Nonlinear Enzymatic Cycling Systems: the Exponential Cycling System

II. EXPERIMENTAL CYCLING SYSTEM*

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SUMMARY
The existence of a previously undescribed class of enzymatic cycling systems, the nonlinear cycling systems, is demonstrated in the form of an exponential cycling system. The system is based on the reactions catalyzed by myokinase and pyruvate kinase. Under the appropriate conditions, the concentrations of ADP, ATP, and pyruvate in the system increase exponentially with time and are linear functions of the initial concentrations of ADP and ATP. The behavior of the experimental cycling system is compared with the explicit mathematical model of the system. Preliminary results with two other exponential cycling systems are briefly discussed.

In an accompanying paper (1) we demonstrated the theoretical existence of a previously undescribed type of enzymatic cycling system, the exponential cycling system. Mathematical models for three such systems were derived and some of the properties of the systems were discussed. The present communication presents a detailed investigation comparing some of the predictions of the model of the myokinase-pyruvate kinase exponential cycling system (shown in Fig. 1) with the behavior of the corresponding experimental cycling system.

MATERIALS AND METHODS
The following reagents were purchased from Sigma: ADP, ATP, P-enolpyruvate, NADH, and crystalline rabbit skeletal muscle enzymes; myokinase (Grade III, 600 to 1000 units per mg), pyruvate kinase (type II, 300 to 500 units per mg), and lactate dehydrogenase (type II, 400 units per mg). The myokinase and pyruvate kinase preparations were further purified to reduce adenine nucleotide contamination. Escherichia coli alkaline phosphatase (salt fractionated, 25 units per mg) was obtained from Armour Inc. AMP was obtained from P-L Biochemicals, and was further purified to remove ADP and ATP contamination. Tris(hydroxymethyl)aminomethane was the Ultrapure grade of Mann Biochemicals. Triethylamine was a product of Matheson, Coleman, and Bell and was redistilled before use. Bio-Gel P-150 (100-200 mesh) was obtained from Bio-Gel TE-2 anion exchange resin was a gift of Mr. Anthony Ross (Brown University).

The purification of pyruvate kinase—Pyruvate kinase was purified by the following modification of the Turtle and Kipnis method (2) which was originally used for the purification of myokinase.

One milliliter of a 3.2 M ammonium sulfate suspension of pyruvate kinase, containing 10 mg of protein, was adjusted to 0.9 to 0.95 saturation in ammonium sulfate by the addition of the solid salt. The precipitate was collected by centrifugation and redissolved in 200 μl of 0.1 M Tris (Cl), pH 8.0, containing 50 μg of E. coli alkaline phosphatase. After incubation for 1 hour at 30°C, the mixture was clarified by centrifugation and applied to a Bio-Gel P-150 column (9 mm x 750 mm) equilibrated at room temperature with 50 mM Tris (Cl), pH 8.0, 100 mM KCl, 1 mM EDTA plus 5% (v/v) glycerol. The column was developed at room temperature with the same buffer. The precipitate was centrifuged and dissolved in 200 μl of 0.1 M Tris (Cl), pH 8.0, containing 50 μg of E. coli alkaline phosphatase. After incubation for 1 hour at 30°C, the mixture was clarified by centrifugation and applied to a Bio-Gel P-150 column (9 mm x 750 mm) equilibrated at room temperature with 50 mM Tris (Cl), pH 8.0, 100 mM KCl, 1 mM EDTA plus 5% (v/v) glycerol. The column was developed at room temperature with the same buffer. Fractons (0.5 ml) were collected and assayed for pyruvate kinase and alkaline phosphatase activities.

Pyruvate kinase activity was assayed spectrophotometrically by a coupled assay. The assay mixture contained 100 mM Tris (Cl) pH 8.0, 100 mM KCl, 4 mM MgSO4, 2 mM P-enolpyruvate, 3 mM ADP, 0.15 mM NADH, and 10 μg per ml of lactate dehydrogenase. Aliquots (10 μl) of each fraction diluted with 50 mM Tris (Cl) pH 8.0 were added to 100-μl portions of the assay mixture, and the rate of reaction was determined from the recorded rate of change in absorbance at 340 nm. Alkaline phosphatase activity was estimated with P-enolpyruvate as the substrate. Aliquots (10 μl) of each fraction were incubated for 5 to 10 min at room temperature in 100-μl portions of an assay mixture containing 100 mM Tris (Cl), pH 8.0, 2 mM MgSO4, 1 mM ZnSO4, 2 mM P-enolpyruvate, 0.15 mM NADH, and 10 μg per ml of lactate dehydrogenase. After incubation, the reaction velocity was determined from the recorded rate of change in absorbance at 340 nm. The incubation period served to increase the sensitivity and reproducibility of the phosphatase assay.

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and the collected fractions were assayed for myokinase and was identical to that for pyruvate kinase except that the buffer coli alkaline phosphatase. The remainder of the purification sulfate suspension were collected by centrifugation and redisolved in 200 ~l of 

kinase. Approximately 4 mg of myokinase in an ammonium preparations used in this procedure; however, AMP and P-enolpyruvate (PEP) are present at relatively high concentrations. The cycling intermediates, ATP and ADP are initially present at very low concentrations, and increase in concentration exponentially with time. Pyruvate, a by-product of the system, also increases its concentration exponentially with time.

Myokinase activity was assayed spectrophotometrically in a coupled assay. The assay mixture contained 100 mM Tris (Cl), pH 8.0, 100 mM KCl, 4 mM MgSO_4_, 2 mM P-enolpyruvate, 2.5 mM ATP, 4 mM AMP, 0.15 mM NADH, 10 µg per ml of pyruvate kinase and 10 µg per ml of lactate dehydrogenase. Samples (10 µl) of each fraction, previously diluted with 50 mM Tris (Cl) pH 8.0 containing 1 mg per ml of bovine plasma albumin, were added to 100-µl portions of the assay mixture. The rate of reaction was determined from the recorded rate of change in absorbancy at 340 nm.

Alkaline phosphatase activity was assayed as in the purification of pyruvate kinase.

**Purification of AMP—**A 12 mm X 120 mm column was packed with Bio-Gel TE-2 anion exchange resin, and washed successively with 100-ml portions of water, 0.1 M NaOH, water, 0.1 M and 0.01 M triethylamine buffer (Buffer A) saturated with carbon dioxide. AMP (200 mg of the free acid) was dissolved in 10 ml of distilled water with the aid of concentrated ammonium hydroxide. The pH of the solution was 8.0 when it was applied to the column. The AMP was eluted with a linear gradient of Buffer A from 0.01 to 0.4 M over 500 ml. The fractions containing AMP were pooled and concentrated to dryness by flash evaporation.

**Dynamic Cycling Procedure, Procedure A—**The dynamic cycling procedure was developed to test the agreement between some of the kinetic predictions of the model cycling system and the behavior of the experimental cycling system. The procedure used lactate dehydrogenase as an indicator enzyme and monitored the behavior of the system by the changing absorbancy of the reaction mixture at 340 nm.

Cycling reaction mixtures were prepared containing 50 mM Tris (Cl), pH 8.0, 100 mM KCl, 2 mM MgSO_4_, 0.50 mM P-enolpyruvate, 1 mM AMP, 50 to 60 µM NADH, appropriate amounts of myokinase and pyruvate kinase and lactate dehydrogenase to give a pseudo-first order rate constant k_L of at least 15 per min with respect to pyruvate. The reaction was initiated by the addition of the AMP. It was not necessary to purify the myokinase and AMP preparations used in this procedure; however, the commercial myokinase kinase preparation contained an inhibitor which was removed by dialysis for 2 hours at room temperature against 50 mM Tris (Cl), pH 7.5, 100 mM KCl, and 1 mM EDTA.

The amount of lactate dehydrogenase required to give the desired rate constant was calculated from the relation

$$V_L = V_{max}/K_m$$

where $V_{max}$ and $K_m$ were determined from a Lineweaver-Burk plot under conditions similar to the cycling reaction conditions except that pyruvate kinase and myokinase were omitted and appropriate concentrations of pyruvate were added to the reaction mixtures. The apparent $K_m$ for pyruvate under those conditions was 1 mM.

**Timed Point Cycling Procedure, Procedure B—**The timed point cycling procedure was developed to test the prediction that the concentration of pyruvate at any given time in the cycling system is a linear function of the initial concentration of ATP in the system. Cycling reaction mixtures were prepared containing appropriate concentrations of ATP and the cycling reaction was allowed to proceed for a constant period of time. The reaction was terminated by the addition of EDTA and the pyruvate generated by the cycling reaction was quantitated by its conversion to lactate with lactate dehydrogenase. The NAD produced in the conversion was quantitated fluorometrically by a modification of the procedure by Lowry et al. (3).

Cycling reaction mixtures were prepared with 25-µl samples of known ATP concentrations, 25 µl of 2 mM AMP and 50 µl of a solution containing 100 mM Tris (Cl), pH 8.0, 200 mM KCl, 4 mM MgSO_4_, 1 mM P-enolpyruvate, 300 µg per ml of bovine plasma albumin, and myokinase and pyruvate kinase to give pseudo-first order rate constants of 7.5 per min and 0.47 per min for ATP and ADP, respectively. The cycling reaction was initiated at timed intervals by the addition of the AMP. After incubation at 30°C for the appropriate cycling time, the reactions were stopped by the addition of 25 µl of 50 mM EDTA (adjusted to pH 8.0 with sodium hydroxide) at the same time intervals used to initiate the reaction. Each reaction then received 25 µl of a mixture containing 25 mM EDTA, pH 8.0, 10 µg per ml of lactate dehydrogenase and approximately 150 µM NADH (made NAD free by the method of Lowry et al.) (3). After incubation for at least 10 min at room temperature, the NAD concentration of the mixtures was determined by a slight modification of the Lowry procedure (3). Hydrochloric acid (25 µl of 1.2 N HCl) was added to each reaction mixture followed by 150 µl of 9 N sodium hydroxide after at least 5 min. The mixtures were heated at 95°C for 10 min, cooled to room temperature, and diluted with 1 ml of distilled water. The fluorescence of the mixtures was determined with a Turner filter fluorometer equipped with a 7-60 primary filter and 2A plus 48 filters as secondary filters. The change in fluorescence was calibrated using the same procedure with known pyruvate concentrations, and was found to be reproducible from day to day.
RESULTS AND DISCUSSION

The mathematical description of the model myokinase-pyruvate kinase cycling system has been given previously (1). For comparison of the model cycling system and its experimental counterpart, only a portion of the original set of equations is necessary:

\[ P(t) = P_0 - D_0 - 2T_0 \]
\[ + \frac{k_2}{R} \left\{ 2k_1T_0 + \left( \lambda_1 + k_1 \right)D_0 \right\} e^{\frac{\lambda_1 t}{k_1}} \]
\[ - \left\{ 2k_1T_0 + \left( \frac{\lambda_2 + k_1}{D_0} \right)k_2 \right\} e^{\frac{\lambda_2 t}{k_2}} \]

where \( k_1 \) and \( k_2 \) are first order rate constants for the reactions catalyzed by myokinase and pyruvate kinase, respectively. \( P \), \( D \), and \( T \) are concentrations of pyruvate, ADP, and ATP in the cycling system, respectively. The subscript zero denotes the initial concentrations of these substances.

The prediction from Equation 1 that the amount of pyruvate generated by the system increases exponentially with time was tested by use of the dynamic cycling procedure (Procedure A). This procedure uses lactate dehydrogenase to convert pyruvate to lactate, simultaneously giving a stoichiometrically equal conversion of NADH to NAD. The use of lactate dehydrogenase introduces an additional kinetic parameter, \( k_L \), the pseudo-first order rate constant with respect to pyruvate, which must be considered in the interpretation of the data. A model of the system based on Equation 1 and including the lactate dehydrogenase reaction is derived in Appendix I and given by the Equation 5.

\[ N_0 + 2T_0 - N = 2T_0 + \left( \lambda_1 + k_1 \right) \frac{2k_1k_2k_L}{\lambda_1(\lambda_2 + k_1)} e^{\frac{\lambda_1 t}{k_1}} \]
\[ - \frac{2k_1k_2k_L}{\lambda_2(\lambda_2 + k_1)} e^{\frac{\lambda_2 t}{k_2}} \]
\[ - \frac{2k_1k_2k_L}{\lambda_1(\lambda_1 + k_1)} e^{\frac{\lambda_1 t}{k_1}} \]

where \( N \) is the NADH concentration in the cycling system and \( -\lambda_2 \neq k_L \). \( N_0 \) is \( N \) when \( t = 0 \). Equation 6 which is used in the interpretation of the experimental data was obtained from Equation 5 by assuming that the terms involving \( e^{\lambda_1 t} \) and \( e^{\lambda_2 t} \) were negligible during the period of data collection. The theoretical validity of these assumptions may be checked by direct calculation from Equation 5. In calculations used for determining the point in time at which the negligibility assumptions became valid, it was assumed that a term was negligible if its magnitude was less than 1% of the magnitude of the term involving \( e^{\lambda_1 t} \). A typical example of calculations of this type indicated that, for a value of \( k_L \) 15 times larger than the largest value of \( \lambda_1 \) in a set of cycling reactions, the assumptions were valid for \( \lambda_L \) greater than or equal to approximately 1.0, regardless of the values of \( k_1 \) and \( k_2 \). For the dynamic cycling reported in this paper, direct calculations with Equation 5 indicated that Equation 6 could be used in the interpretation of the data after the 1st min of cycling. In practice, the data from the 1st min of the cycling reaction in most cases was below the limits of resolution of the spectrophotometric determinations used in the cycling procedure. Equation 6 may be transformed to give a more usable form of the equation:

\[ \ln \left( N_0 - N + 2T_0 \right) = \lambda_1 t + \ln \left( \frac{2k_1k_2k_L}{\lambda_1(\lambda_1 + k_1)} \right) \]

The equation implies that a semilog plot of change in concentration of NADH, corrected for the initial ATP concentration, versus time should give a straight line with its slope equal to \( \lambda_1 \) (Fig. 2). Fig. 2 shows a typical example of data obtained by the dynamic cycling reaction. It was transformed to give a line with slope \( \lambda_1 \) by addition of a small constant absorbancy to the initial absorbancy of the reaction mixture. The value of \( \lambda_1 \) as calculated from the slope of line \( b \) is 0.801 per min.
concentrations of ATP and ADP in the system have become large enough to invalidate the first order kinetic assumptions used in the derivation of the mathematical model. Thus, over a substantial portion of the measurable cycling reaction, pyruvate is generated in exponentially increasing amounts with time.

The simple method for transforming the crude data from the dynamic cycling procedure to give a straight line with its slope equal to $k_1$ provides a way of testing the relationship of $\lambda_1$ to $k_1$ and $k_2$, as predicted by Equation 2. Qualitatively, as one of the reaction rate constants (e.g., $k_1$) becomes much larger than the other ($k_2$), the value of $\lambda_1$ asymptotically approaches the value of the rate limiting rate constant ($k_2$) (Fig. 3).

The asymptotic behavior of $\lambda_1$ as one of the rate constants becomes larger than the other gives a method of quantitating the activities of myokinase and pyruvate kinase in terms of their apparent pseudo-first order rate constants in the cycling reaction; the value of the rate constant for the rate limiting enzyme is approximately equal to $\lambda_1$ when the other enzyme is present in large excess. The method of quantitating the reaction rate constants, $k_1$ and $k_2$, gives a way of testing the quantitative aspects of the equations. In its original form, the equation makes the problem of demonstrating the quantitative aspects of the relation unnecessarily complex. The equation may be algebraically rearranged to give two equivalent equations emphasizing different aspects of the same relation:

$$k_2 = \frac{\lambda_1 (\lambda_1 + k_2)}{k_1 - \lambda_1}$$  \hspace{1cm} (8)

$$k_1 = \frac{\lambda_1 (\lambda_1 + k_2)}{k_2 - \lambda_1}$$  \hspace{1cm} (9)

These equations predict that for a set of cycling reactions in which one of the reaction rate constants is held constant at $k_2$ while the other, $k_1$, is varied, a plot of $\lambda_1 (\lambda_1 + k_2)$ versus $k_1 - \lambda_1$ should give a straight line intersecting the origin and having its slope equal to $k_2$ (Figs. 4 and 5). Taking both sets of data together, Equation 2 quantitatively describes the relationship of $\lambda_1$ to $k_1$ and $k_2$ over more than four orders of magnitude change in the ratio of $k_1$ to $k_2$. The prediction that Equation 3 quantitatively describes the relation between $\lambda_2$ and $k_1$ and $k_2$ over the same interval is consistent with this result.

The apparent validity of Equation 2 gives a method of refining the data somewhat. For example, a preliminary plot of $\lambda_1 (\lambda_1 + k_2)$ versus $k_1 - \lambda_1$ gives a better estimate of $k_2$ than a single asymptotic determination of $k_2$. The refined estimate of $k_2$ can be in turn used to refine the original estimate of $k_1$. The refined estimates of $k_1$ and $k_2$ generally differ from the original asymptotic estimates by less than 10%, so that the results are not materially changed except for a slightly better fit by the experimental data to the predicted relation.

Equation 2 also gives a simple iterative procedure (described in Appendix II) for estimating the reaction rate constants for preparations of myokinase and pyruvate kinase without resorting to asymptotic conditions in the assays. For the data shown...
the initial estimates for $k_1$ and $k_2$ were made by the iterative procedure. These estimates were left unchanged by the procedure described above for refining them.

Aside from the kinetic properties of the myokinase-pyruvate kinase cycling system, the most important remaining predicted characteristic of the system is that the concentrations of pyruvate, ATP, and ADP in the system after a constant cycling reaction period are a linear function of the initial concentrations of ATP and ADP. This prediction was tested for the case of pyruvate as a function of the initial ATP concentration. The results of two such tests are shown in Fig. 6. Taken together, the results indicate that the pyruvate concentration is a linear function of the initial ATP concentration for at least two orders of magnitude variation in the initial ATP concentration. The fact that the pyruvate increased with time in the cycling reaction mixtures prepared with no added ATP suggests that the reagents used to prepare the mixtures still contained traces of contaminating ATP or ADP. The magnitude of the apparent contamination may be estimated from the ATP-axis intercept in Fig. 6b as equivalent to approximately 0.68 pmol pyruvate. The vertical bars through the point are the standard deviations for those points.

Models for two other exponential cycling systems have been derived (1). The first of these, the pyruvate carboxylase system, shown in Fig. 7, is based on the reactions catalyzed by pyruvate carboxylase (4, 5), pyruvate kinase, and oxaloacetate decarboxylase (6). Preliminary results with this system (5) indicate that the system is more complicated than the model...
FIG. 7. The pyruvate carboxylase cycling system. The reactions designated $k_1$, $k_2$, and $k_3$ are catalyzed by pyruvate kinase, pyruvate carboxylase, and oxalacetate decarboxylase, respectively. P-enolpyruvate (PEP), ATP, and bicarbonate are present at relatively high concentrations. The cycling intermediates, ADP, pyruvate, and oxalacetate, are initially present at very low concentrations and their concentrations increase exponentially with time during the cycling reaction. Orthophosphate, a by-product of the cycling reaction, also increases in concentration exponentially with time.

FIG. 8. The P-enolpyruvate synthase cycling system. The reactions designated $k_1$, $k_2$, and $k_3$ are catalyzed by P-enolpyruvate synthase, myokinase, and pyruvate kinase, respectively. ATP and P-enolpyruvate are present at relatively high concentrations. The cycling intermediates, pyruvate, AMP, and ADP, are initially present at very low concentrations and their concentrations increase exponentially with time during the course of the cycling reaction. Orthophosphate, a by-product of the cycling reaction, also increases in concentration exponentially with time.

suggests. The system is a nonlinear cycling system, but apparently cannot be described in terms of simple exponential functions. However, the concentrations of the cycling intermediates at any given time appear to be linear functions of the initial concentrations of the intermediates.

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APPENDIX I

The Myokinase-Pyruvate Kinase Cycling System with Lactate Dehydrogenase as an Indicator Reaction on the Dynamic Cycling Procedure

Consider the myokinase-pyruvate kinase cycling system as a pyruvate generator:

\[
P(t) = \frac{2k_1 k_2 T_0}{\sqrt{k}} \left( e^{\lambda_2 t} - e^{-\lambda_1 t} \right) - 2T_0
\]

where Equation I-1 is equivalent to Equation 1 of the test with $P_0 = D_0 = 0$. With conditions such that the pyruvate to lactate conversion catalyzed by lactate dehydrogenase,

\[
\text{pyruvate}^- \rightarrow \text{NADH}^- + \text{H}^+ \rightarrow \text{lactate}^- + \text{NAD}^+
\]

is describable by

\[
\frac{dP}{dt} = -k_L P
\] (I - 2)

where $k_L$ is a first order rate constant, the rate of change in pyruvate concentration in a system in which pyruvate is being generated in time by Equation I-1, and simultaneously converted to lactate by Equation I-2 is given by

\[
\frac{dP}{dt} = -k_L P + \frac{2k_1 k_2}{\sqrt{k}} (e^{\lambda_1 t} - e^{\lambda_2 t}).
\] (I - 3)

Equation I-3 may be explicitly solved for $P$ as a function of time by the use of Laplace transforms. (See Hildebrand (8) for a discussion of the use of Laplace transforms, and Selby (9) for a useful table of transforms.)

The Laplace transform, $f(s)$, of I-3 is

\[
f(s) = \frac{2k_1 k_2 T_0}{\sqrt{k}} \left[ \frac{1}{(s - \lambda_1)(s - \lambda_2)} - \frac{1}{(s - \lambda_2)(s - \lambda_1)} \right]
\]

with initial condition that $P_0 = 0$.

The inverse transformation of I-4 for the case of $-\lambda_2 \neq k_L$ gives:

\[
P(t) = -k_L P \left[ \frac{1}{(\lambda_1 + k_L)} (e^{\lambda_1 t} - e^{-k_L t}) - \frac{1}{(\lambda_2 + k_L)} (e^{\lambda_2 t} - e^{-k_L t}) \right].
\] (I - 5)

Considering the lactate dehydrogenase reaction, it is evident that

\[
\frac{dN}{dt} = -k_L P
\] (I - 6)
where \( N \) is NADH. Substitution of I-5 into I-6 and integration with respect to time gives

\[
N_o + 2T_o - N = \frac{2k_1 k_2 T_o}{vR} \left[ \lambda_1 e^{\lambda_1 t} \div \frac{1}{\lambda_1 (\lambda_1 + k_L)} \right. \\
\left. - \frac{e^{\lambda_2 t}}{\lambda_2 (\lambda_2 + k_L)} \right] \\
- \frac{vR e^{k_L t}}{(\lambda_1 + k_L)(\lambda_2 + k_L)}. \tag{I - 7}
\]

**APPENDIX II**

**Iterative Procedure for Estimation of \( k_1 \) and \( k_2 \) for values of \( \lambda_1 \) in the Myokinase-Pyruvate Kinase Exponential Cycling System**

Given two cycling reactions mixtures; Mixture A which yields \( \lambda_1 \) for \( k_1 \) and \( n k_2 \), and Mixture B which yields \( \lambda_1 \) for \( m k_1 \) and \( k_2 \), where \( m \) is the ratio of myokinase activity in Mixture B to its activity in Mixture A, and \( n \) is the ratio of pyruvate kinase activity in Mixture A to its activity in Mixture B. Both \( m \) and \( n \) may be greater than or equal to one, but they may not both equal one simultaneously. Technically, setting \( m \) equal to \( n \) is most convenient (e.g. 2 volumes of pyruvate kinase are used in Mixture A and 1 in Mixture B, while 1 volume of myokinase is used in Mixture A and 2 volumes in B to give \( m = n = 2 \).

Let \( (k_1)_i \) and \( (k_2)_i \) be the \( i \)th estimates for \( k_1 \) and \( k_2 \), respectively, where \( i = 1, 2, 3 \ldots \), then let \( (k_1)_1 \) be an arbitrary real number, \( c \).

First estimate:

\[
(k_1)_1 = c \tag{II - 1}
\]

\[
(k_2)_1 = \lambda_1 \left[ \lambda_1 + n (k_2)_1 \right] / [m (k_2)_1 - \lambda_1] \tag{II - 2}
\]

Second estimate:

\[
(k_1)_2 = \lambda_1 \left[ \lambda_1 + n (k_2)_1 \right] / [m (k_2)_1 - \lambda_1] \tag{II - 3}
\]

\[
(k_2)_2 = \lambda_1 \left[ \lambda_1 + n (k_2)_2 \right] / [m (k_2)_2 - \lambda_1] \tag{II - 4}
\]

\[
(k_1)_i = \lambda_1 \left[ \lambda_1 + n (k_2)_1 \right] / [m (k_2)_1 - \lambda_1] \tag{II - 5}
\]

\[
(k_2)_i = \lambda_1 \left[ \lambda_1 + n (k_2)_i \right] / [m (k_2)_i - \lambda_1] \tag{II - 6}
\]

As \( i \) becomes larger, the differences between successive estimates for either \( k_1 \) or \( k_2 \) become smaller. The convergence of the estimation procedure has not been shown rigorously, but calculations performed on a digital calculator programmed for the calculations showed that the sequences do converge, regardless of the initial estimate for \( (k_1)_1 \). Any degree of precision could be obtained by a sufficient number of iterations. In practice only four or five iterations are required to give at least three digit precision in the estimates for \( k_1 \) and \( k_2 \) when the original estimate was arbitrarily set equal to 1 min\(^{-1}\).

**REFERENCES**

1. Kopp, L. E., and Miech, R. P. (1972) J. Biol. Chem. 247, 3558-3563
2. Turtle, J. R., and Kipnis, D. M. (1967) Biochemistry 6, 3970-3976
3. Lowry, O. H., Roberts, N. R., and Kapphan, J. I. (1957) J. Biol. Chem. 234, 1047-1064
4. Steubert, W., and Remberger, U. (1961) Biochem. Z. 334, 401-414
5. Kopp, L. E. (1971) Doctoral dissertation, Brown University
6. Herbert, D. (1955) Methods Enzymol. 1, 875
7. Cooper, R. A., and Kornberg, H. L. (1967) Proc. Roy. Soc. London B Biol. Sci. 168, 263
8. Hildebrand, F. B. (1962) Advanced calculus for applications, Prentice-Hall, Inc. Englewood Cliffs, New Jersey
9. Selby, S. M. (Editor) (1969) Standard mathematical tables, Ed. 17, Chemical Rubber Co., Cleveland, Ohio
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