liver, $\Delta F\ddagger = 28{,}620$ cal.; for phosphorus in rat liver, $\Delta F\ddagger = 25{,}500$ cal. and $\Delta F\ddagger = 26{,}140$ cal.

Equation (22) reveals a limitation on the use of isotopes in the analysis of systems through Eq. (15) since reactions with free energies of activation of the order of a few thousand calories or less (e.g., diffusion processes) will have such large α's that they are prone not to occur in Eq. (15) since the terms containing the exponentials would have fallen to negligible values within a few micro- or milliseconds. It seems plausible, therefore, to look upon the energies calculated from the α's in this manner as being lower limits for the total energy involved and as being the energy associated with the chief rate-determining reactions in the system.

Acknowledgments

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Summary

The integral equation treatment of metabolizing systems is used to discuss the rate of entry, turnover time, and free energies of activation for the normal substance from the results of a tracer experiment. The characteristics and limitations of such a description are discussed in some detail.

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