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*Chemical Oscillations in Enzymatic Cyclic Reactions  
with the Conservation of Mass*

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Oscylacje chemiczne w cyklicznych reakcjach enzymatycznych  
z zachowaniem masy

1. INTRODUCTION

Processes with oscillative kinetics have been the object of interest of researchers in ecology, physiology, immunology and other biological disciplines for many decades [6, 7, 13]. It seems to me that one of the simplest systems leading to the chemical oscillations is the cycle of a few chemical reactions in which the sum of concentration of the reacting substances is conserved. But there are very few, if any, analyses of such a kind of cycles in the literature. The main reason of such a situation is the widespread opinion that oscillations in systems with mass conservation would contradict the second law of thermodynamics and they could not represent any real system. However, in many cases, such an opinion can be erroneous. Let us consider the cycle of reactions shown schematically in Figure 1. Really, if solely interconverting substances  $A, B, C$  and  $D$  take part in the reactions, then the only possible evolution of the system will be a relaxation from initial conditions to the state of stable equilibrium. Any oscillative solution of the respective kinetic equations would contradict the second law of thermodynamics. However, it is possible that some of the reactions shown in

Figure 1 or all of them are coupled with oxidation of reductors (e.g. NADH, NADPH, ascorbic acid) and with reduction of oxidizers (e.g. oxygen). Some reactions can be driven by light. If so, then there is a permanent inflow of the free energy to the system. In consequence, the concentrations of  $A$ ,  $B$ ,  $C$  and  $D$  can oscillate conserving their sum and without the violation of the second law of thermodynamics. Of course, in such whole system, including cofactors, the sum of the masses of all the reacting substances is not more conserved, because of the consumption of cofactors. If, however, the turnover of the cycle is low as compared with the rate of cofactor production and consumption in other metabolic processes, then there is no need to include cofactor concentrations into kinetic equations. They can be treated as constant. An example of such a situation is delivered by xanthophyll cycle in thylakoid membranes, where the consumption of NADPH and molecular oxygen is much smaller than their production in primary photosynthetic processes. The sum of violaxanthin, antheraxanthin and zeaxanthin content rests constant at all nonsaturating light intensities [3, 14].

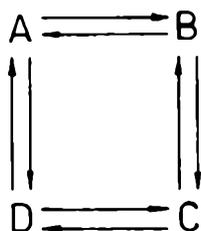


Fig. 1. The scheme of the cyclic reactions of four interconverting substances

Schemat cyklu przemian czterech substancji

Reaction cycles with the conservation of mass, like xanthophyll cycle, can have only regulative and not metabolic role and as such are rather exceptional in metabolic pathways of living cells. I think that this is the second reason of the lack of investigations on mass conserving oscillating systems. On the other hand, it is quite a usual situation, when an initial pool of reacting substances is slowly decreasing and can be treated as constant with a satisfactory approximation. As an example of this kind of systems can serve cyclic reactions of demethylation and methylation of methoxyphenolic compounds in culture of *Nocardia* and *Rhodococcus* [4, 10]. Veratric acid, when introduced to the culture of these bacteria, is converted into vanillic, isovanillic and protocatechuic acid and later it is reproduced to the concentration only slightly lower than the initial one. The period of oscillations amounts to a few hours. The maximum concentration of veratric acid rests almost constant for many periods of oscillations. In this case an assumption of the mass conservation seems to be quite natural and substantially simplifies the kinetic model.

On the other hand, analyses of cyclic systems are quite popular in papers devoted to models of neural nets. In 1970, Dunin-Barkovsky [5] proposed the model of neural oscillator consisting of  $n$  ordered neurons which form a ring. Each neuron inhibits the excitation of all of the others except for the next one. If such a ring of neurons is stimulated by a common source the wave of excitation will propagate around the ring. Later, the neuron oscillator of this kind has been found in medicinal leech [11]. General properties of rings of coupled biological oscillators have been analysed by Collins and Stewart [1, 2]. In this paper I would like to present the simplest cycle of enzymatic reactions whose dynamics is similar to that of neuron oscillator.

## 2. THE KINETIC MODEL OF CYCLIC REACTIONS WITH MASS CONSERVATION LAW

Let us consider the reaction cycle represented by Figure 2a. Kinetic interdependences in this cycle are based on the neuron oscillator (Figure 2b) proposed by Dunin-Barkovsky [5]. The neurons 1, 2 and 3 in the scheme in Figure 2b interact in such a way that each of them inhibits the preceding neuron (1—●3, 2—●1, 3—●2) and does not inhibit the following one. In a similar way in reactions shown in Figure 2a, the high concentration of any reagent ( $x$ ,  $y$  or  $z$ ) inhibits the production of the preceding substance and does not inhibit the production of the following one. Both systems have a prevailing direction of movement, the clockwise direction in Figures 2a and 2b. In the case of neuron ring, asymmetry follows from the lack of inhibition in the 1, 2, 3 direction. In chemical system (Figure 2a) asymmetry follows from different expressions for reaction rates in clockwise and anticlockwise direction. According to Figure 2a the rate of the  $x \rightarrow y$  reaction, should be proportional to the expression  $x/(1+z^m)$  and is a monotonous function of substrate concentration ( $x$ ). The opposite reaction  $y \rightarrow x$  has a rate proportional to  $y/(1+y^m)$ . In this case, the reaction rate can reach a constant value ( $m = 1$ ) or can go through the maximum value and decrease ( $m > 1$ ) on the growing substrate concentration  $y$ .

The changes of concentrations of the substances reacting according to the scheme in Figure 2a can be described by the following equations in dimensionless variables:

$$\frac{dx}{dt} = \frac{z + ky}{1 + y^m} - \frac{kx}{1 + x^m} - \frac{x}{1 + z^m};$$

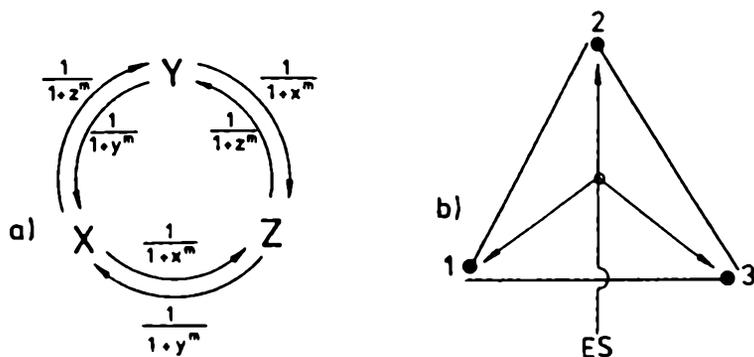


Fig. 2. a) The cycle of three interconverting substances. Reaction rates are proportional to the expressions at respective arrows; b) three neurons with inhibition. *ES* denotes the source of excitation

a) Cykl przemian trzech substancji. Szybkości reakcji są proporcjonalne do wyrażeń umieszczonych przy odpowiednich strzałkach; b) trzy neurony z hamowaniem. *ES* — źródło impulsów

$$\begin{aligned} \frac{dy}{dt} &= \frac{x + kz}{1 + z^m} - \frac{ky}{1 + y^m} - \frac{y}{1 + x^m}; \\ \frac{dz}{dt} &= \frac{y + kx}{1 + x^m} - \frac{kz}{1 + z^m} - \frac{z}{1 + y^m}, \end{aligned} \quad (1)$$

where  $m$  is a natural number. The equations (1) can describe the cycle of enzymatic reactions where each of the enzymes is repressed by the respective reagent, according to the mechanism proposed by Jacob and Monod [9]. If so, then the expression  $1/(1 + z^m)$  at the arrow connecting  $x$  and  $y$  in Figure 2a means that the substance  $z$  is a corepressor of the enzyme which catalyzes reaction  $x \rightarrow y$ . The concentration of this enzyme is approximately proportional to the expression  $1/(1 + z^m)$  if two following conditions are satisfied: 1)  $m$  molecules of corepressor are used to produce one molecule of active repressor, 2) The rates of synthesis and decay of the enzyme is essentially higher than the rate of the reaction catalyzed by the enzyme [6, 8, 13]. As it is evident from equations (1), it has been assumed that all of the parameters characterizing repression are identical for the all enzymes. I would like to note that the equations (1) can describe also the cycle of reactions catalyzed by allosteric enzymes without referring to the regulation of enzyme synthesis. Parameter  $k$  is the ratio of rate constants for backward and direct reactions. It has been introduced in order to examine the significance of the reversibility of the reactions under consideration.

The dynamical system described by equations (1) has an obvious point of equilibrium with coordinates  $x = y = z = C$ , where  $C$  is equal to one third of the sum of concentrations of the reagents  $x, y$  and  $z$ . The kind of the equilibrium is determined by eigenvalues of the system (1), that is by the solutions of the equation (2):

$$[\partial F_i / \partial x_j - \lambda \delta_{ij}] = 0, \quad (2)$$

where  $F_i$  are functions in the right of equations (1),  $x_j = x, y, z$ ,  $\lambda$  is an eigenvalue and  $\delta_{ij}$  is the Cronecker's symbol. At  $x = y = z = C$ , equation (2) gives the following solutions for  $\lambda$ :

$$\begin{aligned} \lambda_1 &= 0 \\ \lambda_{2,3} &= \frac{3[kb - (k+1)a] \pm i\sqrt{3}[(k-1)a - (k+2)b]}{2}, \end{aligned} \quad (3)$$

where

$$a = \frac{1}{1 + C^m}; \quad b = \frac{m C^m}{(1 + C^m)^2}.$$

The eigenvalue  $\lambda_1 = 0$  points out to the existence of the integral

$$x + y + z = 3C \quad (4)$$

The couple of complex eigenvalues  $\lambda_2$  and  $\lambda_3$  means that the equilibrium under consideration is a focus. Stability of this focus is determined by the sign of the real part of  $\lambda_2$  and  $\lambda_3$ . It appears that the real part of  $\lambda_2$  and  $\lambda_3$  can be positive only at enough high values of  $C$  and  $k$ . Neither  $C$  nor the dynamical variables can be negative as concentrations. If so, then it follows from (3) that real part of the complex eigenvalues is positive when the following two conditions are satisfied:

$$C^m > \frac{k+1}{km - k - 1} \quad \text{and} \quad k > \frac{1}{m-1}. \quad (5)$$

If any of the conditions (5) is not satisfied the singularity under consideration is a stable focus. When conditions (5) are satisfied, then the point of equilibrium  $x = y = z = C$  is an unstable focus. Any deviation from such equilibrium will produce oscillations with growing amplitude and we can expect that there is a limit cycle corresponding to autooscillations.

Due to the mass conservation (4) all of phase trajectories belong to the plane defined by equation (4). Even more, each trajectory must belong to the triangle portion of this plane corresponding to positive values of  $x, y$  and  $z$ ,

since concentrations cannot be negative. In order to present phase portrait of the system in one plane I transform dynamical variables according to the relations (6):

$$\begin{aligned} \xi &= (-y + z)/\sqrt{2}; & x &= (2\eta/\sqrt{6}) + C; \\ \eta &= (2x - y - z)\sqrt{6}; & y &= -(\xi/\sqrt{2}) - (\eta/\sqrt{6}) + C; \\ \zeta &= (x + y + z)/\sqrt{3} = C\sqrt{3}; & z &= (\xi/\sqrt{2}) - (\eta/\sqrt{6}) + C. \end{aligned} \quad (6)$$

The axes  $\xi$  and  $\eta$  are parallel to the plane containing phase trajectories. The axis  $\zeta$  is perpendicular to this plane. As it follows from (4) and (6), the coordinate  $\zeta$  has a constant value equal to  $C\sqrt{3}$  and determines the distance of the phase trajectory plane from the origin of coordinates. Let us consider an example with  $m = 3$ . Then, according to (5),  $k$  should be higher than 0.5. I put  $k = 1$ . Under accepted values of  $m$  and  $k$ , the bifurcation value of  $C$  is equal to  $2^{1/3} \cong 1.26$ . Figure 3 presents numerical solution and phase portrait of the system (1) for  $C = 2$ . It is clear from Figure 3 that the limit cycle does really exist. Numerical solutions of equations (1) for  $m = 3$ ,  $k = 2$  and different values of  $C$  show that the higher is  $C$  the bigger is the oscillation amplitude and the more close to the edges of the accessible triangle is the limit cycle.

Solutions presented in Figure 3 allow us to predict qualitatively the behaviour of some systems without mass conservation which are similar to one described by equations (1). Let us consider, for example, the system described by the following equations:

$$\begin{aligned} \frac{dx}{dt} &= \frac{z + y}{1 + y^3} - \frac{x}{1 + x^3} - \frac{x}{1 + z^3}; \\ \frac{dy}{dt} &= \frac{x + z}{1 + z^3} - \frac{y}{1 + y^3} - \frac{y}{1 + x^3} - k_1 y; \\ \frac{dz}{dt} &= \frac{y + x}{1 + x^3} - \frac{z}{1 + z^3} - \frac{z}{1 + y^3}. \end{aligned} \quad (7)$$

In this system there is an outflow of the substance from the pool of  $x, y$  and  $z$  to some other pathways. If the outflow is slow enough ( $k_1 \ll 1$ ), then at  $C > 2^{1/3}$  oscillations with diminishing amplitude take place in the system. The oscillation amplitude decreases because of the decreasing sum of concentrations of  $x, y$  and  $z$ , which is, in turn, caused by the outflow ( $-k_1 y$  in the equation for  $dy/dt$ ). On decreasing the value of  $C$  below

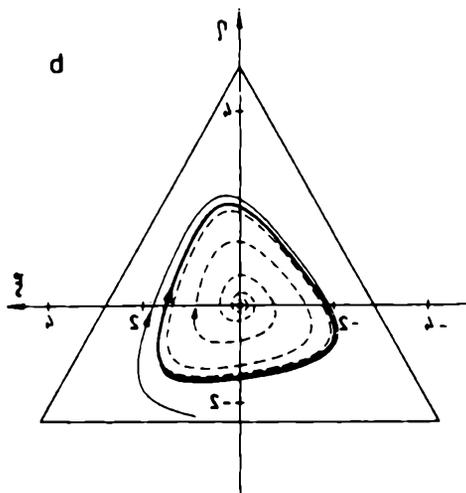
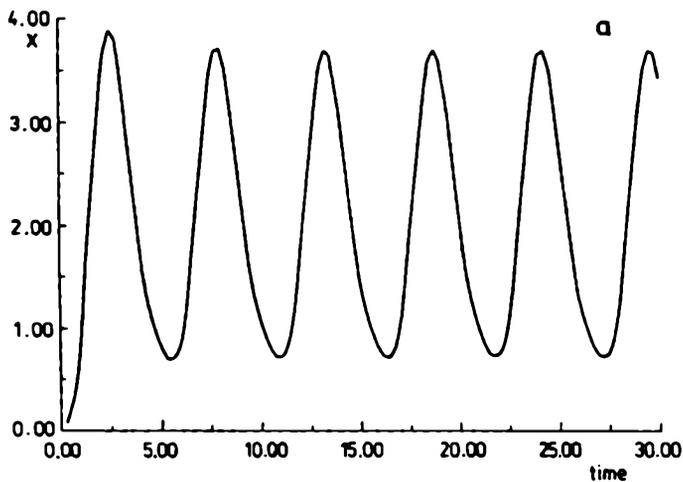


Fig. 3. a) Dependence  $x(t)$  obtained as a numerical solution of equations (1) at  $m = 3$ ,  $k = 1$  and  $C = 2$ ; b) phase portrait of the system (1) with the same values of  $m, k$  and  $C$   
 a) Zależność  $x(t)$  otrzymana z numerycznego rozwiązania równań (1) dla  $m = 3$ ,  $k = 1$  i  $C = 3$ ; b) portret fazowy układu (1) dla tych samych wartości  $m, k$  i  $C$

$2^{1/3}$ , the damping of oscillations appears in the system. The smaller is  $C$  the bigger is logarithmic decrement of damping. In phase space, the triangle containing trajectory becomes smaller and smaller and approaches the origin of coordinates. The systems of such a kind are quite common in experiments, when we add to the bacterial culture a substance and we observe its extinction.

*In vivo*, we rather expect to find cycles of reactions where the pool of reacting substances is a balance between inflow and outflow. One of the simplest system of this kind can be described by equations (8):

$$\begin{aligned}\frac{dx}{dt} &= \frac{z+y}{1+y^3} - \frac{x}{1+x^3} - \frac{x}{1+z^3} + f; \\ \frac{dy}{dt} &= \frac{x+z}{1+z^3} - \frac{y}{1+y^3} - \frac{y}{1+x^3} - k_1y; \\ \frac{dz}{dt} &= \frac{y+x}{1+x^3} - \frac{z}{1+z^3} - \frac{z}{1+y^3};\end{aligned}\tag{8}$$

where  $f$  is a constant inflow of  $x$ . It follows from (8) that

$$\frac{d}{dt}(x+y+z) = f - k_1y.$$

Hence, in equilibrium,  $y = f/k_1$ . If  $f$  is small in comparison with other items in equations (8) and  $k_1 \ll 1$ , we will obtain a system with  $C$  of the order of  $f/k_1$ . This time, however, the value of  $C$  oscillates because the inflow is constant and outflow is proportional to the oscillating value of  $y$ . The phase trajectory should be similar to that presented in Figure 3b but dimensions of the accessible triangle and its distance from the coordinates origin should oscillate with the same frequency as  $y$ . In spite of the fact that the system represented by equations (8) is explicitly open, the oscillations are not caused by the in- and outflow of substances but they are due to the internal dynamics of cyclic reactions.

### 3. SIGNIFICANCE OF THE REVERSIBILITY OF REACTIONS

Oscillating chemical systems are postulated to be far from thermodynamical equilibrium. It is also often postulated that some reactions go on in one direction. Such an assumption usually simplifies calculations. The system under consideration must be essentially remote from thermodynamical equilibrium because reactions described by equations (1) do not satisfy the

principle of detailed equilibrium. In spite of this, we have to assume that reactions can go on in both directions being catalyzed by two different enzymes. The possibility of interconversions in both directions is a necessary condition for the existence of oscillative solutions. The word "reversibility" means here that reactions in both directions can have place. If clockwise reactions in the scheme of Figure 2a were irreversible, then we should put in equations (1)  $k = 0$ . At  $k = 0$ , the real part of eigenvalues  $\lambda_{2,3}$

$$Re(\lambda_{2,3}) = -\frac{3}{2(1 + C^m)}$$

and is negative at any value of  $C$ . So, the equilibrium  $x = y = z = C$  is then a stable focus and only damped oscillations are possible in the system. In this way, we demonstrate that reversibility of reactions constitutes the necessary condition for the existence of autooscillative regime in the cycle of three interconverting substances like that represented by Figure 2a and equations (1). As it states inequality (5), autooscillations are possible only on condition that the ratio of rate constants for backward and direct reactions is higher than  $1/(m - 1)$ .

However, the instability of focus  $x = y = z = C$  not always means the existence of autooscillations. At high enough values of  $k$ , a few new points of equilibria appear in the system. At least, some of them are stable. As it follows from (5) oscillations are possible at  $m = 2$ ,  $C = 2$  and  $k > 1$ . Numerical solutions of respective equations show that at  $k = 14$  we can still obtain oscillations, but at  $k = 15$  there is only aperiodic relaxation to approximate values of  $x = 4.796$ ,  $y = 1$  and  $z = 0.204$ . The cyclic permutation  $x \rightarrow y \rightarrow z \rightarrow x$  will give us two other points of stable equilibrium. Additional singularities do not belong to any of symmetry axes of the triangle containing phase portrait of the system and it is difficult or even impossible to find their coordinates in analytical form. For given values of parameters  $m$ ,  $k$  and  $C$ , stable equilibria can be found by solutions of differential equations. But the system can also have some points of unstable equilibrium which cannot be identified in this way. In order to give at least an approximate shape of the phase portrait of the system at high values of  $k$ , I will describe the limit case with  $m = 2$  and infinitely high  $k$ . The infinite value of  $k$  corresponds to the case, when only anticlockwise reactions take place in the system shown in Figure 2a. In this case, there are no oscillations in the system. The unstable focus at  $x = y = z = C$  is then surrounded by three stable nodes and three saddle points. So, we have to do with a trigger system having three points of stable equilibrium. Additional singularities appear at  $C = 2\sqrt{2}/3$ , when the central point of equilibrium is still stable.

The equilibrium points of such a system are shown in coordinates  $\xi, \eta$  (6) in Figure 4. For high finite values of  $k$ , general picture of the system should be similar to that in Figure 4. However, the points of stable equilibria (3, 5 and 7 in Figure 4) should be displaced by a certain small angle in respect to those shown in Figure 4. I cannot say anything about the localization of unstable equilibria.

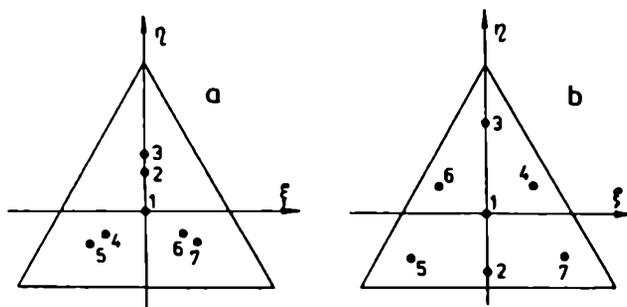


Fig. 4. Points of equilibria for anticlockwise reactions of the cycle shown in Figure 2a (equations (1) with infinitely high  $k$ ). Points 2, 4 and 6 are saddle points while points 3, 5 and 7 are stable nodes; a)  $C$  is slightly smaller than 1; point 1 is then a stable focus;

b)  $C$  higher than 1; point 1 is then an unstable focus

Punkty równowagi dla wstecznych reakcji cyklu z Ryc. 2a. (układ dynamiczny (1) przy  $k$  dążącym do nieskończoności). Punkty 2, 3 i 6 są punktami siodłowymi. Punkty 3, 5 i 7 są węzłami trwałymi; a)  $C$  nieznacznie mniejsze od 1 — punkt 1 jest ogniskiem trwałym;

b)  $C$  większe od 1 — punkt 1 jest ogniskiem nietrwałym

#### 4. RELAXATION OSCILLATIONS AT HIGH VALUES OF $M$

If  $m$  is big enough, then relaxation oscillations can take place in the system represented by Figure 2a and equations (1). In a limit case, when  $m$  becomes infinitely high, expressions of the type  $1/(1+x_i^m)$  can be simplified as follows:

$$1/(1+x_i^m) \rightarrow f(x_i) = \begin{cases} 1 & \text{for } 0 < x_i < 1 \\ 0.5 & \text{for } x_i = 1 \\ 0 & \text{for } x_i > 1 \end{cases} \quad (9)$$

where  $x_i = x, y, z$ .

Insertion of the approximation (9) into equations (1) makes of them a set of linear equations. The equations, however, change their particular shape each time any dynamical variable crosses the value 1. Let us suppose, for

example, that  $x < 1$ ,  $y > 1$  and  $z < 1$ . Then, at  $k = 1$ , the equations (1), with accounting (9), take a form:

$$\begin{aligned} dx/dt &= -2x; \\ dy/dt &= x + z - y; \\ dz/dt &= y + x - z. \end{aligned} \quad (10)$$

As can be seen from (10), the mass conservation law (4) is still valid. Solution of the system (10) consists of monotonous functions of time, which can attain their highest and lowest values only at limits of the interval, where equations (10) are valid. The limits of such intervals are determined by the moments, when any of variables  $x$ ,  $y$  or  $z$  crosses the value 1. Let us accept, for example, that at  $t = 0$ ,  $x = 1$ ,  $y > 1$  and  $z < 1$ . It is easy to check, taking into account (1) and (9), that at such conditions  $dx/dt < 0$  and except for the very initial moment the equations (10) describe our system. The solution of (10) in form:

$$\begin{aligned} x &= \exp(-2t) \\ y &= A \exp(-2t) + 1.5C \\ z &= -(1 + A) \exp(-2t) + 1.5C \end{aligned} \quad (11)$$

where  $A$  is an integration constant, satisfies the accepted initial conditions and conservation law (4). The reactions will be properly described by (10) and (11) until the variable  $z$  reaches the value of 1, that is, till the moment  $t_1$  defined by the following relation:

$$\exp(-2t_1) = \frac{3C - 2}{2(1 + A)} \quad \text{or} \quad t_1 = 0.5 \ln \frac{2(1 + A)}{3C - 2}. \quad (12)$$

Later, we will have  $z > 1$  and as previously  $x < 1$  and  $y > 1$  and instead of (10) the system will be described by equations (13):

$$\begin{aligned} dx/dt &= -x; \\ dy/dt &= -y; \\ dz/dt &= y + x. \end{aligned} \quad (13)$$

As initial conditions for equations (13) I accept the values given by functions (11) at  $t = t_1$  (12). Then, equations (13) have a solution:

$$x = \frac{3C - 2}{2(1 + A)} \exp(-t);$$

$$y = \frac{3(2AC + C) - 2A}{2(1 + A)} \exp(-t); \quad (14)$$

$$z = (1 - 3C) \exp(-t) + 3C.$$

The moment  $t = 0$  in formulae (14) coincides with  $t = t_1$  in formulae (11). The equations (13) and their solution (14) are valid till the variable  $y$  becomes equal to 1. This moment of time ( $t_2$ ) is determined by relation:

$$\exp(-t_2) = \frac{2(1 + A)}{A(3C - 2) + 3C(1 + A)}$$

or (15)

$$t_2 = \ln \frac{A(3C - 2) + 3C(1 + A)}{2(1 + A)}$$

When the variable  $y$  becomes smaller than 1, then we will have  $x < 1, y < 1$  and  $z > 1$  and then we will be able, using (9), to give equations (1) the shape:

$$\begin{aligned} dx/dt &= z + y - x \\ dy/dt &= -2y \\ dz/dt &= y + x - z. \end{aligned} \quad (16)$$

It is easy to see that equations (16) can be obtained from equations (10) by a circular substitution according to the scheme:  $x \rightarrow y \rightarrow z \rightarrow x$ . Of course, initial conditions for equations (16) are given by functions (14) at  $t = t_2$  which is defined by (15). Let us now suppose that the point with coordinates  $x_0, y_0, z_0$  obtained from (11) at  $t = 0$  belongs to the limit cycle. Then the point  $x_2, y_2, z_2$ , obtained from (14) at  $t = t_2$ , will also belong to the same limit cycle. Moreover, the circular substitution I have mentioned above will be valid not only for equations (10) and (16) but also for their initial conditions. So, the following relations should be satisfied:

$$\begin{aligned} x_0 &= y_2; \\ y_0 &= z_2; \\ z_0 &= x_2. \end{aligned} \quad (17)$$

The first of the above equalities is a tautology ( $1=1$ ). The two other lead to the same expression for the integration constant  $A$ :

$$A = \frac{9C^2 - 12C + 2 + [3(27C^4 - 36C^3 + 16C - 4)]^{1/2}}{4(3C - 1)}. \quad (18)$$

At a given value of  $C$ , the formula (18) allows us to calculate  $A$  and then calculate coordinates of three points belonging to the limit cycle.

It would be useful to demonstrate how this formalism works in one exemplary case. Let us put  $C = 2$ . Then one obtains from (18)  $A = 1.8358$ . The insertion of this value and  $t = 0$  into (11) gives  $x_0 = 1$ ,  $y_0 = 4.8358$  and  $z_0 = 0.1642$ . From (12) we obtain  $t_1 = 0.1746$ . At this moment, equations (10) and (11) are replaced by equations (13) and (14). Introducing into (11) the value of  $A$  and  $t = t_1$  we obtain coordinates of another point belonging to the limit cycle:  $x_1 = 0.7053$ ,  $y_1 = 4.2947$  and  $z_1 = 1$ . The same values of  $x_1$ ,  $y_1$  and  $z_1$  can be obtained from (14) at  $t = 0$ . The equations (13) and (14) are valid till  $t = t_2 = 1.4574$ . The letter figure follows from (15). Insertion of  $t = t_2$  in (14) results in coordinates of one more point belonging to the limit cycle:  $x_2 = 0.1642$ ,  $y_2 = 1$  and  $z_2 = 4.8358$ . Having applied transformation (6) to these three points one obtains their coordinates in the plane  $\xi, \eta$ , which can be respectively denoted as  $(\xi_0, \eta_0)$ ,  $(\xi_1, \eta_1)$  and  $(\xi_2, \eta_2)$ . The rest of the characteristic points of the limit cycle can be obtained from  $(\xi_0, \eta_0)$  and  $(\xi_1, \eta_1)$  after rotation the coordinates  $\xi, \eta$  by 120 or 240 degree. In particular, the point  $(\xi_2, \eta_2)$  can be obtained from the point  $(\xi_0, \eta_0)$  after rotation the coordinates by 120 degrees. It can be demonstrated that functions (11) as well as those of (14) give linear dependences between  $x, y$  and  $z$ . Respective dependences between  $\xi$  and  $\eta$  must be also linear. So, the obtained characteristic points of the limit cycle can be joined by straight lines. The limit cycle arising from this procedure and corresponding to relaxation oscillations is shown in Figure 5b. For comparison, Figure 5a presents another limit cycle obtained from computer solution of equations (1) at  $m = 10$  which is the value substantially higher than the minimum  $m = 2$ , but still far from infinity. The period of relaxation oscillations is equal to  $3(t_1 + t_2)$ .

It seems to me that this kind of relaxation oscillations can be realized in neuron oscillators, like the one shown in Figure 2b, where threshold phenomena play an important role. However, in this case, dynamic variables should be interpreted as the probability (or frequency) of the spike generation by neurons 1, 2 and 3 in Figure 2b.

#### 4. CONCLUSIONS

The presented analysis of equations (1) and related equations (7, 8, 10, 13 and 16) allows to formulate tentatively minimum criteria, which are to be satisfied by a mass conserving system in order to produce chemical oscilla-

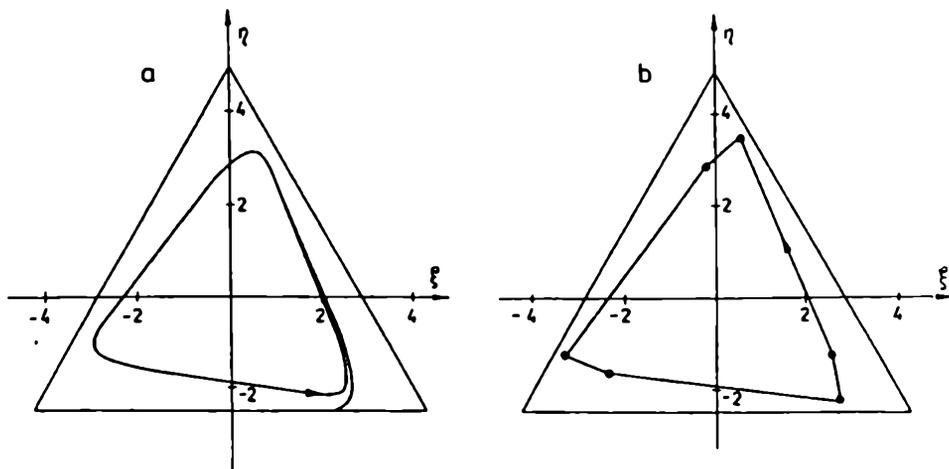


Fig. 5. The limit cycle of the dynamical system (1) at  $k = 1$  and  $C = 2$ ; a) computer solution for  $m = 10$ ; b) relaxation oscillations at infinitely high  $m$  (analytical solution)  
 Cykl graniczny układu dynamicznego (1) dla  $k = 1$  i  $C = 2$ ; a) rozwiązanie numeryczne dla  $m = 10$ ; b) oscylacje relaksacyjne dla bardzo dużych  $m$  — rozwiązanie analityczne

tions. The system should contain at least three interconverting substances. In a system consisting of two reagents, for any singularity, one eigenvalue must be equal to 0 (because of mass conservation) and another will be a solution of a linear algebraic equation. Consequently, this second eigenvalue cannot be complex. So, in such a system, there are no equilibria of the focus type and oscillations are impossible. By the way, at least three forms of protein should take part in oscillations of enzymatic activity *in vitro* [12].

The nonlinearity of kinetics should be sufficiently strong. In the case of the cycle of three reagents, the parameter  $m$  (1) has to be higher than 1 (see (5)).

Each of three reactions constituting a cycle should be reversible. More precisely this criterion has been formulated in relation (5).

Autooscillations can appear if the sum of the concentrations of reagents is sufficiently high (see (5)).

It also follows from the above consideration that the stream of substances is not a necessary condition of autooscillations. Even more, the case presented by equations (8) demonstrates that oscillations in a stream of reagents can be caused by the internal kinetics of the cyclic reactions and not by the stream of substances. Most probably, the stream of reagents can evoke oscillations in cyclic systems with multiple equilibria, like that presented in Figure 4. This question has yet to be investigated.

Finally, it is necessary to note that autooscillations are a dissipative phenomenon and, especially in mass conserving systems, the source of free energy has to be specified in each particular case. It seems to me that the source of free energy is a crucial problem for any explanation of the enzymatic activity oscillations *in vitro*.

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## STRESZCZENIE

W niniejszym artykule przeanalizowana została możliwość pojawienia się oscylacji samowzbudnych w zamkniętym cyklu przemian trzech reagentów. Sformułowane zostały warunki konieczne wystąpienia reżimu autooscylacyjnego w takim układzie. Równania kinetyczne powinny charakteryzować się dostatecznie silną nieliniowością, a przemiany dowolnej pary z trzech reagentów muszą zachodzić w obie strony. ZadeMONstrowana została możliwość oscylacji relaksacyjnych przy bardzo silnej nieliniowości równań kinetycznych.