65. J. Klotz and J. Frakzen, J. Am. Chem. Soc., 84, No. 18, 3461-3466 (1962).
66. V. Jenks, Catalysis in Chemistry and Enzymology [Russian translation], Mir, Moscow (1972), Chapters 6-9.
67. L. Schafer, C. Van Alsenoy, and J. N. Scarsdall, J. Chem. Phys., 76, No. 3, 1439-1444 (1982).
68. A. Wlodawer, J. Walter, R. Huber, and L. Sjolin, J. Mol. Biol., 180, No. 2, 301-329 (1984).
69. J. Cheney, B. V. Cheney, and W. G. Richards, Biochim. Biophys. Acta, 954(P47), No. 1, 137-139 (1988).
70. M. V. Vol'kenshtein, I. B. Golovanov, A. K. Grenader, G. L. Ermakov, and V. V. Nauchitel', Dokl. Akad. Nauk SSSR, 287, No. 4, 986-990 (1987).
71. M. V. Vol'kenshtein, I. B. Golovanov, A. K. Grenader, and G. L. Ermakov, Biofizika, 33, No. 6, 968-972 (1988).
72. M. A. Basharov, M. V. Vol'kenshtein, I. B. Golovanov, A. K. Grenader, and G. L. Ermakov, Biofizika, 31, No. 5, 741-745 (1986).
73. M. F. Peruts, G. Fermi, D. J. Abraham, G. Poyart, and E. Bursauk, J. Am. Chem. Soc., 108, No. 5, 1064-1078 (1986).
74. M. Levitt and M. F. Peruts, J. Mol. Biol., 201, No. 4, 751-754 (1988).

A REFINED MATHEMATICAL MODEL OF AN ALMOST IDEAL BIOCHEMICAL RELAXATION OSCILLATOR BASED ON THE COVALENT MODIFICATION OF AN ENZYME

I. I. Goryanin, E. E. Sel'kov, and K. I. Serdyuk

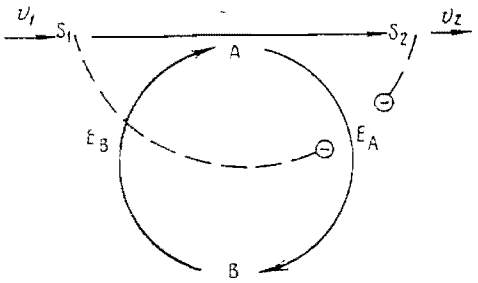
UDC 577.152

A refined mathematical model (M2) was developed and analyzed; the model describes the generation of relaxation auto-oscillations in the open reaction $\xrightarrow{E(A,B)} S_1 \rightleftharpoons S_2 \xrightarrow{}$ in which the enzyme E(A, B) is covalently modified by the modifying enzymes E_A and E_B in such a way that the active A form of enzyme is converted to the inactive B form by enzyme E_A, and the B form in reactivated to the A form by enzyme E_B. The M2 model assumes that the substrate S_1 and the product S_2 competitively inhibit the inactivating enzyme E_A. The system described by M2, like the previously described phenomenological model M1 (E. E. Sel'kov and I. I. Goryanin, Mol. Biol., 20, 1550-1562 (1986)), was shown to be able to undergo relaxation auto-oscillation. Asymptotic equations for the quasi-stationary rate of the reaction $S_1 \rightarrow S_2$ were derived, taking all enzyme—ligand complexes of enzymes E, E_A, and E_B into consideration, and asymptotic expressions for the period and amplitude of the relaxation oscillations were also deduced. Good qualitative and quantitative agreement was demonstrated between experimentally measured oscillation periods for the M1 and M2 models and values obtained by numerical integration of the M2 model in conditions in which the total enzyme concentration E(A, B) was significantly greater than the total concentrations of enzymes E_A and E_B.

Covalent modification of enzymes [1-6] is a significantly more efficient way of controlling enzyme activity than methods based on equilibrium conformational transitions [7-10]. This explains the fact that the most important key reactions in cell metabolism are regulated by covalent enzyme modification [1-6]. Sel'kov and Goryanin [11] investigated a mathematical model of the flow-through reaction $\rightarrow S_1 \rightarrow S_2 \rightarrow$, which is catalyzed by the enzyme E(A, B), which is in turn subject to covalent modification by enzymes E_A and E_B according to scheme (1).

Institute of Theoretical and Experimental Biophysics, Russian Academy of Sciences, Pushchino, Moscow Region 142292. Translated from Molekulyarnaya Biologiya, Vol. 26, No. 2, pp. 404-417, March-April, 1992. Original article submitted October 17, 1991.

 0026-8933/92/2602-0310$12.50



(1)

In scheme (1), A is the active form and B is the inactive form of enzyme E(A, B); S_1 and S_2 are the substrate and product respectively of the reaction $S_1 \rightarrow S_2$, and are also inhibitors of the modifying enzyme E_A, inactivating the active A form; E_B is the activating modifying enzyme.

Analysis of a mathematical model of system (1) showed that in certain conditions it can be an almost ideal generator of relaxation oscillations, whose period and amplitude of oscillation can be calculated analytically.

The possibility of analytically calculating the parameters of the oscillatory regime in systems of type (1) opens wide vistas in the theoretical analysis of the mechanisms by which cell metabolism is organized in time [12, 13], which up to now has rested solely on very difficult numerical calculations [14]. Sel'kov and Goryanin [11] derived an asymptotic mathematical model of system (1) that permits analytical calculations of its oscillatory regime to be carried out, and the derivation was based on a series of simplifying assumptions; phenomenological expressions for the rates of the reactions catalyzed by enzymes E(A, B), E_A, and E_B were used. However, the conditions required for deducing the rate reactions from molecular interactions between the enzymes and their ligand remained unknown.

In this report we describe a kinetic model of system (1), taking into account all possible enzyme—ligand complexes, and we determine the conditions in which the mathematical model of system (1) approximates the previously described phenomenological model [11].

KINETIC MODEL

The complete scheme of all possible interactions of ligands with the three enzymes E(A, B), E_A, and E_B in scheme (1) is very complex, and the system can only be analyzed numerically. However, if certain assumptions are made about the mechanisms of the interactions between the ligands and the enzymes, and if large differences in the concentrations of the various components and the rates of the elementary stages are used, i.e., as usually occur in real biochemical systems, the mathematical description of system (1) becomes considerably simpler, and in particular conditions allows analytical investigations to be carried out. For the purposes of deducing a mathematical model of system (1) we use the following simplifying assumptions on the mechanisms of action of enzymes E(A, B), E_A, and E_B, and on the nature of the interactions of the ligands with these enzymes.

We assume that enzyme E(A, B) has two different binding centers: a catalytic center to which the substrate S_1 attaches, and an allosteric center whose modification by the modifying enzymes E_A and E_B leads to cyclical interconversion of the two forms, i.e., $A \rightleftarrows B$. Events in the catalytic center of forms A and B are independent of attachment of enzymes E_A and E_B, though the forms themselves have different affinities for S_1 and different catalytic efficiencies. The mechanism of action of forms A and B can be described as a Michaelis—Menten mechanism:

$$S_1 + A \underset{k_{-1}}{\overset{k_{+1}}{\rightleftarrows}} S_1A \rightarrow A + S_2, \tag{2}$$

$$S_1 + B \underset{k'_{-1}}{\overset{k'_{+1}}{\rightleftarrows}} S_1B \rightarrow B + S, \tag{3}$$

where $k_{\pm 1}$, $k'_{\pm 1}$, k_{+2}, and k'_{+2} are the rate constants of the elementary stages.

We further propose that form B has a low efficiency:

$$k'_{+2} \ll k_{+2}. \tag{4}$$

Supporting the independence of events in the catalytic and allosteric centers, mechanism (2) holds both for the free A form and for its complexes with enzyme E_A, and for complexes of E_A with the allosteric inhibitors of this enzyme S_1 and S_2. Similarly, mechanism (3) also holds for the complex of the B form with E_B. Again supporting this independence, the modifying enzymes act in identical ways on forms A and B free and bound with S_1:

$$A'+E_A \underset{a_{-1}}{\overset{a_{+1}}{\rightleftharpoons}} A'E_A \xrightarrow{a_{+2}} E_A+B', \tag{5}$$

$$B'+E_B \underset{b_{-1}}{\overset{b_{+1}}{\rightleftharpoons}} B'E_B \xrightarrow{b_{+2}} E_B+A', \tag{6}$$

where $a_{\pm 1}$, $b_{\pm 1}$, a_{+2}, and b_{+2} are the rate constants of the early stages. Here and subsequently, a prime (') is used to designate mixtures of free and S_1-bound molecules. The concentrations of such mixtures are given by the sums:

$$A'=A+S_1A,\ A'E_A=AE_A+S_1AE_A, \tag{7}$$
$$B'=B+S_1B,\ B'E_B=BE_B+S_1BE_B.$$

We also assume that substrate S_1 and product S_2 of the major reaction $S_1 \rightarrow S_2$ (equations 2, 3) are allosteric inhibitors of the modifying enzyme E_A, are competitive in relation to each other, and are non-competitive in relation to molecules of A and S_1A attached to the catalytic center of E_A. This type of inhibition is described by a system of chemical equations:

$$\begin{aligned}
&S_1+E_A \underset{l_{-1}}{\overset{l_{+1}}{\rightleftharpoons}} E_AS_1,\\
&S_1+A'E_A \underset{l_{-1}}{\overset{l_{+1}}{\rightleftharpoons}} A'E_AS_1,\\
&S_2+E_A \underset{l_{-2}}{\overset{l_{+2}}{\rightleftharpoons}} E_AS_2,\\
&S_2+A'E_A \underset{l_{-2}}{\overset{l_{+2}}{\rightleftharpoons}} A'E_AS_2.
\end{aligned} \tag{8}$$

Here, $\ell_{\pm 1}$ and $\ell_{\pm 2}$ are rate constants, and the concentration of the mixture is determined by the sums:

$$\begin{aligned}
&A'E_A=AE_A+SAE_A,\ A'E_AS_1=AE_AS_1+S_1AE_AS_1,\\
&A'E_AS_2=AE_AS_2+S_1AE_AS_2.
\end{aligned} \tag{9}$$

System (1) is open with respect to S_1 and S_2 and closed with respect to all forms of E(A, B), E_A, and E_B. The exchange of S_1 and S_2 with the medium occurs with rates:

$$v_1=V_1-k_1S_1,\ v_2=-V_2+k_2S_2, \tag{10}$$

where v_1, is the rate of S_1 input, V_1 is the rate of input at $S_1 = 0$, v_2 is the rate of S_2 output, V_2 is the rate of S_2 input, and k_1 and k_2 are the exchange rate constants.

The closed nature of system (1) for the enzymes means that the total concentration of enzymes

$$E_0=A'+B'+A'E_A+B'E_B+A'E_AS_1+A'E_AS_2, \tag{11}$$
$$E_{A0}=E_A+A'E_A+A'E_AS_1+A'E_AS_2, \tag{12}$$
$$E_{B0}=E_B+B'E_B \tag{13}$$

is constant. Here, E_0, E_{A0}, and E_{B0} are the total concentrations of E(A, B), E_A, and E_B. For the purposes of deducing the mathematical model, we will, as previously [11], propose that the reactions of system (1) occur in a perfectly mixed medium with controlled temperature and pH.

MATHEMATICAL MODEL

Deduction of a mathematical model for system (1) involves the proposition that the system contains a hierarchy of concentrations described by the conditions:

$$S_1, S_2 \gg E_0 \gg E_{A0}, E_{B0}. \tag{14}$$

As shown by an analysis of the extensive experimental data (more than 6000 publications), carried out using the DBEMP enzymes and metabolic pathways data bank [15, 16], this kind of hierarchy is typical for cells in vivo. In fact, the DBEMP data show that the mean concentration of intermediates in cellular metabolism, taken as the mean log of the Michaelis constant for intermediates, is -3.6 ± 1.1, and the mean log enzyme and modifying enzyme (various types) concentrations are -7.4 ± 1.3 and -8 ± 0.8 respectively. The low concentrations of enzymes E(A, B), E_A, and E_B in relation to S_1 and S_2 allows the analysis to be significantly simplified: in this case, the concentrations of S_1 and change much more slowly than the concentration of all enzyme forms. Thus, system (1) rapidly establishes a quasi-stationary state in which static conditions are established for all enzyme forms, but not for S_1 and S_2. In this state, the relationships between the quasi-stationary concentrations of all enzyme molecules (a total of 16 different types) can be determined by four linear reactions (Eqs. 15-18) and one non-linear reaction (Eq. 19). In reaction (19), v_A and v_B are quasi-stationary rates of covalent modification.

$$A \underset{k_{-1}+k_{+2}}{\overset{k_{+1}S_1}{\rightleftharpoons}} S_1A, \tag{15}$$

$$B \underset{k'_{-1}+k'_{+2}}{\overset{k'_{+1}S_1}{\rightleftharpoons}} S_1B, \tag{16}$$

$$\begin{array}{ccccc} E_AS_1 & \overset{l_{+1}S_1}{\underset{l_{-1}}{\rightleftharpoons}} & E_A & \overset{l_{+2}S_2}{\underset{l_{-2}}{\rightleftharpoons}} & E_AS_2 \\ \Big\| a_{-1}+a_{+2} \quad \Big\| a_{+1}A' & & \Big\| a_{-1}+a_{+2} \quad \Big\| a_{+1}A' & & \Big\| a_{-1}+a_{+2} \quad \Big\| a_{+1}A' \\ A'ES_1 & \overset{l'_{+1}S_1}{\underset{l_{-1}}{\rightleftharpoons}} & A'E_A^- & \overset{l'_{+2}S_2}{\underset{l_{-2}}{\rightleftharpoons}} & A'E_AS_2 \end{array} \tag{17}$$

$$E_B \underset{b_{-1}+b_{+2}}{\overset{b_{+1}B'}{\rightleftharpoons}} B'E_B, \tag{18}$$

$$A' \underset{v_B}{\overset{v_A}{\rightleftharpoons}} B'. \tag{19}$$

The rate of formation of product S_2 by enzyme form A is given by the sum of the rates of degradation of all types of molecule carrying S_1:

$$w = k_{+2}(S_1A + S_1AE_A + S_1AE_AS_1 + S_1AE_AS_2). \tag{20}$$

Similarly, the rate of formation of S_2 by form B is given by:

$$w' = K_{+2}(S_1B + S_1BE_B). \tag{21}$$

The rate of conversion A → B is:

$$v_A = a_{+2}A'E_A \tag{22}$$

and the rate of conversion B → A is:

$$v_B = b_{+2}B'E_B. \tag{23}$$

The concentrations of the enzyme–substrate complexes are determined using Eqs. (15) and (16):

$$S_1A = \frac{S_1}{K_m + S_1}A'_1, \qquad S_1B = \frac{S_1}{K'_m + S_1}B'_1, \tag{24, 25}$$

where

$$K_m=(k_{-1}+k_{+2})/k_{+1}, \quad (26)$$
$$K'_m=(k'_{-1}+k'_{+2})/k'_{+1} \quad (27)$$

are the Michaelis constants for S_1 of forms A and B.

The independence of the catalytic and allosteric centers allows similar equations for the concentrations of the remaining complexes of A and B carrying S_1 in the active center to be written:

$$S_1AE_A=\frac{S_1}{K_m+S_1}A'E_A, \quad (28)$$

$$S_1AE_AS_1=\frac{S_1}{K_m+S_1}A'E_AS_1. \quad (29)$$

$$S_1AE_AS_2=\frac{S_1}{K_m+S_1}A'E_AS_2. \quad (30)$$

From scheme (17) we find:

$$A'E_A+A'E_AS_1+A'E_AS_2=E_{A0}\frac{A'}{K_A+A'}, \quad (31)$$

where

$$K_A=(a_{-1}+a_{+2})/a_{+1} \quad (32)$$

is the Michaelis constant for E_A. Considering Eqs. (28)-(30), this gives:

$$S_1AE_A+S_1AE_AS_1+S_1AE_AS_2=E_{A0}\frac{S_1}{K_m+S_1}\cdot\frac{A'}{K_A+A'}. \quad (33)$$

Substituting Eqs. (24) and (33) into the sum (20) gives

$$w=k_{+2}\,A'\frac{S_1}{K_m+S_1}+k_{+2}\,E_{A0}\frac{S_1}{K_m+S_1}\cdot\frac{A'}{K_A+A'}. \quad (34)$$

Following the same argument for reaction (18), we obtain

$$w=k_{+2}\,B'\frac{S_1}{K_m+S_1}+k_{+2}\,E_{B0}\frac{S_1}{K_m+S_1}\cdot\frac{B'}{K_B+B'}. \quad (35)$$

For calculating rates v_A and v_B using reactions (17) and (18), we find

$$A'E_A=E_{A0}\frac{A'}{(K_A+A')\left(1+\frac{S_1}{K_{i1}}+\frac{S_2}{K_{i2}}\right)}, \quad (36)$$

$$B'E_B=E_{B0}\frac{B'}{K_B+B'}, \quad (37)$$

where

$$K_{i1}=\frac{l_{-1}}{l_{+1}}, \quad K_{i1}=\frac{l_{-2}}{l_{+2}}, \quad K_B=\frac{b_{-1}+b_{+2}}{b_{+1}} \quad (38\text{-}40)$$

are the inhibition constants of enzyme E_A and the Michaelis constant of enzyme E_B for B'. From reaction (19), it follows that

$$v_B=v_A, \quad (41)$$

which, considering Eqs. (22), (23), (36), and (37), gives the relationship between the concentrations A' and B':

$$\frac{B'}{K_B+B'}=r\frac{A'}{(K_A+A')(1+S_1/K_{i1}+S_2/K_{i2})}, \quad (42)$$

where

$$r = a_{-2}E_{A0}/b_{-2}E_{B0} \tag{43}$$

is the relative maximum rate for E_A.

In [11], an important role in the analysis of system (1) was assigned to the ratio function:

$$q \equiv \frac{1}{r}\left(1+\frac{S_1}{K_{i1}}+\frac{S_2}{K_{i2}}\right) = \frac{A'}{K_A + A'} \Big/ \frac{B'}{K_B + B'}. \tag{44}$$

Here, using Eqs. (31) and (37), it is easy to obtain the ratio:

$$\frac{A'E_A + A'E_AS_1 + A'E_AS_2}{B'E_B} = \frac{A'}{K_A + A'} \Big/ \frac{B'}{K_B + B'}, \tag{45}$$

which is the ratio of concentrations of E_A enzyme–substrate complexes to the concentrations of E_B enzyme–substrate complexes. This ratio in the present model is equivalent to the ratio in Eq. (44) from the previously published model [11]. Considering Eqs. (33) and (37), Eq. (11) for the total concentration E_0 takes the form:

$$E_0 = A' + B + E_{A0}\frac{A}{K_A + A} + E_{B0}\frac{B}{K_B + B}. \tag{46}$$

To simplify further analysis, we introduce dimensionless variables and parameters:

$$\begin{gathered}
\sigma_1 = \frac{S_1}{K_{i1}}, \quad \sigma_2 = \frac{S_2}{K_{i2}}, \quad \alpha = \frac{A'}{E_0}, \quad \beta = \frac{B'}{E_0}, \\
\nu = \omega + \omega', \quad \omega = \frac{w}{k_{+2}E_0}, \quad \omega' = \frac{w'}{k_{+2}E_0}, \quad \tau = \frac{k_{+2}E_0}{K_{i1}}t, \\
\nu_{1m} = \frac{V_1}{k_{+2}E_0}, \quad \nu_{2m} = \frac{V_2}{k_{+2}E_0}, \quad \kappa_1^* = \frac{k_1K_{i1}}{k_{-2}E_0}, \\
\kappa_2 = \frac{k_2K_{i2}}{k_{+2}E_0}, \quad \kappa_m = \frac{K_m}{K_{i1}}, \quad \kappa_m' = \frac{K_m'}{K_{i1}}, \quad \kappa_A = \frac{K_A}{E_0}, \\
\kappa_B = \frac{K_B}{E_0}, \quad \varepsilon = \frac{k_{+2}'}{k_{+2}}, \quad \varepsilon_A = \frac{E_{A0}}{E_0}, \quad \varepsilon_B = \frac{E_{B0}}{E_0}, \quad \varepsilon_2 = \frac{K_{i2}}{K_{i1}},
\end{gathered} \tag{47}$$

where t is the time dimension. Calculating the substitutions in Eqs. (47), the behavior of system (1) in dimensionless time τ is described by the following system of mass balance equations:

$$\begin{aligned}
\frac{d\sigma_1}{d\tau} &= \nu_{1m} - \kappa_1\sigma_1 - \nu, \\
\varepsilon_2\frac{d\sigma_2}{d\tau} &= \nu - \kappa_2\sigma_2 - \nu_{2m};
\end{aligned} \tag{48}$$

where

$$\begin{gathered}
\nu = \omega - \omega', \\
\omega = \frac{\sigma_1}{\kappa_m + \sigma_1}\left(\alpha + \varepsilon_A\frac{\alpha}{\kappa_A + \alpha}\right), \\
\omega' = \varepsilon\frac{\sigma_1}{\kappa_m' + \sigma_1}\left(\beta + \varepsilon_B\frac{\beta}{\kappa_B + \beta}\right), \\
\alpha + \beta + \varepsilon_A\frac{\alpha}{\kappa_A + \alpha} + \varepsilon_B\frac{\beta}{\kappa_B + \beta} = 1, \\
\frac{\beta}{\kappa_m + \beta} - r\frac{\alpha}{(\kappa_A + \alpha)(1 + \sigma_1 + \sigma_2)} = 0.
\end{gathered}$$

*κ is the Greek letter kappa.

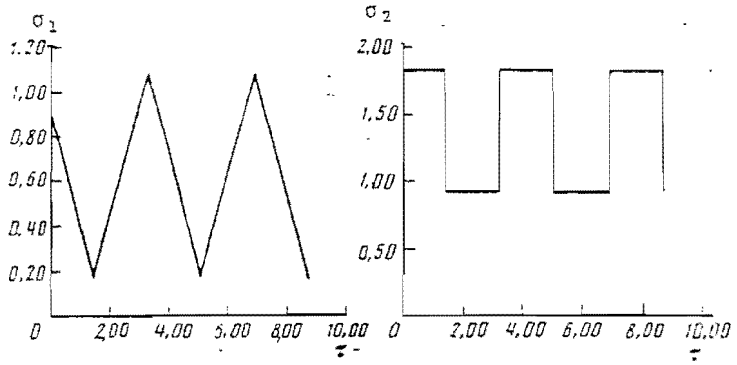


Fig. 1. Relaxation auto-oscillation of the dimensionless substrate concentration of substrate $S_1(\sigma_1)$ and product $S_2(\sigma_2)$ in reaction (1), τ is dimensionless time. Plots were obtained by integration of model (49) by a modified Colahan method for the solution of rigid systems [17, 18]. Parameter values were: $\varepsilon = 10^{-3}$, $\varepsilon_2 = 9.01 \cdot 10^{-4}$, $\varepsilon_3 = 1.0 \cdot 10^{-4}$, $\kappa_1 = 0$, $\nu_{1m} = 0.5$, $\kappa_A = \kappa_B = \kappa_m = \kappa_m' = 1.0^{-5}$, $r = 3$, $\nu_{2m} = 1$, and $\nu_2 = 1.1$.

Here, σ_1, σ_2, α, and ß are the dimensionless concentrations of S_1, S_2, A', and B'; ν is the dimensionless rate of conversion $S_1 \to S_2$ catalyzed by enzyme E(A, B); ω and ω' are analogous parameters for forms A and B separately; ν_{1m} and ν_{2m} are the maximum rates of S_1 and S_2 input; κ_1 and κ_2 are rate constants for the metabolism of S_1 and S_2; κ_m, κ_m', κ_A, and κ_B are dimensionless Michaelis constants for A, B, E_A, and E_B; ε is the relative activity of form B; ε_A and ε_B are the relative concentrations of the modifying enzymes E_A and E_B; and ε_2 is the relative inhibition constant of E_A by S_2. If we consider that in model (48) the relative concentrations of enzymes E_A and E_B can be made very small (ε_A, $\varepsilon_B \to 0$), then it takes the form:

$$\left.\begin{aligned} \frac{d\sigma_1}{dt} &= \nu_{1m} - \kappa_1 \sigma_1 - \nu, \\ \varepsilon \frac{d\sigma_2}{dt} &= \nu - \kappa_2 \sigma_2 + \nu_{2m}, \end{aligned}\right\} \tag{49}$$

where

$$\nu = \frac{\sigma_1}{\kappa_m + \sigma_1}\alpha - \varepsilon \frac{\sigma_1}{\kappa_m + \sigma_1}(1-\alpha),$$

$$\frac{1-\alpha}{\kappa_m + 1 - \alpha} - r\frac{\alpha}{(\kappa_A + \alpha)(1 + \sigma_1 + \sigma_2)} = 0.$$

Finally, neglecting the low activity of form B ($\varepsilon \to 0$) leads (Eq. (49)) to the previously studied phenomenological model [11].

APPROXIMATION EQUATIONS FOR THE AMPLITUDES OF RELAXATION OSCILLATIONS

We will consider model (49) in conditions in which parameters κ_m, κ_A, κ_B, ε, and ε_2 are small. In these conditions this model can generate almost ideal relaxation of the oscillation of variable σ_1 and square-wave oscillations in the fast variable σ_2 (Fig. 1). Using the limiting transition $\varepsilon_2 \to 0$, we can bring model (49) to a confluent first order model with a discontinuous right hand side:

$$\frac{d\sigma_1}{dt} = \nu_{1m} - \kappa_1 \sigma_1 - \bar{\nu}(\sigma_1), \tag{50}$$

in which $\bar{\nu}(\sigma_1)$ is the quasi-stationary value of the rate of reaction $S_1 \to S_2$, which satisfies the system of algebraic equations:

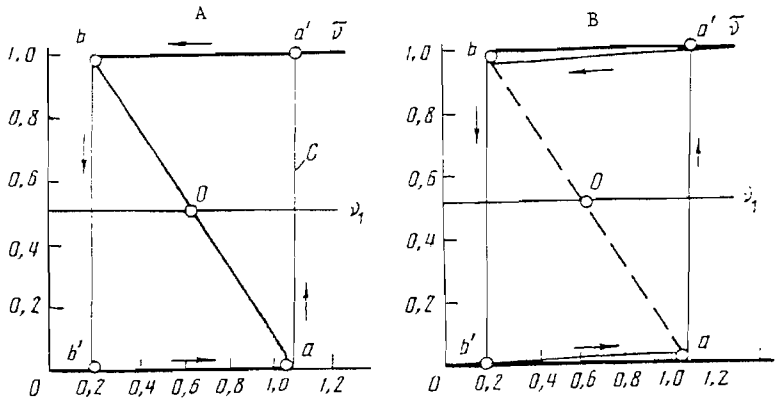


Fig. 2. (A) Quasi-stationary hysteretic relationship between the dimensionless rate of reaction $S_1 \to S_2(\tilde{v})$ and the dimensionless concentration of substrate $S_1(\sigma_1)$ (bold line) and the limit cycle C, embracing the hysteretic part of the plot of $\tilde{v}(\sigma_1)$ (thin closed curve). Arrows show the direction of changes in time τ, 0 is the unstable stationary state, the rate of the source of substrate (bold line). The behavior of $\tilde{v}(\sigma_1)$ was determined by numerical integration of model (49) by the Colahan method [17, 18]. Parameters had the same values as in Fig. 1. (B) Approximation of the limit cycle C by the rectangle aa'bb'. The non-linear parts of entry characteristic $\tilde{v}(\sigma_1)$ (bold line) are replaced by the chords b'a and ba' (thin lines). a and b are break points, and a' and b' are "slowing" points, sections aa' and bb' are regions of slow movement, and sections b'a and a'b are regions of rapid movement.

$$\left.\begin{aligned} &v-\frac{\sigma_1}{\kappa_m-\sigma_1}-\varepsilon\frac{\sigma_1}{\kappa_m+\sigma_1}(1-\alpha)=0\\ &\frac{1-\alpha}{\kappa_m+1-\alpha}-r\frac{\alpha}{(\kappa_A-\alpha)(1+\sigma_1+\sigma_2)}=0,\\ &v-\kappa_2\sigma_2+v_{2m}=0. \end{aligned}\right\} \tag{51}$$

Relaxation oscillations in model (50) (Fig. 2), as in the original model (49), arise because of the hysteresis function $\tilde{v}(\sigma_1)$. Such oscillations in the phase plane of variables (σ_1, $\tilde{v}$) correspond to the discontinuous limit cycle C, shown in Fig. 2 (cycle ab'ba'). This cycle C embraces the hysteretic part of the plot of $\tilde{v}(\sigma_1)$ and has two regions of slow movement (sections a'a and bb') and two regions where discontinuities occur from break points a (or b) to "slowing" points b' or a'. The amplitude and period of oscillation of the variables as we go around the cycle C are determined by the coordinates of the break points (a, b) and the "slowing" points (a', b'). In the general case these points cannot be determined analytically. However, this type of determination can be carried out when κ_A and κ_B are small.

Using the two previously proposed approximate solutions [11] of the second Eq. (51), we get

$$\alpha \equiv \frac{q\kappa_A}{1+\kappa_B-q}, \quad \text{for } \alpha \to 0, \tag{52}$$

$$\alpha \equiv \frac{1+\kappa_B-(1-\kappa_A)q}{1-(1+\kappa_A)q}, \quad \text{for } \alpha \to 1, \tag{53}$$

where

$$q=(\sigma_1+\sigma_2+1)/r$$

is the ratio function (44), and we obtain a solution for system (51). This requires an equation equivalent to system (51) to be solved, for each case separately:

$$\frac{(1+\sigma_1+\sigma_2)\kappa_A}{r(1+\kappa_A)-1-\sigma_1-\sigma_2}-\kappa_{2e}\sigma_2+\nu_{2me}=0 \tag{54}$$

and

$$\frac{r(1+\kappa_A)-(1+\kappa_A)(1+\sigma_1+\sigma_2)}{r-(1+\kappa_A)(1+\sigma_1+\sigma_2)}-\kappa_{2e}\sigma_2+\nu_{2me}=0 \tag{55}$$

for the asymptote $\alpha_1 \to 0$ and $\alpha_1 \to 1$ respectively. Here we use the designation of functions:

$$\kappa_{2e}=\kappa_2/f_1,\ \nu_{2me}=(\nu_{2m}+f_2)/f_1, \tag{56-57}$$

$$f_1=\frac{\sigma_1}{\kappa_m+\sigma_1}-f_2, \tag{58}$$

$$f_2=\varepsilon\frac{\sigma_1}{\kappa_m+\sigma_1}. \tag{59}$$

Solving the quadratics of Eqs. (54) and (55) relative to σ_2, and using the conditions of multiple roots for these equations, we obtain equations determining the abscissas of the extreme points a and b of the function $\tilde{\nu}(\sigma_1)$ (Fig. 2):

$$\sigma_{1a}=\frac{\kappa_A-\nu_{2me}}{\kappa_{2e}}+r(\kappa_B+1)-1-2\sqrt{\frac{r\kappa_A(\kappa_A+1)}{\kappa_{2e}}}, \tag{60}$$

$$\sigma_{1b}=\frac{r}{\kappa_A+1}-1-\frac{1+\nu_{2me}}{\kappa_{2e}}+\sqrt{\frac{r\kappa_B}{(1+\kappa_A)\kappa_{2e}}}. \tag{61}$$

These equations cannot be solved relative to σ_{1a} and σ_{1b}, since κ_{2e} and ν_{2me} are the functions $\sigma_1 = \sigma_2$ or $\sigma_1 = \sigma_{2b}$ respectively (Eqs. (56)-(59)). However, at small values of κ_m and κ_m', equations (60) and (61) can be used, simultaneously with expressions (56)-(59), as iteration formulae for analytical or numerical calculation of the abscissas of σ_{1a} and σ_{1b}. In the limiting conditions $\kappa_m \to 0$ and $\kappa_m' \to 0$, as shown by Eqs. (56)-(59),

$$f_1=1,\ f_2=\varepsilon,\ \kappa_{2e}=\kappa_2,\ \nu_{2me}=\nu_{2m}+\varepsilon, \tag{62}$$

the right hand sides of (60) and (61) do not bridge with σ_1 and point values σ_{1a} and σ_{1b}. In another boundary pair $k_m \to 0$ and $k_m \to \infty$.

$$f_1=1,\ f_2=0,\ \kappa_{2e}=\kappa_2,\ \nu_{2me}=\nu_{2m}, \tag{63}$$

and the right hand sides of Eqs. (60) and (61) again give the point values of σ_{1a} and σ_{1b}, as found previously [1].

At low values of κ_A, κ_B, and κ_m, iteration of Eqs. (60) and (61) converge rapidly, so a first approximation is adequate for calculation of the roots, having taken a null approximation of the value:

$$\sigma_{1a}=r-1-\frac{\nu_{2m}}{\kappa_2},\ \sigma_{1b}=r-1-\frac{1+\nu_{2m}}{\kappa_2}, \tag{64)-(65}$$

obtained from Eqs. (60) and (61) at $\kappa_A = \kappa_B = \kappa_m = \varepsilon = 0$. However, with increases in κ_A and κ_B, iterations of Eqs. (6) and especially (61) converge poorly or not at all, and then some other method must be used to improve the convergence of the iterations (for example, Aitken's δ^2 process [17]). In this case, the analytical expressions for σ_{1a} and σ_{1b} become too laborious and their use becomes inappropriate. If σ_{1a} and σ_{1b} are determined, the corresponding multiple values of σ_2 and $\tilde{\nu}$ can be determined analytically using the equations:

$$\sigma_{2a}=\frac{1}{2}\left\{r(\kappa_B+1)-1+\frac{(\nu_{2me}-\kappa_A)}{\kappa_{2e}}-\sigma_{1a}\right\}, \tag{66}$$

$$\sigma_{2b}=\frac{1}{2}\left\{\frac{(1+\nu_{2me})}{\kappa_{2e}}+\frac{r}{\kappa_A+1}-1-\sigma_{1b}\right\}, \tag{67}$$

$$\tilde{\nu}_a = \kappa_{2e}\sigma_{2a} - \nu_{2me}, \tag{68}$$

$$\tilde{\nu}_b = \kappa_{2e}\sigma_{2b} - \nu_{2me}. \tag{69}$$

The abscissas of the slowing points a' and b' on the limit cycle C (Fig. 2, A) agree with the abscissa of the break points. Thus, using the values of σ_{1a} and σ_{1b} already found, we can use the quadratic Eqs. (54) and (55) respectively to calculate values of σ_2 and the ordinates of $\tilde{\nu}_{a'}$ and $\tilde{\nu}_{b'}$:

$$\sigma_{2a'} = \frac{1}{2}\left\{\left(\nu_{2me} - \kappa_A - R_a\right)/\kappa_{2e} + r\left(\kappa_B + 1\right) - 1 - \sigma_{1b}\right\}, \tag{70}$$

$$\sigma_{2b'} = \frac{1}{2}\left\{\left(1 + \nu_{2me} + R_b\right)/\kappa_{2e} + r\left(\kappa_A + 1\right) - 1 - \sigma_{1a}\right\}, \tag{71}$$

$$\tilde{\nu}_{a'} = \kappa_{2e}\sigma_{2a'} - \nu_{2me}, \tag{72}$$

$$\tilde{\nu}_{b'} = \kappa_{2e}\sigma_{2b'} - \nu_{2me} \tag{73}$$

where

$$R_b = \sqrt{(\nu_{2me} - 1 + \kappa_{2e}C_1)^2 - 4R\kappa_B\kappa_{2e}}, \tag{74}$$

$$R_a = \sqrt{(-\nu_{2me} + \kappa_A - \kappa_{2e}C_2)^2 - 4\kappa_{2e}\left(\kappa_A(1 + \sigma_{1a}) + \nu_{2me}C^2\right.}, \tag{75}$$

$$C_1 = 1 + \sigma_{1a} - r\kappa'_A,\ C_2 = r\kappa'_B - 1 - \sigma_{1b}$$

$$\kappa'_A = 1 + \kappa_A,\ \kappa'_B = 1 + \kappa_B.$$

The amplitudes of oscillations of the variables are determined as the difference between their extreme values:

$$A_{\sigma_1} = \sigma_{1a} - \sigma_{1b}, \tag{76}$$

$$A_{\sigma_2} = \sigma_{2b'} - \sigma_{2a'}, \tag{77}$$

$$A_{\tilde{\nu}} = \tilde{\nu}_{b'} - \tilde{\nu}_a = \kappa_{2e}A_{\sigma_2}. \tag{78}$$

EQUATIONS FOR THE PERIOD OF RELAXATION OSCILLATION

Approximating the region of slow movement on the limit cycle C (Fig. 2) with chords ab' and ba', we obtain expressions for the period of oscillation:

$$\tau_0 = \ln\left[\left\{\frac{\sigma_{1b} - (A_1 - \nu_{1me})/B_1}{\sigma_{1a} - (A_1 - \nu_{1me})/B_1}\right\}^{\frac{1}{B_1}}\left\{\frac{\sigma_{1b} + (A_2 - \nu_{1me})/B_2}{\sigma_{1a} + (A_2 - \nu_{1me})/B_2}\right\}^{\frac{1}{B_2}}\right] \tag{79}$$

where

$$B_1 = \frac{\tilde{\nu}_{b'} - \nu_b}{\sigma_{1b} - \sigma_{1a}} - \kappa_1,\ B_2 = \frac{\tilde{\nu}_a - \tilde{\nu}_{a'}}{\sigma_{1b} - \sigma_{1a}} - \kappa_1,$$

$$A_1 = \tilde{\nu}_{a'} - B_1\sigma_{1a},\ A_2 = \tilde{\nu}_{a'} - B_2\sigma_{1a}.$$

At small values of κ_A, κ_B, and ε_2, Eq. (79) gives the period τ_0 with an error of a few percent (Table 1).

In the important special case of an irreversible source of S_1, in which $\kappa_1 = 0$ approximating the regions of slow movement of characteristic $\nu(\sigma_1)$ with sectors $\tilde{\nu} = \tilde{\nu}_a$ and $\tilde{\nu} = \tilde{\nu}_{b'}$ $(\sigma_{1a} \leqslant \sigma_1 \leqslant \sigma_{1b})$ leads to a simpler equation for the period:

$$\tau_0 = \frac{A_{\sigma_1}A_\nu}{\left(\tilde{\nu}_{b'} - \nu_{1m}\right)\left(\nu_{1m} - \tilde{\nu}_{a'}\right)}. \tag{80}$$

This equation is further simplified in the limiting conditions in which κ_A, κ_B, $\kappa_m \to 0$:

$$\tau_0 = \frac{1}{\kappa_2\left(1 + \varepsilon - \nu_{1m}\right)\left(\nu_{1m} - \varepsilon\right)}. \tag{81}$$

Transforming to dimensional values, we obtain:

TABLE 1. Comparison of the Extreme Points of the Variable σ_1 and the Period of Auto-Oscillation in Model (49) Obtained by Numerical Integration and by Analytical Calculation

Value of parameter k_2	Numerical integration of model (49)			Calculated by asymptotic Eqs. (64), (65), and (79)			Relative error in τ_0, %
	σ_{1max}	σ_{1min}	τ_{0N}	σ_{1a}	σ_{1b}	τ_0	
1.1	1.081	0.174	3.637	1.080	0.187	3.606	—0.8
1.2	1.158	0.326	3.342	1.156	0.339	3.404	—1.1
1.3	1.224	0.454	3.093	1.221	0.467	3.049	—1.4
1.4	1.280	0.563	2.880	1.276	0.576	2.831	—1.7
1.5	1.328	0.658	2.695	1.324	0.671	2.641	—2.0
1.6	1.371	0.741	2.535	1.366	0.754	2.475	—2.4
1.7	1.409	0.815	2.393	1.403	0.828	2.329	—2.7
1.8	1.442	0.880	2.268	1.436	0.893	2.199	—3.1
1.9	1.472	0.938	2.156	1.465	0.951	2.083	—3.4

Notes. Numerical integration of model (49) was carried out using the more detailed model (84) for calculation purposes. Integration was carried out using the Colahan method to the fourth order of precision [17, 18] with a step error of 10^{-8}. Parameter values were: $\varepsilon = 10^{-3}$, $\varepsilon_2 = 9.01 \cdot 10^{-4}$, $\varepsilon_3 = 1.0 \cdot 10^{-4}$, $\kappa_1 = 0$, $\nu_{1m} = 0.5$, $\kappa_A = \kappa_B = \kappa_m = \kappa_m' = 1.0^{-5}$, $r = 3$, and $\nu_{2m} = 1$.

$$T_0 = \frac{K_{i1}}{k_2 K_{i2}\left(1+\frac{k_{+2}}{k_{+2}}-\frac{V_1}{V}\right)\left(\frac{V_1}{V}-\frac{k_{+2}}{k_{+2}}\right)}, \tag{82}$$

where $V = k_{+2}E_0$, which is the maximum rate of form A, and

$$\frac{k_{+2}'}{k_{+2}} < V_1 < 1+\frac{k_{+2}'}{k_{+2}}. \tag{83}$$

EVALUATION OF THE ERROR OF THE ASYMPTOTIC EQUATIONS

Control calculations were performed to evaluate the error with which the asymptotic Eqs. (76)-(79) estimate the amplitude and period of oscillation of the variables in model (49). For this purpose the hybrid model (49), including both differential and algebraic equations was replaced with the following third-order model, which is more suitable for integration:

$$\begin{aligned} \frac{d\sigma_1}{dt} &= \nu_{1m} - \kappa_1\sigma_1 - \nu, \\ \varepsilon_2\frac{d\sigma_2}{dt} &= \nu - \kappa_2\sigma_2 + \nu_{2m}, \\ \varepsilon_3\frac{d\alpha}{dt} &= \frac{1-\alpha}{\kappa_B+1-\alpha} - r\frac{\alpha}{(\kappa_A+\alpha)(1+\sigma_1+\sigma_2)}. \end{aligned} \tag{84}$$

where

$$\nu = \frac{\sigma_1}{\kappa_m+\sigma_1}\alpha + \varepsilon\frac{\sigma_1}{\kappa_m'+\sigma_1}(1-\alpha), \ \varepsilon_3 << \varepsilon_2 << 1.$$

Table 1 shows the extreme values of the variable σ_1 (σ_{1max} and σ_{1min}) and the period of auto-oscillation τ_{0N}, calculated by direct integration of model (84) at different values of parameter κ_2. For comparison, Table 1 also shows values for σ_{1a}, σ_{1b}, and τ_0 calculated using the asymptotic Eqs. (60), (61), and (79).

Comparison of these values shows that the asymptotic equations allow, at ε, ε_2, κ_m, κ_A, and $\kappa_B \ll 1$, to estimate the parameters of the auto-oscillation regime in system (84) with very low error levels (on the order of a few percent).

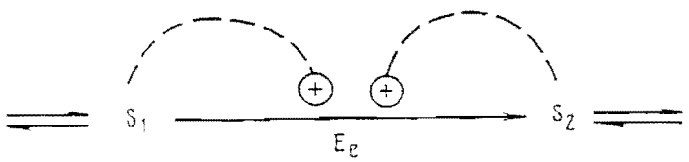
DISCUSSION

This report describes the development and analysis of mathematical model (49), which provides a more accurate description of the auto-oscillatory regime in reaction (1), as compared with a previously published phenomenological model [11]. The previous model [11] suggested that the kinetics of action of the modifying enzymes E_A and E_B could be described by equations of type (36) and (37), though the mechanisms of the interactions of E_A and E_B with ligands, required to satisfy these kinetics, were not identified. In the present paper, it is shown that the mechanism of elementary interactions of enzymes E, E_A, and E_B, represented by Eqs. (15)-(19), actually can be described by the kinetic Eqs. (36) and (37), which were postulated earlier [11].

During the development of model (49) and the asymptotic Eqs. (76) and (79), we avoided a number of simplifying assumptions used for the development and analysis of the previous model [11]: the enzyme form E_B was considered to have small, but nonetheless some activity ($\varepsilon \neq 0$), and the relative Michaelis constants for both forms of the enzyme also had non-zero values (though small), i.e., $\kappa_m \neq 0$, $\kappa_m' \neq 0$. The consequence of this was that, unlike the simpler model [1], the extreme values of the variable σ_1 can be calculated only by iteration. However, in the limiting conditions $\varepsilon = \kappa_m = \kappa_m' = 0$, the asymptotic Eqs. (60), (61), (66), (76)-(79) agree with the previous conclusions.

This comparison of the asymptotically calculated values for σ_{1a}, σ_{1b}, and τ_0 with the corresponding values obtained by direct integration of model (49) showed that the asymptotic equations were very accurate. Thus, the laborious operation of calculation of the parameters of the auto-oscillatory regime by direct integration can be replaced with the asymptotic equations. This substitution is extremely important for analyzing the behavior of the relaxation biochemical auto-generators in complex multi-contour regulatory systems that exist in real conditions – such as in carbohydrate energy metabolism [14].

The system of regulatory relationships, as shown in scheme (1), has not yet been experimentally observed. However, it should be noted that the reaction system in scheme (1) is qualitatively equivalent to a single enzymatic reaction.



(85)

Its equivalent enzyme E_e is cooperatively activated by substrate S_1 and product S_2. In fact, as shown previously [11], the relationship between the rate of conversion $S_1 \rightarrow S_2$ in (1) and the S_1 (σ_2 = constant) and S_2 (σ_1 = constant) concentrations are sigmoidal in nature. This type of kinetics is characteristic of oligomeric enzymes which are activated allosterically by their products and substrates [7].

An analysis of experimental data thus far published, using a data bank on enzymes and metabolic pathways [14, 15], shows that there are more than ten different oligomeric enzymes which are regulated in this way (Eq. (85)). Among these enzymes is the well-studied key enzyme of the glycolytic system of animal tissues, phosphofructokinase (E.C.2.7.1.11), which is activated by its substrate D-fructose-6-phosphate and by its product D-fructose-1,6-diphosphate [19, 20]. Other enzymes of this type include rat liver mitochondrial enzyme glutaminase (E.C.3.5.1.2), which is cooperatively activated by its substrate, L-glutamine and by its product, NH_3 [21].

Regulation of the type shown in Eq. (85) apparently plays an important role in generating the auto-oscillation required for the organization of metabolism in time, and especially for reducing parasitic recirculation of substrates in futile cycles [6, 13, 16, 22, 23]. Futile cycles include those catalyzed by antagonizing enzymes: fructose diphosphatase (E.E.3.1.3.11) simultaneously with phosphofructokinase [6, 13, 16, 22, 23], and glutamine synthetase (E.C.6.3.1.2) simultaneously with glutaminase [7].

Theoretical analysis of the role of reactions of the kind shown in (84) in the temporal organization of cellular metabolism involve very laborious numerical studies of complex mathematical models, which consist of non-linear differential equation systems [6, 7, 13, 16, 22]. The equivalence of reaction (85) with the system studied here (scheme (1)), and the

possibility of carrying out analytical calculations of the parameters describing the oscillatory regime system (1) to be used as a phenomenological model of reactions of the type shown in (85), catalyzed by oligomeric enzymes. This substitution of the simple reaction (85) for the significantly more complex system (1), which at first sight seems absurd, allows numerical studies of the models to be replaced with analytical methods, which radically simplified the problem.

In conclusion, it should be noted that apart from reactions of types (1) and (85), which are susceptible to the asymptotic equations developed here, there is a great variety of equivalent reactions [14], many of which are often met in the metabolism of a variety of organisms. Thus, the asymptotic equations developed in [11] and in the present work may find wide use in the theoretical analysis of the mechanisms involved in the time organization of multi-enzyme systems.

LITERATURE CITED

1. S. J. Pilkis, T. Chrisman, B. Burgress, M. McGrane, A. Colosia, J. Pilkis, H. C. Thomas, and M. R. El-Magharabi, Adv. Enzyme Regul., 21, 147 (1963).
2. H. Holzer and W. Duntze, Ann. Rev. Biochem., 40, 345-374 (1971).
3. P. B. Chock, S. G. Ree, and E. R. Stadtman, Ann. Rev. Biochem., 49, 813-819 (1989).
4. H. G. Hers, Biochem. Soc. Trans., 12, 729 (1984).
5. E. Van Schaftingen, L. Hue, and H. G. Hers, Biochem. J., 192, 897 (1980).
6. E. L. Shevelev and E. E. Sel'kov, Biofizika, 32, 429-433 (1987).
7. E. E. Sel'kov and S. N. Dynnik, Mol. Biol., 12, 1122-1138 (1978).
8. A. Goldbeter and D. E. Koshland, Jr., Proc. Natl. Acad. Sci. USA, 78, 6840-6844 (1981).
9. A. Goldbeter and D. E. Koshland, Jr., Quart. Rev. Biophys., 15, 555-591 (1982).
10. A. Goldbeter and D. E. Koshland, Jr., J. Biol. Chem., 259, 1441-1447 (1984).
11. E. E. Sel'kov and I. I. Goryanin, Mol. Biol., 20, 1550-1562 (1986).
12. G. R. Ivanitskii, V. I. Krinskii, and E. E. Sel'kov, Mathematical Biophysics of the Cell [in Russian], Nauka, Moscow (1978).
13. E. E. Sel'kov and S. N. Dynnik, Biokhimiya, 41, 1677 (1976).
14. E. E. Selkov, I. I. Goryanin, N. P. Kaimachnicov, E. L. Shevelev, and I. A. Yanus, Studia Biophysica, 129, 155-165 (1989).
15. I. I. Goryanin, E. L. Shevelev, and I. A. Yanis, Studia Biophysica, 129, 166-172 (1989).
16. N. V. Avseenko, L. Ya. Lisinchuk, and E. E. Sel'kov, Biofizika, 32, 248 (1987).
17. A. A. Samarskii and A. V. Gulin, Numerical Methods [in Russian], Nauka, Moscow (1989), p. 198.
18. L. Lapidus and J. H. Seinfeld, Numerical Solution of ODE, Academic Press, New York (1971), p. 267.
19. K. Tornheim and J. M. Lowenstein, J. Biol. Chem., 251, 7322-7328 (1976).
20. D. P. Bloxham and E. A. Lardy, The Enzymes, P. D. Boyer (ed.), Academic Press, New York (1973), Vol. 8, pp. 239-278.
21. L. I. Szweda and D. E. Atkinson, J. Biol. Chem., 265, 20869-20873 (1990).
22. E. E. Selkov, Ber. Bunsenges. Phys. Chem., 84, 399-402 (1980).
23. A. Boitex, B. Hess, and E. E. Selkov, Curr. Top. Cell. Reg., 17, 171-203 (1980).
24. E. E. Selkov, Analysis and Simulation of Biochemical Systems: 8th FEBS Meeting, North-Holland, Amsterdam (1972), Vol. 25, pp. 145-161.