Theorem in Chemical Kinetics

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We consider a "double isotope" experiment in which a precursor A_1 and an expected intermediate A_2 labeled with two different isotopes, say ^{14}C and ^3H, are simultaneously subjected to an enzymic preparation. Assuming a first order kinetic reaction scheme $A_1 \rightarrow A_2 \rightarrow A_3 \rightarrow A_4 \ldots$, we prove certain inequalities for the expected ratios of ^{14}C to ^3H in the products A_i which are valid for all time.

1. Introduction

The availability of ^{14}C and ^3H labeled steroids and of techniques for their simultaneous determination has encouraged the study of steroid metabolism using the "double isotope" technique. In this approach a precursor A_1 labeled say with ^{14}C and an expected intermediate A_2 labeled say with ^3H are simultaneously subjected to an enzymic tissue preparation. The isotope ratio of ^{14}C to ^3H is then determined in the succeeding products A_3, A_4, etc. (In the more standard nomenclature $A_1, A_2, A_3, \ldots$, are called A, B, C,...).

Let $x_i(t)$ and $y_i(t)$, $i = 2, 3, \ldots$, be the amounts of A_i at time t labeled with ^{14}C and ^3H, the label of A_1 and A_2 at $t = 0$ in our example, respectively. It has frequently been accepted that in an ideal homogeneous irreversible consecutive reaction scheme $A_1 \rightarrow A_2 \rightarrow A_3 \rightarrow A_4 \ldots$ in which one assumes complete mixing of the introduced intermediate with that formed from the

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preceding reactant, the isotope ratio in A_i, $R_i(t) \equiv x_i(t)/y_i(t)$ for $i = 3, 4, \ldots$, should closely approximate the isotope ratio in A_2, $R_2(t)$, if there are no other pathways involved (Matsumoto & Samuels, 1969). Any marked deviation of the isotope ratio in the products A_3, $A_4, \ldots$ from that found in the intermediate A_2 would then be considered to indicate the formation of a given product A_3, $A_4, \ldots$ from A_1 by a route not involving A_2. Such conclusions appear to have been based entirely on the intuitive notion that the ^{14}C appearing in A_2 instantaneously mixes with the ^3H already introduced in A_2 to give A_2 a certain isotope ratio, and this in turn leads to A_3 and succeeding products $A_4, \ldots$ possessing this same isotope ratio.

In a recent study of enzymic irreversible consecutive reaction sequences involving the conversion of cholesterol to pregnenolone (Burstein, Kimball & Gut, 1971) it became necessary to compute the theoretical isotope ratios $R_i(t)$ of the members of such a homogeneous first order irreversible reaction sequence. It was then found by numerical computation for various values of the rate constants of the first order sequence that the ratios $R_2(t)$, $R_3(t)$, etc. were not equal. Rather the inequality $R_2(t) > R_3(t) > R_4(t)$ always obtained. It was conjectured that these inequalities hold for all $t > 0$ for arbitrary values of the rate constants. However, a general proof of this conjecture was not provided.

It is the purpose of the present communication to provide a general analytical proof of this inequality for first order reactions.

2. First Order Irreversible Homogeneous Reactions

Consider a set of n first order irreversible homogeneous reactions. Let the reactants be enumerated sequentially as shown in the following diagram:
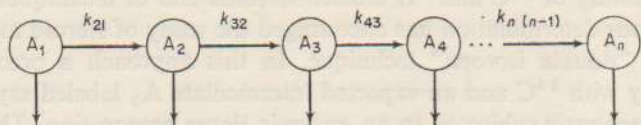
The directed arrows leaving a given reactant A_j signify that the associated reactant may either disappear to the exterior of the system or be transformed into the succeeding reactant. The quantity $k_{(j+1)j}$ represents the fractional rate at which reactant j is transformed into the succeeding reactant $(j+1)$. We shall let k_{jj} represent the net rate of disappearance of reactant j. By definition, all the k_{ij} are positive numbers, and $k_{jj} \geqq k_{(j+1)j}$.

The differential equations governing this reaction system are as follows, and will be recognized as a simple example of compartment equations of the catenary type. Let $x_j(t)$ represent the labeled amount of reactant j at time t.

Then

$$\frac{dx_1}{dt} = -k_{11}x_1,$$

$$\frac{dx_j}{dt} = k_{j(j-1)}x_{j-1} - k_{jj}x_j, \qquad j = 2, 3, \ldots, n. \qquad (1)$$

There are two particular choices of initial conditions which represent the conditions in a "double isotope" experiment. In one, a unit amount of material is contained in compartment 1, and all other compartments are empty. Thus $\{x_1, x_2, \ldots x_n\} = \{1, 0, \ldots, 0\}$ at $t = 0$. In the second choice of initial condition, a unit amount of labeled material is contained in compartment 2 and all other compartments are empty. We shall designate the solution to equation (1) for this initial condition as $\{y_j\}$ to distinguish it from the solution to equation (1) for the first initial condition, which is designated as $\{x_j\}$. For the second solution, $\{y_1, y_2, \ldots, y_n\} = \{0, 1, 0, \ldots, 0\}$ at $t = 0$.

In the "double labeling" experiment discussed in the Introduction, unit amounts of differently labeled precursor A_1 and intermediate A_2 are introduced simultaneously at $t = 0$. However, due to the assumed linearity of the reactions (i.e. they are first order), the differently labeled materials evolve independently of each other. Thus the labeled amounts in $A_1, A_2, A_3, \ldots$ at any time $t > 0$ is the same as the $x_i(t)$ and $y_i(t)$ given here. We shall show that the ratios $R_i(t)$ satisfy the inequalities

$$\frac{d}{dt} R_i(t) > 0, \qquad t > 0, \qquad i = 2, 3, \ldots, n, \qquad (2)$$

and

$$R_2(t) > R_3(t) > \ldots > R_n(t). \qquad (3)$$

We first note that, under the conditions on k_{ij} given, it is known (Bellman, 1960) that x_j is non-negative for all values of j and all $t > 0$ provided x_j is non-negative at $t = 0$, but otherwise arbitrary. This result is intuitively obvious in view of the physical significance of x_j. Because x_j and y_j are always positive, so is R_j. The solution to equation (1) for the first initial condition can be written explicitly as

$$x_1(t) = e^{-k_{11}t}, \qquad (4a)$$

$$x_j(t) = k_{j(j-1)} e^{-k_{jj}t} \int_0^t e^{k_{jj}t'} x_{j-1}(t')\, dt', \qquad j = 2, 3, \ldots, n. \qquad (4b)$$

Equation (4b) demonstrates that the amount of x_j at time t depends only on the past history of x_{j-1} and the two rate constants $k_{j(j-1)}$ and k_{jj}. Similarly,

for the second initial condition,

$$y_1(t) = 0, \tag{5a}$$

$$y_2(t) = e^{-k_{22}t} \tag{5b}$$

and

$$y_j(t) = k_{j(j-1)} e^{-k_{jj}t} \int_0^t e^{k_{jj}t'} y_{j-1}(t') \, dt', \qquad j = 3, 4, \ldots, n. \tag{5c}$$

From equations (4b) and (5c), it follows that $R_j(t)$ may be expressed as follows:

$$R_j(t) = \int_0^t \varphi_j(t'; t) R_{j-1}(t') \, dt', \qquad j = 3, 4, \ldots, n, \tag{6}$$

where

$$\varphi_j(t'; t) = \frac{e^{k_{jj}t'} y_{j-1}(t')}{\int_0^t e^{k_{jj}t'} y_{j-1}(t') \, dt'}, \qquad 0 \leq t' \leq t. \tag{7}$$

From (7) it is seen that

$$\int_0^t \varphi_j(t'; t) \, dt' = 1. \tag{8}$$

We note also that $\varphi_j(t'; t)$ is positive for all $t, t' > 0$ because $y_j(t)$ is positive for all $t > 0$.

We now show that R_j is a strictly increasing function for all $t > 0$. From equation (6), we calculate

$$\frac{dR_j(t)}{dt} = \varphi_j(t; t) R_{j-1}(t) + \int_0^t \frac{\partial \varphi_j}{\partial t}(t'; t) R_{j-1}(t') \, dt', \tag{9}$$

and from equation (7) it follows that

$$\frac{\partial \varphi_j(t'; t)}{\partial t} = -\varphi_j(t'; t) \varphi_j(t; t). \tag{10}$$

Substituting equation (10) into equation (9), there results

$$\frac{dR_j(t)}{dt} = \varphi_j(t; t) \int_0^t \varphi_j(t'; t) \{R_{j-1}(t) - R_{j-1}(t')\} \, dt'. \tag{11}$$

Therefore, $dR_j/dt > 0$ and $R_j(t)$ is a strictly increasing function of t provided $R_{j-1}(t)$ is a strictly increasing function of t. Thus, if $R_2(t)$ has this property, then so does R_j for all $j \geq 3$. $R_2(t)$ does indeed have this property, as may be seen by explicit calculation from equations (4) and (5):

$$R_2(t) = \begin{cases} \dfrac{k_{21}}{k_{22} - k_{11}} \left[e^{(k_{22} - k_{11})t} - 1 \right], & k_{22} \neq k_{11}, \\ k_{21} t, & k_{22} = k_{11}. \end{cases} \tag{12}$$

From equations (6) and (8) it follows directly that

$$R_{j+1}(t) - R_j(t) = \int_0^t \varphi_{j+1}(t'; t)\{R_j(t') - R_j(t)\}\, dt', \quad j = 2, 3, \ldots, n-1. \quad (13)$$

Inasmuch as $\varphi_{j+1}(t'; t)$ is always positive and the bracket is always negative (except at the endpoint $t' = t$) because of the strictly increasing nature of $R_j(t)$, the right-hand side of equation (13) is a negative number so long as $t > 0$. Hence the desired result is proven:

$$R_j(t) > R_{j+1}(t), \quad j = 2, 3, \ldots, n-1, \quad t > 0. \quad (14)$$

The actual values of $R_j(t)$ depend of course on the rate constants. It is easy to find a set of rate constants for which $R_j(t)$ is very much greater than $R_{j+1}(t)$. Indeed, we can have cases where $R_j(t)/R_{j+1}(t) \to \infty$ exponentially as $t \to \infty$.

3. Discussion

Here we have concerned ourselves with the ideal, first approximation approach in which homogeneous reaction conditions were presumed. The situation, of course, becomes much more complicated if such conditions do not prevail. If a product produced *in situ* behaves differently from that introduced into the medium, then interpretation of data on merely intuitive grounds may prove to be grossly misleading.

For reactions obeying first order kinetics, if the isotope ratios of the intermediates and products are found to disobey the inequalities proven herein, then we may infer the existence of other pathways, and/or "non-ideal" reaction conditions. The determination of the "ideality" of the reaction will require independent means other than the determination of the individual reaction rates. An attempt in this direction has been described by Matsumoto & Samuels (1969).

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