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The Contribution to the Theory of Isotopic Metabolic Experiments

VÁCLAV KORÁL

The dynamics investigations of the biological systems metabolism are enabled by the isotopic metabolic experiments. Some quantitative aspects of these experiments are presented in this paper and the factors operating the metabolism kinetics in the living organism are discussed.

The use of labelled radioactive compounds is quite common in the biological sciences. As an example the examination of distribution and kinetics of the labelled compound in organisms, or the application of radioisotope for the therapeutical use may be presented. In this paper only the special case of biological use of radioisotopes is elaborated, the so-called *isotopic metabolic experiments* (IME).

As IME only those cases are taken into account where the labelled radioactive compounds subject to chemical transformations are administered into the organism; the bahevior of these compounds or of the products of these compounds due to the chemical transformations is followed in time and space.

A few notes must be added to this general definition of IME.

1. The administration of the labelled compound may be done only once, repeatedly, or continuously and may be introduced into the examined biological system by different ways. (E.g. the labelled compound may be administered to the laboratory animal either per os or by injection into the blood system etc.). The way of administration is dependent on the aim of experiment and on the technical possibilities of the experimentator.

2. As "biological system" is meant here not only the living organism but even its parts, e.g. the surviving organ, tissue culture etc. It is difficult to trace the boundary between the biological and non-biological system. As biological system is meant, in this work, a system having an adequate metabolism in adequate conditions. In this work the general biological system is elaborated.

3. The "labelled compound" is a chemical individual or a mixture of chemical individuals labelled on one or more atoms of the same or different atomic index.

Only one compound is administered in most cases but sometimes a simultaneous use of more compounds is more advantageous. In this paper the application of one single compound is presented labelled on one atom located on the same place in all labelled molecules.

4. "Compound being subject to chemical changes" is a matter reacting with other compounds present in the organism or, being destructed (enzymatically or non-enzymatically) in a biological system. Not all the compounds used in this biological isotopic experiments are chemicaly reactive. As an example for this fact the radioactive krypton-85 may serve, being used in the pulmonary circulation studies.

5. As "behavior of labelled compound in the space" are meant the transitions among the various compartments of the organism, e.g. between the blood and tissues. As a result of the space transitions the distribution of the compound in an organism may be observed with the time t_i. The behavior of the labelled compound with time is the time-dependence of the labelled molecules number in the same compartment of an organism which is called "kinetics". In the IME even distribution and kinetics or only one of these characteristics, is used to be followed. The distribution and kinetics are not the independent actions even because the definition of the term "compartment" of the biological system is dependent on the aim of IME.

The quantity of administered labelled compound is a serious circumstance. The quantity being very low so that the concentration of the same unlabelled compound in the given biological system stays unchanged, the administered matter has not perceptible influence on the development of chemical actions in the organism. The administered activity not being negligible, the changes will appear in the chemical actions. In further only the first of the above described cases will be discussed, being called the tracer experiment (experiment using the tracer dose of labelled compound). Each IME investigates the chemical actions in the biological system following only those molecules labelled by the radioactive atoms instead of all molecules in the system. The labelled molecules are supposed to be the representative selection of all molecules of the same kind present in an organism. It must be supposed that the labelled molecules have the same chemical characteristics as the unlabelled ones or, in other words, that the organism does not distinguish the labelled or unlabelled molecules.*

* Being satisfied enough in practice this assumption is not valid absolutely. It is known that different isotopes of the same element (and their compounds) may have rather different chemical reactivity. This fact is more significant in the elements with low atomic numbers and in the compounds having more atoms substitued by isotopes. The most striking case is the "heavy water" D_2O or T_2O having different physical, chemical, and biological properties in comparison with the ordinary water H_2O. It is not necessary to consider the different chemical reactivity at the common more complicated molecules labelled by one heavier radioactive atom; it will not be taken into account in further development of this paper. Nor the alteration of metabolic actions in the organism due to the radiation of labelled molecules will be considered; the tracer dose of the radioisotope is so futile that any metabolic changes are practically unprovable.

The living organism has the character of a very complicated self-regulated system. Its properties may be taken as output variables. The output variables may be divided into the essential and non-essential ones. Among the essential variables belongs the concentration of some matters in the biological system; as non-essential e.g. the length of hair, color of eyes etc. may be taken. It is known that the concentration of certain matters in the living organism is constant in the given range and on the "normal conditions". A state of the biological system where the concentration of matters (or other physiological quantities not considered here) is non-changing with time, is called the steady-state. The steady-state (further *S*) in this sense of the word is, of course, an abstract conception irrespecting the physiological fluctuations (variations) of matter concentrations nearby the average value.

If the matter concentration is constant (except the physiological variations) i.e. if it changes systematically in one direction the organism is in the state of dynamic inequilibrium being called as "not-steady-state" (further *N*). *N* a is more general state than *S* because *S* may be taken as the limiting case of *N* when systematic changes of matter concentrations are coming near zero. Not every fluctuation of matter concentration means state *N* as will be shown later on.

The concentration change of each metabolite is given by the difference of the rate of its creation and the rate of its decrease. The rate of creation is proportional to the concentrations of matters giving origin to the followed metabolite (precursors, further *P*). The constants of proportionality k are called rate constants. The same is valid for the decrease of the matter: the rate of decrease is given by its concentration, by the concentration of matters chemically reacting with it and by the proper rate constant. These relations will be dealt further on. The very serious fact is that the rate constants in relatively unchanging physicall conditions which are altogether fulfilled in the living organism are not systematically changing their values in dependence on time. The state *S* may thus be defined as a state where neither the concentrations of matters nor the rate constants are changing with time, the state *N* as a state where the rate constants being unchanged the concentrations are changing.*

The state *N* may be further generalized supposing the time-dependent quantitites instead of rate constants. It may be proved that the variation of concentrations is general in such a case. The state when the rate constants are changing with time, which means that even the concentration of matters in the organism is changing, is called *I*. It may be seen that the state *N* is the special case of the state *I* setting the quantities independent on time instead of the functions giving the time-dependency of rate "constants".

* Even here and in the further text, we suppose the metabolic events are acting in the liquid phase only and the volume conditions of the biological system stay unchanged during the examination. The concentration of matter is thus proportional to its mass (weight, number of molecules etc.).

Metabolism is the multiple of chemical actions in the organism. Its typical property is the continuality and the tendency, in physiological conditions, to keep the concentration of metabolites constant in the given range (quasi-constant, state S). The equality of the total income and output of matters and energies is the necessary condition. In physiology the state of this kind is called the zero metabolic balance. Nevertheless the metabolic balance is non-zero in general conditions.

All chemical reactions may be divided, from the view of chemical reaction kinetics, considered from different standpoints:

1. According to the number of molecules (or atoms) entering the reaction. If only one molecule is entering the reaction (e.g. "spontaneously" decaying) then the reaction is monomolecular. In case more molecules are entering the reaction, it is polymolecular.
2. According to the number of molecules determining the course of the reaction. The reaction of zero, first, second, or higher orders are existing. The order of the reaction need not agree with its molecularity.
3. According to the direction of reaction course one-directional or both-directional (reversal) reactions are distinguished. The one-directional reactions are the limiting case of the reversal reactions if the output from one direction is negligible in comparison to the output from the contrary one.
4. According to the shape of the reaction scheme. The reactions may be divided into the linear (not branched and branched = side-), and the cyclic ones. The simple reverse reaction is not regarded as cyclic.

It may be supposed that all above mentioned types of reactions are presented in the metabolism. Suggesting the ideal chemical action, including those reactions, we

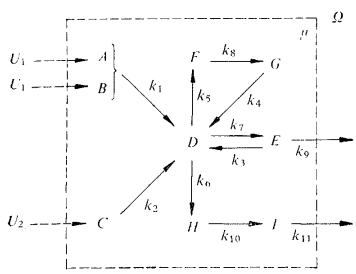

Fig. 1. Model of the metabolism μ. The quantities $A, B, \ldots, I$ belonging to the nodes are representing the metabolite concentrations; the quantities $k_1, k_2, \ldots, k_{11}$ belonging to the edges (arrows) are representing the rate constants. Ω is the environment of the system μ. U_1, U_2 are the quantities (constants) representing the input of matters and energies into the biological system.

can obtain the homomorphious model system with the real exchange of organism compounds under the above described circumstances (Fig. 1). The behavior of the

respective metabolites under the different conditions may be analysed on this model. Since the detailed study of this real metabolism (original) is technically impossible the work with the model system seems to be the necessity.

If the model contains only the monomolecular reactions, the course of concentration of each compound M may be described by the equation

$$\frac{dM}{dt} = \sum_{i=1}^{n} k_i P_i - M \sum_{j=n+j}^{m} k_j , \tag{1}$$

where: M = arbitrary metabolite,
P_i = i-th precursor,
k_i, k_j = rate constants ($i = 1, 2, \ldots, n$; $j = n + 1, n + 2, \ldots, m$).

It is necessary to note that one and the same matter may be simultaneously both the precursor and the product of compound M. In the scheme (Fig. 1) the precursors of matter D are, e.g. A, B, C, E and products E, F, H. Here and further on we consider only direct precursors and products, i.e. those compounds connected in the scheme (Fig. 1) by arrow with the metabolite under investigation.

In fact, however, the metabolites do not arise and are not destroyed by way of monomolecular reactions only. As it is known, the rate of increase of the product of polymolecular reaction is equal to the concentrations of the r_i matters entering the reaction multiplied by the respective rate constant. Therefore it is necessary in the equation (1) to replace the precursor P_i by the expression

$$\prod_{l=1}^{r_i} P_{il} = P_{i1} P_{i2} \ldots P_{ir_i} . \tag{2}$$

If the matter M is decomposed by the polymolecular reactions, the rate of decrease of the metabolite M is equal to its concentration multiplied by s_j matters L_{jl} which react with it and by the respective rate constant. The rate constant k_j in the equation (1) must also be replaced by the expression

$$k_j \prod_{l=1}^{s_j} L_{jl} = k_j (L_{j1} L_{j2} \ldots L_{js_j}) . \tag{3}$$

After substitution of the left sides of equations (2), (3) into the equation (1) we obtain the equation

$$\frac{dM}{dt} = \sum_{i=1}^{n} k_i \prod_{l=1}^{r_i} P_{il} - \sum_{j=n+1}^{m} k_j \prod_{l=1}^{s_j} L_{jl} . \tag{4}$$

The equation (4) describes the course of concentration of each metabolite under a more general situation where the reactions creating and destroying the metabolite are not monomolecular.

If in the equation (4) resp. (1) is valid that $(dM/dt) = 0$ for all $t \geqq t_0$, the metabolic system is in the state S, as defined. In the opposite case the system is in the state N. The transition from the state S into the state N can be provoked only by some intervention from the surroundings.

The course of the concentrations in the state N is evidently different according to the caracter of this intervention, i.e. either unique (e.g. in biological experiment usual rapide intravenous injection of a certain metabolite) or durable, e.g. during the infusion. In the first case the system returns to its stabilized state where the concentrations, however, need not be identical with those before the deviation, in the other case the course of concentrations is more complicated and depending on the duration, intensity and course of the intervention. Except the analoque modelation of the experiment, where the labelled matter is delivered to the organism continually and at a constant rate, in the following the unique deviation of the system will only be considered.

The differences between the states S, N will appear markedly during the solution on the analogue computer (Fig. 2). On the analogue model under the given rate constants both states N, S differ only in the initial conditions on the integrators. To the

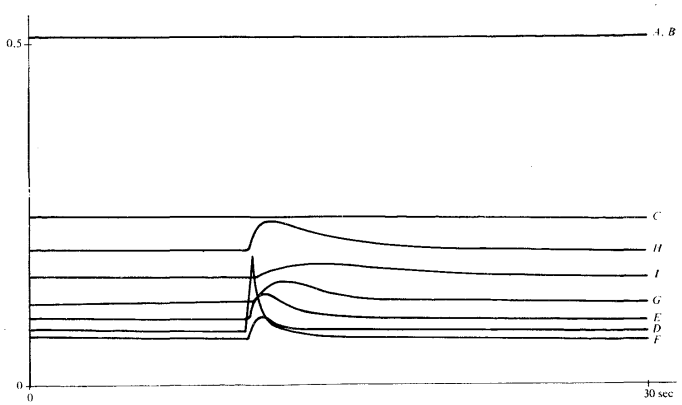

Fig. 2. Analogue representation of the states S and N on the system μ (x axis: time of solution in seconds; y axis: voltage in the scale of computer unit (± 50 V)). Initial conditions of the quantities $A, B, \ldots, I$ were chosen so that they are suiting, in the given values of rate constants ($k_1 = 2{\cdot}0$; $k_2 = 1{\cdot}6$; $k_3 = 0{\cdot}6$; $k_4 = 0{\cdot}8$; $k_5 = 1{\cdot}2$; $k_6 = 1{\cdot}4$; $k_7 = 1{\cdot}0$; $k_8 = 1{\cdot}2$; $k_9 = 0{\cdot}4$ $k_{10} = 0{\cdot}4$; $k_{11} = 0{\cdot}5$; $C_1 = 0{\cdot}5$; $C_2 = 0{\cdot}4$), to the state S. During the solution the quantity D was deviated by a unique impulse from outside so that the system μ was transferred to the state N.

state S suit certain combinations only, while the state N is satisfied by the arbitrary combinations of their possible values.

The development of metabolite concentration after its unique deviation from the value appertaining to the state S depends on the type of metabolite transition into its products. In the sequel we assume that this deviation has no influence on the precursor concentration values. Only some special cases will now be discussed:

1. If the metabolite transits to its products by the monomolecular reactions only, if it is not a member of the cyclic reaction, and if none of its products is simultaneously its precursor.

If in the equation (1) will be $\sum_{i=1}^{n} k_i P_i = C$, $\sum_{j=n+1}^{m} k_j = k$ then the equation $\mathrm{d}M/\mathrm{d}t = C - kM$ may be written.

Its solution gives the course of M in the form

$$M = \frac{C}{k} + \mathrm{e}^{-kt}\left(M_0 - \frac{C}{k}\right) \tag{5}$$

which is the single exponential. It may be seen from the equation (5) that if $M_0 = C/k$ then the value of M is not changing with time, the latter being the reduction into the state S.

2. If the metabolite transits to its product by a bimolecular reaction other conditions leaving the same as in the case 1.

If M will be the concentration of deviated metabolite and Q the concentration of the compound entering the bimolecular reaction with it then:

$$\frac{\mathrm{d}M}{\mathrm{d}t} = C - kMQ\,; \quad \frac{\mathrm{d}Q}{\mathrm{d}t} = C - kMQ\,. \tag{6}$$

Let's put M_S and Q_S the concentration of M and Q respectively belonging to the state S before the deviation of M. Thus $Q_S = M_S + r$, where r is a constant having its value in the interval $(-M_S, Q_S)$. If the moment of deviation will be t_0 then $M_0 = M_S + \Delta$, $Q_0 = Q_S = M_S + r$. The left members of equations (6) are positive for $\Delta < 0$ and negative for $\Delta > 0$ for every $t \geqq t_0$. M will be constant if $MQ = M_S Q_S = C/k$. Further from the equations (6) follows that, $Q = M + r - \Delta$ for $t \geqq t_0$. Setting into (6) and rewriting one thus obtains

$$M^2 + (r - \Delta)\, M - \frac{C}{k} = 0$$

as a condition of constancy of M, from where

$$M_{1,2} = \left(\frac{r - \Delta}{2}\right) \pm \sqrt{\left[\left(\frac{r - \Delta}{2}\right)^2 + \frac{C}{k}\right]}.$$

For all Δ fulfilling the physical assumption $\Delta > -M_S$ the positive solution of the expression under the root sign only is suiting. The solution is giving the value on which M will stabilize again in the given conditions. It is interesting that the newly stabilized values of M and Q respectively are not identical with M_S and Q_S.

If the kinetics of bimoleculary removing M – denoted by M_b – is compared with the kinetics of monomoleculary destructed M – denoted by M_m – and introducing

$$M_{b0} = M_{m0}\,, \quad \left.\frac{\mathrm{d}M_b}{\mathrm{d}t}\right|_{t=t_0} = \left.\frac{\mathrm{d}M_m}{\mathrm{d}t}\right|_{t=t_0}\,,$$

then

$$\frac{\mathrm{d}M_b}{\mathrm{d}t} = C - kM_bQ_b\,; \quad \frac{\mathrm{d}M_m}{\mathrm{d}t} = C - k'M_m\,,$$

where $k' = kQ_{b0}$. For $t > t_0$ it then follows for all Δ:

$$\left|\frac{\mathrm{d}M_b}{\mathrm{d}t}\right| < \left|\frac{\mathrm{d}M_m}{\mathrm{d}t}\right| \Rightarrow |M_b - M_S| > |M_m - M|_S$$

because Q_b is increasing for $\Delta < 0$ and decreasing for $\Delta > 0$.

It may be proved that the course of M_b (in contradistinction to the exponential course of M_m) is not symmetrical by the axis M_S if the values of M_S deviation was $+\Delta$ or $-\Delta$ respectively.

3. If M transits to its products by the bimolecular reaction and if in addition to M even its reaction partner Q is at once deviated all other conditions leaving unchanged as in cases 1 and 2.

First of all the analytical solution of the equations (6) will be done:

The first integral of (6) is $M - Q = A \Rightarrow M = A + Q$. Setting for M into the second equation (6) one thus obtains the non-linear equation

$$\frac{\mathrm{d}Q}{\mathrm{d}t} = C - kAQ - kQ^2\,.$$

Using a first substitution $Q = -z/k$ and rewriting we obtain

$$\frac{\mathrm{d}z}{\mathrm{d}t} = z^2 - Akz - Ck\,,$$

and using a second substitution $z = y + (Ak/2)$ and rewriting we obtain

$$\frac{\mathrm{d}y}{\mathrm{d}t} = y^2 - \left(\frac{Ak}{2}\right)^2 - Ck\,,$$

where

$$\left(\frac{Ak}{2}\right)^2 + Ck = B > 0\,.$$

Introducing to the first equation we obtain

$$\frac{dy}{dt} = y^2 - B .$$

The solution may be obtained by the separation of variables having thus

$$y = \sqrt{B} \cdot \frac{1 + R \exp(2t\sqrt{B})}{1 - R \exp(2t\sqrt{B})} .$$

And returning to the original function $Q(t)$ then

$$Q = -\frac{\sqrt{B}}{k} \cdot \frac{1 + R\, e^{2t\sqrt{B}}}{1 - R\, e^{2t\sqrt{B}}} - \frac{A}{2} ; \quad M = -\frac{\sqrt{B}}{k} \cdot \frac{1 + R\, e^{2t\sqrt{B}}}{1 - R\, e^{2t\sqrt{B}}} + \frac{A}{2} .$$

The constants A, R may be found from the initial conditions

$$A = M_0 - Q_0 ; \quad R = \frac{k(M_0 + Q_0) + 2\sqrt{B}}{k(M_0 + Q_0) - 2\sqrt{B}} .$$

Evidently the expression for R is valid only if $k(M_0 + Q_0) - 2\sqrt{B} \neq 0$ which is supposed at present. From the form of the solution for M, Q may be seen yet that

$$\lim_{t \to \infty} M = \frac{\sqrt{B}}{k} + \frac{A}{2} ; \quad \lim_{t \to \infty} Q = \frac{\sqrt{B}}{k} - \frac{A}{2} .$$

Now let's pay attention to the case when $k(M_0 - Q_0) - 2\sqrt{B} = 0$. Rewriting this one can obtain $M_0 Q_0 = C/k$ meaning that $M = M_0$, $Q = Q_0$, i.e. there is no deviation from the state S.

Now let's find the dependence of M and Q kinetics respectively on the value and deviation direction of these quantities in t_0: Let's introduce $M_0 = M_S + \Delta$, $Q_0 = Q_S + \varepsilon$, $Q_S = M_S + r$.

The function $T = -\Delta^2 + (r + M_0)\Delta + M_0\varepsilon$ may be defined so that its value determines the sign of first derivation of the quantities M, Q. For $T = 0$, M and Q are constant for every $t \geqq t_0$; for $T < 0$ are M and Q increasing, and for $T > 0$ are M and Q decreasing functions of t. As it may be seen for example M may be, after the deviation up, increasing which means withdrawing from the values belonging to the state S etc.

Now let's compare again the kinetics of M_b in the case when M is removed by a monomolecular mechanism. Let's introduce again

$$M_{b0} = M_{m0}, \quad \left.\frac{dM_b}{dt}\right|_{t=t_0} = \left.\frac{dM_m}{dt}\right|_{t=t_0} ,$$

$$M_{b0} = M_{bS} + \Delta , \quad Q_{b0} = Q_{bS} + \varepsilon , \quad M_{bS} = M_{mS} .$$

The physical sense of the comparison in this case may be found only if the derivation of M and the deviation Δ have opposite signs. Since for $t > t_0$ is $Q \neq Q_0$ the course of M_b will be different from M_m. It may be proved that for arbitrary combinations of the possible values Δ, $\varepsilon(\Delta > M_{bS}, \varepsilon > Q_{bS})$ it is fulfilled again the relation

$$\left|\frac{dM_b}{dt}\right| < \left|\frac{dM_m}{dt}\right| \Rightarrow |M_b - M_S| > |M_m - M_S| .$$

The course of M is, similarly to the case 2 not symmetrical by the axis M_S choosing the deviations $+\Delta$, $+\varepsilon$ firstly and $-\Delta$, $-\varepsilon$ secondly. Making evidence is satisfied by proving the unsymmetry for t_0. The course in t_0 being symmetrical the following equation should be valid:

$$\frac{C - k(M_S + \Delta)(Q_S + \varepsilon)}{C - k(M_S - \Delta)(Q_S - \varepsilon)} = -1 .$$

But this formula is equal to -1 only if at least one of the deviations Δ, ε is equal to zero. In the case $\Delta \neq 0$, $\varepsilon = 0$ our problem changes into that one discussed in the case 2.

4. If M is a member of cyclic reaction, then it is valid without any other limiting conditions that after the single deviation Δ of M_S the oscillations having the character of damped vibrations may be found in the course of M. Their frequency and amplitudes are determined by the values of rate constants, number of compounds in the cycle, molecularity of the respective reactions etc.

The analytical solution of the kinetics of M situated in the cyclic reaction will not be presented; instead of this the analogue solution of the special case is given (Fig. 3).

The state I may be obtained replacing at least one rate constant in the system μ by an arbitrary non-negative time-dependent function. I is the general state of the metabolic system. The state N or S respectively may be derived from the same by introducing certain supplementary conditions. The equation for the metabolite kinetics in the state I, i.e. the general equation for the compound concentration development in the biological system may be obtained by introducing the respective time-dependent functions instead of the rate constants into the equation (4). The course of compound concentrations is quite general here in the range of non-negative values.

Originating from the previous considerations the following mathematical model of the metabolism may be formulated:

Let us have a certain system μ given by a multiple of nodes A and a multiple of edges K.

$$A = \{A_1, A_2, \ldots, A_n\} ; \quad K = \{K_1, K_2, \ldots, K_m\} .$$

To each node A_i in the system belongs a certain quantity $a_i(t)$ having a certain time-development. (This quantity shows the metabolite concentration on the place A_i). Even every edge K_i is characterized by a time-dependence of a certain quantity

$k_i(t)$. The quantities $a_i(t)$ and $k_i(t)$ are reaching in the time $t = t_0$ the so-called initial values marked a_i^0, k_i^0.

Due to the structure of the system μ the variations of quantities $a_i(t)$ with time are dependent on the initial values a_i^0 and k_i^0 and on the course of quantities $k_i(t)$. The

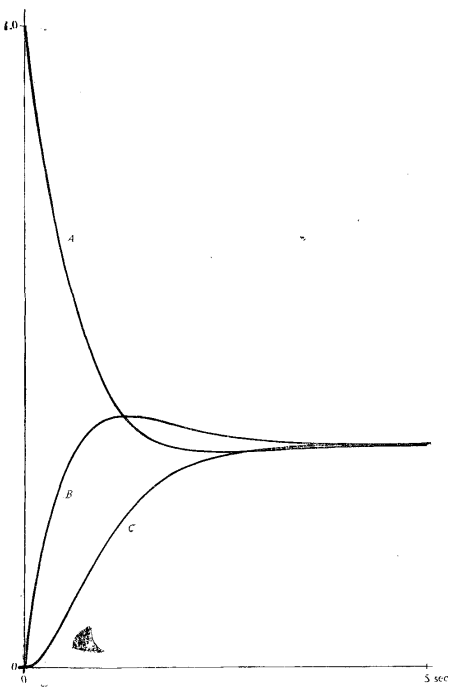

Fig. 3. Analogue representation of the oscillation of matter concentrations in the metabolic cycle $A \xrightarrow{k_1} B \xrightarrow{k_2} C \xrightarrow{k_3} A$ in the state N (x axis: time of solution in seconds; y axis: voltage in the scale of computer unit). Initial conditions: $A = 1$; $B = C = 0$; $k_1 = k_2 = k_3 = 1{\cdot}0$.

sudden changes of some quantities $a_i(t)$ resulting from impulse outside of the system μ are also admittable.

The considered system may be found in three states:

a) if the initial values a_i^0, k_i^0 are arbitrary (in the range of possible values) and if the course of quantities $k_i(t)$ is quite general then the system appears in the state I.

b) if the initial conditions a_i^0, k_i^0 are arbitrary and if $k_i(t) = k_i^0$ for all t, then the system appears in the state N.

c) if the values a_i^0 are chosen by a certain system in accordance with the values k_i^0 and if $k_i(t) = k_i^0$ as in sub b) then the system is in the state S being $a_i(t) = a_i^0$.

ISOTOPIC EXPERIMENTS IN THE DIFFERENT STATE OF THE ORGANISM

It was introduced on the beginning of this paper what is meant as IME. It must be emphasised that only those cases are investigated where the mass of administered labelled compound is negligible. Further we must suppose all reactions (metabolic paths) of compound M transition into its products are known so that the aim of IME is only the estimation of rate constants or derived quantities and not the discovery of still unknown metabolic paths. (The discovery of metabolic paths is very often the main problem in practice but such experiments are not discussed in our paper.)

Two quantities connected with the radioactivity of administered compound appear in IME; it is the "activity" and the "specific activity". The activity is proportional to the number of labelled molecules p (in the investigated compartment) while the specific activity is proportional to the activity divided by the number of unlabelled molecules P of the same compound. Thus

$$A(M) = \lambda p\,, \tag{7}$$

$$a(M) = \frac{A(M)}{\varkappa P}\,, \tag{8}$$

where $A(M)$ = activity,
$a(M)$ = specific activity,*
$\lambda, \varkappa$ = constants.

Since M is constant the development of $a(M)$ and $A(M)$ is conform if the investigated system is in the state S. Now let us try to find the course of activity for the single administration of labelled compound. It is evident that $A(M)$ reaches its maximum value in the time t_0. Further development of $A(M)$ depends on the types of reactions transiting M to its products. In case that none of the products is simultaneously the precursor and M is not the member of metabolic cycle, the course of $A(M)$ is exponential regardless on their molecularity. In case that at least one of these reactions is reversal and M is not the member of a metabolic cycle then the course of $A(M)$ decrease is slower than it is in exponential with the same value of M_0 and $\mathrm{d}M/\mathrm{d}t|_{t=t0}$. Finally in case that M is the member of cyclic reaction then the oscillations appear in the course of $A(M)$ irrespective of any other conditions.

* The specific activity is often defined rather differently so that the number of labelled atoms (in a certain unit) is related to certainly defined number of molecules of different kinds expressed in the unit of weight. In this paper the term "specific activity" is used in the meaning given by the equation (8).

The activities of all products of the labelled matter M are equal to zero in the time t_0; later they are rising to their maximum values and then decreasing again. The value of maximum and its position on the time-axis is dependent on a number of circumstances but it is always evaluable if the values of rate constants and the course of precursors of the followed metabolite are known. On the other side the values of the respective rate constants may be estimated from the position of maximum if other

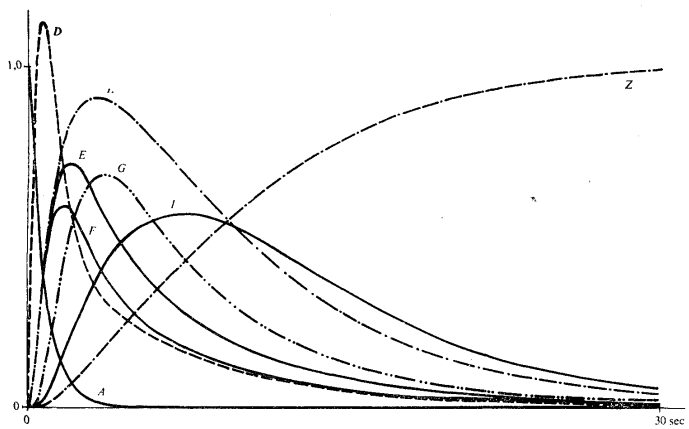

Fig. 4. Analog representation of the activity course on the system μ after the unique dose of labelled compound A (x axis: time of solution in seconds; y axis: voltage in the scale of computer unit). The values of all rate constants are the same as on the Fig. 2. The amplitudes of the quantities B, C, ..., I are four times increased for the higher intuitiveness. The quantity Z represents the accumulation of products of the labelled compound A in the environment of the system μ.

factors from the differential equation describing the course of the followed compound are known. In the course of activity of the matter M products, except the direct ones, the inflex points may be found.

If the labelled matter M is administered persistently with the constant rate then activities of M and all its products are equal to zero in the time t_0 and then increasing to a certain limited value. In the course of activities the inflex points may be found in all products. The limiting activities of all matters are proportional to the concentrations of the respective unlabelled matters. The possible course of activities in the state S for the single and persisted administration may be seen on Figs. 4 and 5.

Since the concentration of matters standing in the denominator of the equation (8) is time-dependent the conformity of activities and specific activities courses is not

valid in the state N. Nevertheless the course of activities in the state N is the same as in the state S because the decrease of the labelled molecules number is (in the given rate constants) dependent on their actual number and independent on the stage of "dilution" of the labelled molecules by the unlabelled ones. In this way having a knowledge of the specific activity courses in the state N the courses of activities and thus even the rate constant values may be estimated only if the courses of unlabelled compound concentrations are known simultaneously.

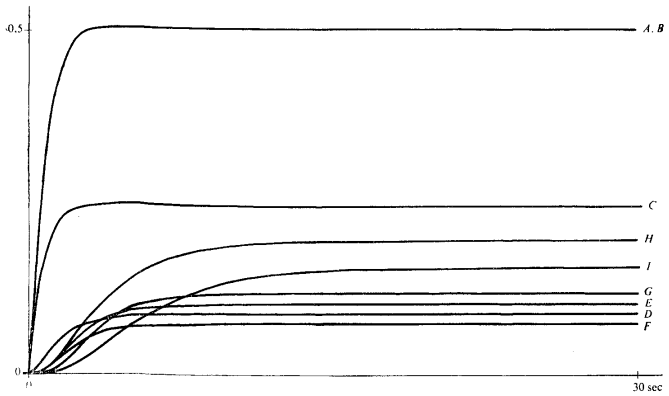

Fig. 5. Analogue representation of the course of activity on the system μ after the persistent constant administration of the labelled compound A (x axis: time of solution in seconds; y axis: voltage in the scale of computer unit). The values of all rate constants are the same as on Figs. 2, 4. All quantities $A, B, \ldots, I$ are drawn in the same scale.

In the state I where the rate constants themselves are changing the activities of compounds may be estimated only if the courses of the respective unlabelled compounds are known, in addition to the specific activity courses. The estimation of rate constant values is, however, in this case much more difficult than in the state N as it will be shown later on.

The course of activity of any arbitrary matter in each metabolic state (S, N, I) is given by the same equation as the course of the unlabelled matter concentration but the following modifications must be respected:

a) the concentrations of all matters including labelled atoms must be replaced by their activity,

b) the concentrations of all matters not including labelled atoms but reacting with labelled compounds must stay unchanged,

c) the concentration of all matters not including labelled atoms and not reacting with labelled compounds must be put equal to zero.

DISCUSSION AND CONCLUSION

The factors determining the activity course of labelled compounds in the biological system were elaborated in a certain type of experiment being called IME. For this purpose the behavior of the mathematical model μ homomorphious with the metabolism of the living organism was examined. Obviously model μ is considerably simplificed against the reality not only containing much less compartments than any biological system but also omitting a number of specific biological properties of an organism. Nevertheless it contains basic types of chemical interactions existing in the biological system so that it may be supposed the results obtained from the system μ are prudently employable in the metabolism study of the living organism.

Three states of the system μ, called S, N, I may be distinguished according to the course of rate constant values and its relations with the matter concentrations in the time t_0 (i.e. with the initial conditions of the matters). It is necessary to note at present how these states determining the matter concentration courses in the system μ are realised in a real biological system. This problem being specifically biological will be dealt only briefly.

Since it is sure that the rate "constants" of metabolic actions are in the reality quantitities changing their values with time, the state I is realised in any biological system. At least some reasons for this assertion will now be introduced. One of the reasons is the fact that the internal temperature of a biological system has fluctuations in a certain degree and it is known from the physical chemistry that the value of rate constant is dependent on a temperature. Another group of reasons is connected with the fluctuation of actual enzymatic activities in the organism. E.g. it is known that the secretion of digestive tract liquids rich in the enzymes is not fluent. Consequently the course of rate constants of matter decomposition in the digestive tract is irregularly periodical. A broad scale of further examples and reasons might be mentioned as e.g. the processes of the physiological adaptability, induction of enzymatic activity (from the word induction the state I was called), etc.

Considering the previous text one must query if the states N or S respectively are real at all from the biological point of view. To say exactly they are not. But they may be fulfilled approximatively if the fluctuation of rate constants is small enough. This fact may be arranged in an experiment by the desirable methodical adjustment. If then the rate constants are supposed to be the real constant values the conditions of state N are fulfilled. Now about the real existence of the state S. Neither this state exists exactly. Even supposing the stability of rate constants the concentrations of metabolites are never time-independent just because the income of foodstuffs into the organism is not a continuous action. Even in this case the oscillations

in the matter concentrations course may be minimalized by a desirable arrangement of an experiment so that the state S may be realised in practice.

Considering the previous text the decision of the state of an observed biological system is dependent on the conditions and aims of the experiment and on experience of the experimentator. The state S is most advantageous for all IME as will be shown later on. The state S is always a simplification; it is an experimentator's own problem how he is able to stabilize the rate constants and the matter concentrations.

The main goal of IME is the estimation of values (or courses in the state I) of the rate constants. Doing IME the measurement of specific activities of the compounds is relatively simple; the measurement of activities is more difficult for the concentrations of the respective unlabelled compounds must be known furthermore. Nevertheless the courses of activities particularly, enable the estimation of rate constant values.

The peculiarity of the state S is that the rate constants may be estimated directly from the specific activity courses, the latter being linearly proportional to the activities in this case. Due to this fact the state S is thus most advantageous for IME.

During the state N the courses of activities are the same as in the state S but the courses of measured specific activities are different due to the different course of the respective unlabelled matters. To estimate the values of the rate constants the courses of matter concentrations must be measured together which means that the experiment is more complicated. It is necessary to realize that the theoretical values of rate constants in the state N may be estimated from the courses of unlabelled matter concentrations. Having the more outstanding course the observation of activity is more advantageous in practice than the measurement of concentration of the unlabelled compounds.

During the state I the courses of specific activity and activity may be very miscelaneous. The courses of rate constants are observable from the simultaneous measurement of the specific activity courses and the courses of concentrations in the respective unlabelled compounds but this observation requires a great mathematical effort. Nowadys biology can hardly set such a high aim as the calculation of the rate constants course in the state S. If the biologist should want to know them he might use the theoretic estimation from the course of unlabelled compound concentrations without using the IME technics.

We may summarise that IME is a fully adequate method for the estimation of rate constant values in the state S. If the organism is in the state N the IME is still a useful method its value being, however, problematical if the organism is in the state I.

Note. The representations on figs. 2—5 were obtained by means of the Czechoslovak analogue differential analyser MEDA.

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VÝTAH

Příspěvek k teorii izotopových metabolických pokusů

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Práce se zabývá problematikou určitého typu izotopových metabolických pokusů. Byl formulován matematický model metabolismu, obsahující základní typy chemických reakcí, probíhající v živém organismu. Podle průběhů rychlostních „konstant" resp. koncentrací látek v okamžiku t_0 byly zavedeny metabolické stavy I, N, S. Dále byly studovány některé průběhy značených i neznačených látek v biologickém systému a diskutovány faktory, kterými jsou tyto průběhy určovány. Závěrem je hodnocena užitečnost izotopových metabolických pokusů za různých stavů biologického systému.

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