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Metabolic Pathways from Tracer Experiments

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INTRODUCTION

It is the purpose of this paper to consider the details of the individual metabolic pathways for a system whose metabolizing function has been found through a tracer experiment to be

$$F(t) = \sum_{i=1}^{\kappa} \beta_i e^{-\alpha_i t}. \tag{1}$$

It is recognized, of course, that what one has done is to fit a series of exponentials to the experimental data. Thus it may be that the actual physiological processes are not as pictured. This limitation is inherent in all such mathematical descriptions of physical phenomena. What we have in all such instances are possible systems consistent with the experimental information.

THEORY

Resumé of the Mathematical Description

The mathematical treatment of tracer experiments revealed that if for the behavior of the tagged metabolite,

$$M(t) = \Sigma_\kappa A_K e^{-\alpha_\kappa t};$$

then in the equation

$$\bar{M}(t) = \int_0^t R(\theta)\, F(t - \theta)\, d\theta$$

one could number the α_κ's so that those occurring with positive A_K's would be assigned to $F(t)$ with K running from l to n. All others would belong to $R(t)$. This distribution was based upon the fact that the negative terms in $\frac{d\bar{M}}{dt}$ should belong to $F(t)$ and the positive ones to $R(t)$. We would have then

$$F(t) = \sum_{i=l}^{n} \beta_i e^{-\alpha_i t} \text{ with } \sum_{i=l}^{n} \beta_i = 1,$$

[1] This work has been done under the sponsorship of the Atomic Energy Commission contract AT(30-1)-892.

as the metabolizing function for the normal metabolite with explicit expressions available for determining the β's from the tracer data.

The β's were shown to be distribution coefficients indicating the fraction of the total normal metabolite which goes along each of the first-order reaction paths. Specifically, β_i gave the fraction along the ith path with the reaction rate constant α_i. If the system is in equilibrium, the normal metabolite follows the equation

$$M(0) = M(0)\, F(t) + \int_0^t R(\theta)\, F(M_0,\, t - \theta)\, d\theta. \tag{2}$$

This representation suggested a system consisting of $(n - l + 1)$ metabolic pathways which were numbered from 1 to K. The rate of entrance into the ith pathway is $\alpha_i\beta_i M_0$ from the solution of Eq. (2).

General Characteristics of the Systems

What we are trying to do is to look into the details of each of the pathways, to find interrelations of the pathways of a single metabolite, and interrelations of the pathways of different metabolites. The number

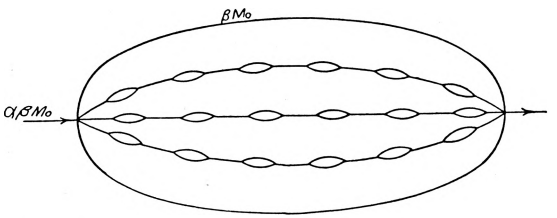

FIG. 1. A possible system for the metabolizing function, $F(t) = e^{-\alpha t}$.

of possible combinations is quite large. We shall consider a few suggested by the data now available.

In our discussion a single metabolic pathway will be the basic unit. Such a pathway is characterized by a constant rate of entry and a first-order metabolizing function.

The first system, a single metabolic pathway determined as described above, we shall consider is schematized in Fig. 1.

This system is characterized by the fact that the metabolite after entering has l possible paths which it might follow with a certain fraction γ_i along the ith. Along all of those paths the metabolite maintains its identity so that a chemical analysis would give the total amount present as βM_0.

The over-all reaction here is described by $F(t) = e^{-\alpha t}$ and $R(t) = \alpha\beta M_0$. Along the ith path, for example, let us consider the first sub-

system, A, the second would be B, etc. Then

$$A_i(t) = A_iF_{Ai}(t) + \int_0^t \gamma_i\alpha M_0F_{Ai}(t - \theta)\, d\theta. \tag{3}$$

Equation (3) makes the assumption that a certain constant fraction, γ_i, of the metabolite which enters the system follows the ith pathway. We assume too that the equilibrium extends to these sub-systems so that $A_i(t) = A_i(0)$. Thus $A_i(0)$ will be a certain fraction of the total so that $A_i(0) = K_{Ai}(\beta M_0)$. With these values Eq. (3) gives

$$F_{Ai}(t) = e^{-\left(\frac{\alpha\gamma_i}{K_{Ai}}\right)}.$$

For any other sequential member in the ith pathway, we have

$$F_{Li}(t) = e^{-\left(\frac{\alpha\gamma_i}{K_{Li}}\right)t} \tag{4}$$

since the equilibrium requires the same amount to enter and leave each of the sub-systems along a given sub-pathway per second.

In the same manner, we have for the jth sub-system of the mth path

$$F_{Jm}(t) = e^{-\left(\frac{\alpha\gamma_m}{K_{Jm}}\right)t},$$

where

$$\sum_{i=1}^{l} \gamma_i = 1 \text{ and } \sum_{i=1}^{l}\sum_{J} K_{Ji} = 1. \tag{5}$$

The summation over J in the K's must include all sub-systems along each of the l paths.

Discussion of System 1

For specificity let us consider the data of Sato and Tyler for inorganic phosphorus (P) in the liver. In the preceding paper, we showed that

$$F(t) = 0.655e^{-0.0031t} + 0.345e^{-0.00017t}.$$

Since the entire liver was analyzed, the data present the organ as a homogenous structure of mass W grams. We shall consider 1 g. of wet liver tissue as our unit. In this gram, there are 3.62 μg. of inorganic P. Hence, 3.62 $\times$ 0.655 μg. follows one path with rate constant 0.0041/min., the remaining 3.62 $\times$ 0.345 μg. follows the second pathway with rate constant 0.00017/min.

Each of these over-all pathways could represent a system as shown in Fig. 1 with l sub-pathways, each with a number of metabolic sub-pools. This analysis reveals then how limited are our present data in deciding explicitly on the details of such pathways, since we need a great deal of additional information before we may place sufficient restrictions on Eqs. (5) and (4) to reveal unique pathways.

In the absence of such detailed information, we may still use our results in conjunction with available data to set limits upon the amount of material which may be in these sub-pools. As an example, let us assume one of the sub-pools ($\beta = 0.345$, $\alpha = 0.00017$) through which the inorganic P passes by diffusion. A probable value for the energy of activation for diffusion is 3000 cal./mole. From Eyring's relation at 37°C.

$$\Delta F\ddagger = \left(19{,}260 + 1510 \log_{10} \frac{K}{\alpha\gamma}\right) \text{cal.},$$

with α in sec.$^{-1}$. For $\Delta F\ddagger = 3000$ cal./mole,

$$\log_{10} \frac{K}{\alpha\gamma} = -5.480$$

or

$$\frac{\alpha\gamma}{K} = 3.02 \times 10^5$$

$$\frac{17 \times 10^{-5}}{60} \frac{\gamma}{K} = 3.02 \times 10^5$$

$$\frac{\gamma}{K} = 1.05 \times 10^{11}.$$

Since $\gamma \leqq 1$, then $K \leqslant 9.4 \times 10^{-12}$. K is the ratio of inorganic P in the sub-pool to the amount in the system, so that the upper limit for the amount of inorganic P in μg./g. in this sub-pool is

$$9.4 \times 10^{-12} \times 3.62 \times 0.345 = 1.18 \times 10^{-11} \mu\text{g./g.}$$

If the passage of this inorganic P through the liver is only a sequence of diffusions across membranes, we see that to account for the rate constant ($\alpha = 0.00017$/min.) along a single group of sub-pools would require, for $\Delta F\ddagger = 3000$ cal./mole for each diffusion process $\left(nK = 1;\ n = \frac{1}{9.4} \times 10^{12} = 1.03 \times 10^{11}\right)$ 10^{11} such diffusion steps. This is an

extreme value which forces us to conclude that diffusion processes are not exclusively involved.

There is one other special case of the system schematized in Fig. 1 which is of interest: when there are l sub-pathways ($\gamma_1 \cdots \gamma_l$) with only one sub-pool each. Here we would have

$$F_1(t) = e^{-\frac{\alpha\gamma_1 t}{K_1}}$$

$$\vdots$$

$$F_l(t) = e^{-\frac{\alpha\gamma_l t}{K_l}}.$$

We observe that if $\gamma_i = K_i$, then each of these sub-pathways would have the same α as the whole. We shall prove that it is necessary that $\beta_i = \gamma_i = K_i$.

The metabolizing function for the system would be

$$F(t) = \sum \beta_i e^{-\frac{\alpha\gamma_i t}{K_i}} = e^{-\alpha t}$$

$$= \sum K_i e^{-\frac{\alpha\gamma_i t}{K_i}} \text{ since } \beta_i = K_i.$$

Then

$$\frac{dF}{dt} = -\alpha\sum\gamma_i e^{-\frac{\alpha\gamma_i t}{K_i}} = -\alpha e^{-\alpha t};$$

$$\therefore \beta_i = \gamma_i.$$

It is interesting to note, however, that it is not necessary for all cells or units to have the same value of γ. Thus the tissue may be inhomogenous but still have a single first-order rate constant for the transferring of the metabolite.

This example represents a membrane or layer of tissue consisting of a single layer of cells or units consisting of many cells so that the amount diffusing into each unit is $\gamma_i\alpha\beta M_0$ and the amount of the metabolite present in each unit is $\gamma_i\beta M_0$ (Fig. 2).

Precursor Relations

Despite the inability of the data from the usual tracer experiment to permit us the detailed insight into the reactions in a biological system

that we would like, the data are still most useful in deciding among various proposed interrelations of metabolites in such systems. This application is easily illustrated in the following examples.

In the passage of such material as inorganic P through the liver, some of this P goes into other fractions: desoxyribonucleic acid (DNA),

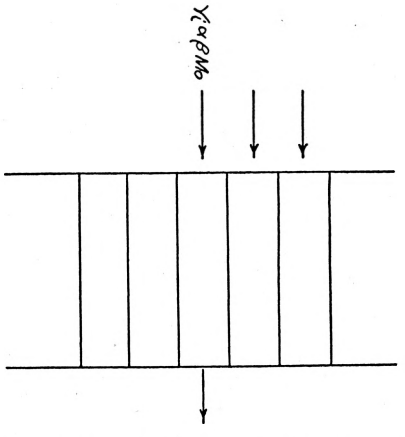

FIG. 2. Schematic representation of a membrane or tissue for which the over-all reaction is first order.

ribonucleic acid (RNA), etc. We are led, therefore, to examine a system shown in Fig. 3.

In this system a certain fraction, γ, of the material from the pool, $\beta_i M_0$, which is one of the chief pathways of Eq. (1), is diverted into compartment A. The systems are in equilibrium, hence

$$A(0) = A(0)\, F_A(t) + \int_0^t \gamma\alpha_i\beta_i M_0 F(t - \theta)\, d\theta.$$

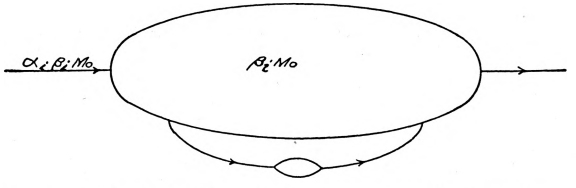

FIG. 3. A metabolic pool with a single compartment where the metabolite will be in a different chemical combination.

As before, we set $\frac{\beta_i M_0}{A(0)} = K$; hence,

$$K = K F_A(t) + \int_0^t \gamma\alpha_i F(t - \theta)\, d\theta$$

so that

$$F_A(t) = e^{-\frac{\gamma\alpha_i t}{K}}.$$

A specific example of this system could be the P of DNA as being derived from the inorganic P of the liver. Sato and Tyler give data which showed two pathways for the inorganic P $[F(t) = 0.345e^{-0.00017t} + 0.655e^{-0.0041t}]$. Their data gave for DNA a single term $F(t) =$

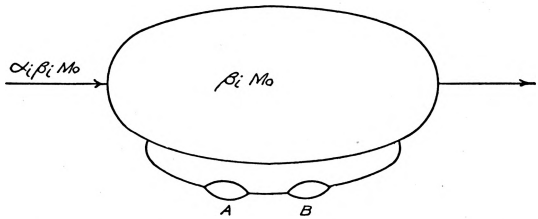

FIG. 4. A metabolic pool with a single branch leading to sequential compartments where the metabolite will be in a different chemical combination in each.

$e^{-0.00034t}$. The amount of P in DNA per gram of wet tissue equals 0.33 μg. (A_0), the inorganic P = 3.62 μg.; hence $\beta_i M = 0.345 \times 3.62$ μg. = 1.25 μg. Then $\frac{1}{K} = \frac{1.25}{0.33} = 3.8$. Since $\gamma \leqq 1$, the DNA could come exclusively from the second pathway ($\alpha_2 = 0.0041$) or it could be derived exclusively from the first ($\alpha_1 = 0.00017$) or partly from one and partly from the other. We shall assume that these slow turnover reactions are derived from the slower turnover pathway. So we have

$$0.00017 \times 3.8 \times \gamma = 0.00034; \gamma = \frac{2}{3.8} = 0.531.$$

These calculations reveal that these data are consistent with the view that the P in DNA is formed from the inorganic P which follows the metabolic pathway through the liver with the smaller α. Of this inorganic P over one-half (53%) eventually passes through the P bound to DNA. However, had we assumed the first pathway, only 1.8% of the inorganic P would pass through the DNA.

A modification of Fig. 3 to allow precursors along the branch path is given in Fig. 4.

As before we assume equilibrium,

$$A(0) = A(0)\,F_A(t) + \int_0^t \gamma\alpha_i\beta_i M_0 F_A(t-\theta)d\theta$$

$$B(0) = B(0)\,F_B(t) + \int_0^t \gamma\alpha_i\beta_i M_0 F_B(t-\theta)d\theta.$$

Let

$$A(0) = K_A\beta_i M_0 \text{ and } B(0) = K_B\beta_i M_0,$$

then $F_A(t) = e^{-\frac{\gamma\alpha_i t}{K_A}}$ and $F_B(t) = e^{-\frac{\gamma\alpha_i t}{K_B}}$. Thus if we determine the metabolizing function and the relative amounts present, we may determine whether or not A is the exclusive precursor of B in such a sequence.

Again we use the data of Sato and Tyler who give for the P of liver RNA with

$$F_A(t) = e^{-0.000059t}$$

and for the P of liver DNA

$$FB(t) = e^{-0.00034t}$$

so that $\frac{K_B}{K_A} = \frac{0.000059}{0.00034} = 0.17$. The amount of P in the DNA is 0.33 μg./g. of wet tissue; that of RNA is 1.2 μg./g. of wet tissue, from which we get $\frac{K_B}{K_A} = \frac{0.33}{1.2} = 0.275$. These values are sufficiently divergent for us to conclude that the P of liver RNA is not the exclusive precursor of the P of DNA in the rat.

The behavior of liver RNA and DNA suggests a fourth system which

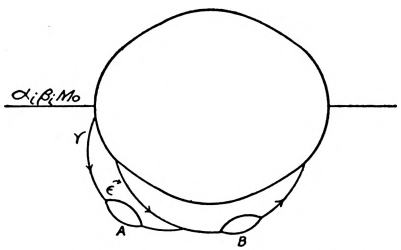

FIG. 5. A metabolic pool with two branches which combine before the second compartment for a single return.

has an additional parameter which will enable us to fit the mechanism to the data. It is, therefore, unsatisfactory in its predictiveness in comparison with the preceding example. The system is shown in Fig. 5. This system states that a fraction, γ, of the amount entering the main pool passes through A; another fraction, ϵ, joins it from the pool and the two continue through B. The equations are

$$A(0) = A(0)\, F_A(t) + \int_0^t \gamma\alpha_1\beta_i M_0 F_A(t-\theta)d\theta$$

$$B(0) = B(0)\, F_B(t) + \int_0^t (\epsilon+\gamma)\alpha_1\beta_i M_0 F_B(t-\theta)d\theta.$$

Since, as before, $K_A\beta_i M_0 = A(0)$ and $K_B\beta_1 M_0 = B(0)$. Then $F_A(t) = e^{-\frac{\gamma\alpha_1}{K_A}t}$ and $F_B(t) = e^{-\frac{(\epsilon+\gamma)\alpha_1 t}{K_B}}$.

Letting A be RNA and B stand for DNA as before and using the data given ahead, we have $\frac{\gamma}{(\gamma+\epsilon)} \times \frac{K_B}{K_A} = 0.17$; $\frac{K_B}{K_A} = 0.275$.

$$K_A = \frac{1.2}{3.62 \times 0.345} = \frac{\gamma\alpha_1}{K_B} = 0.000059$$

$$K_B = \frac{0.33}{3.62 \times 0.345}; \qquad \gamma = \frac{5.65}{17} = 0.33.$$

Since

$$\frac{\gamma}{(\gamma+\epsilon)} = 0.62$$

$$\frac{0.33}{0.62} = 0.33 + \epsilon$$

$$\epsilon = 0.53 - 0.33 = 0.20.$$

We may conclude, therefore, that the data of Sato and Tyler are consistent with the representation in Fig. 4 for the relation between the metabolic pool for the inorganic P with the smaller α (and the larger turnover time) where the P of DNA is derived from the pool. Thirty-three per cent of the inorganic P of the pool passes through the RNA fraction and continues through the DNA. The remainder of the P of the DNA is derived from the pool as shown, amounting to 20% of the pool content. Thus 53% of the inorganic P of the pool would pass through the DNA.

It is easy to show, however, that the system of Fig. 6 represents the data obtained from the tracer experiment equally as well as Fig. 5,

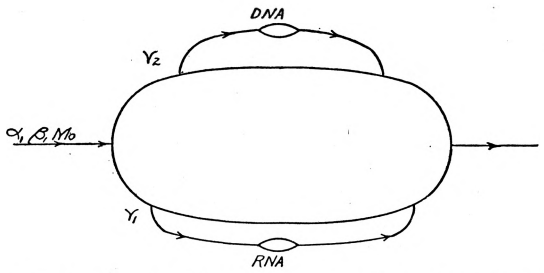

FIG. 6. A metabolic pool with two distinct branches.

without the P of RNA passing through DNA. In Fig. 5, both fractions get their P from the metabolic pool along independent pathways with $\gamma_1 = 0.33$ and $\gamma_2 = 0.53$. Thus some of the P in RNA would find its way to DNA and vice versa but there is no necessity for a direct transfer of P from RNA to DNA or for the two to occupy places on the same pathway. Much more refined experiments will be necessary in order to choose between the representations of Figs. 5 and 6 for this P transfer.

DISCUSSION

The situation encountered in these research problems is akin to the difficulties stressed by Ussing (2) in the interpretation of data on active ion transport across membranes. What we have in the analysis of an organ are the interactions of a host of complicated reactions which are so inextricably combined that it is questionable whether present experimental tracer techniques are sufficiently refined to supply the detailed knowledge essential for the working out of the physiological processes as desired. In the liver, for instance, one can safely assume that the essential reactions are occurring within the cells. We have then for the transfer of P: active transport, diffusion, and self-diffusion at the cell membrane on entering; diffusion, self-diffusion and chemical reaction within the cell; followed by active transport, diffusion, and self-diffusion across the cell membrane on leaving. If we consider the nucleus, we have the same sequence of steps repeated within the cell. The α's determined in the tracer experiment represent some sort of averaging out of these physiological processes and of any other which may be occurring. Eyring's equation for the free energy of activation gives us, in principle, a second relation for determining the parameters of the true physiological processes. This information is helpful as we have shown ahead but the necessary independent data do not exist.

In the study of simpler phenomena, e.g., ion transfer across a membrane not involving active ion transport, the mechanisms proposed on the basis of the integral equation should be closer approximations to the physiological process.

These detailed examples have been presented to show the efficacy of the integral-equation treatment in suggesting possible specific pathways for metabolites on data from tracer experiments. The encouraging fact is that present data enable one to decide in favor of one representation over another in some instances. It is possible, however, to show that still other more complicated systems will account for the present experimental facts. The systems here described seemed the simplest consistent with the information—another use of Occam's razor.

Acknowledgment

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Summary

Some metabolic pathways consistent with the findings of many tracer experiments where the data are represented as a series of exponential terms are presented. Specific examples for data on phosphorus metabolism in the rat liver are discussed in detail. The inability of existing tracer data to furnish sufficiently restrictive conditions is contrasted with the efficacy of such data in deciding against certain suggested interrelations.

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