Biochemistry (Moscow), Vol. 67, No. 3, 2002, pp. 293-311. Translated from Biokhimiya, Vol. 67, No. 3, 2002, pp. 353-376.

REVIEW

Nitric Oxide Cycle in Mammals and the Cyclicity Principle

V. P. Reutov

Institute of Higher Nervous Activity and Neurophysiology, Russian Academy of Sciences, ul. Butlerova 5a, Moscow, 117485 Russia; fax: (095) 338-8500; E-mail: valreutov@mtu-net.ru

Received February 22, 2001
Revision received September 4, 2001

Abstract—This paper continues a series of reports considering nitric oxide (NO) and its cyclic conversions in mammals. Numerous facts are summarized with the goal of developing a general concept that would allow the statement of the multiple effects of NO on various systems of living organisms in the form of a short and comprehensive law. The current state of biological aspects of NO research is analyzed in term of elucidation of possible role of these studies in the system of biological sciences. The general concept is based on a notion on cyclic conversions of NO and its metabolites. NO cycles in living organisms and nitrogen turnover in the biosphere and also the Bethe nitrogen–carbon cycle in star matter are considered. A hypothesis that the cyclic organization of processes in living organisms and the biosphere reflects the evolution of life is proposed: the development of physiological functions and metabolism are suggested to be closely related to space and evolution of the Earth as a planet of the Solar System.

Key words: nitric oxide, NO synthases, nitrites, nitric oxide cycle, cyclicity principle

Each generalization suggests to some degree belief in unity and simplicity of nature. As to unity we cannot meet here any difficulties... We should ask ourselves the question "how is nature united?" rather than the question "is nature united?"

A. Poincaré

We often ignore the fact that all processes in the body are cyclic processes, and each of them has its own cycling...

L. A. Orbeli

During the last two decades, the history of biology and medicine has been significantly influenced by the discovery of a new physiologically active molecule, nitric oxide (NO) [1-8]. NO has a wide spectrum of biological activity [2, 9-18]. Being one of the messengers [19-22], NO is involved in the regulation of intra- and extracellular signaling [20-26]. Good evidence exists that NO is identical to endothelium derived relaxing factor [1, 3-5, 27, 28] which relaxes smooth muscles [1-8, 28-32] and prevents platelet aggregation [33-36] and neutrophil adhesion to endothelium [37, 38]. In addition to regulatory functions [39-58] NO exhibits cytotoxic [59-73], cytostatic [74-77], and many other properties [78-96].

Each epoch is characterized by its own interests, subjects, and objects of increased attention in science [97-101]. Exponential growth of publications on the role of NO in biological systems (more than 40,000 publications over a twenty year period) prompted the American Association for the Advancement of Science to declare NO as the "molecule of the year" in 1992. Many papers (~900) have been published on NO in Russian, Ukrainian, and Belorussian. Some references are cited here [9-23, 28-30, 33-58, 60-63, 65, 70, 71, 78-83, 85-88, 91-96]. In 1998 three American scientists, R. Furchgott, L. Ignarro, and F. Murad, were awarded the Nobel Prize in Physiology and Medicine for elucidation of the role of

0006-2979/02/6703-0293$27.00 ©2002 MAIK "Nauka/Interperiodica"

NO as a signaling molecule in the regulation of the cardiovascular system. Thus, at the end of the XXth century biochemistry, physiology, and medicine were under the sign of NO [102-112]. This raises a natural question: whether peak of interest in NO was culminated in the Nobel Prize for three American scientists or this area is still waiting for the most intriguing discoveries? To answer this question we ought to analyze peculiarities of the current period in NO research in biology and to determine the role of this problem in the system of biological sciences.

PROBLEM OF NO IN THE SYSTEM OF BIOLOGICAL SCIENCES

During the last two decades of the XXth century, the NO problem became one of the hottest subjects in terms of number of published papers. Many experimental methods, facts, and results were accumulated. NO donors and inhibitors of NO synthases (NOS) are used in medical practice. However, very often experimental results are interesting only for some specialists and not interesting for the majority of biologists and clinicians. At a certain stage of the development of science, such results may be useful or useless. The results become useless when researchers do not try to fit them into a general system of our knowledge. However, inclusion of some facts into a general theory or concept often requires the development of new notions summarizing all the facts. Some scientists consider such an approach as boring and worthless for researchers. That is why among many research papers only in a few of them the authors summarize their own results and data obtained in other laboratories. This is one of the reasons for excessive accumulation of non-summarized or poorly analyzed experimental data on NO in biology and medicine. A natural question arises whether this situation is unique for the NO problem in biology or this situation is also typical for other areas of modern science.

The period from the end of the XVIIIth and up to the XXth century was characterized by emphasized respect for empirical data. There was a widespread notion that first of all science needs experimental facts. However, accumulation of huge amounts of empirical material required systemic analysis and summarization, and C. Bernard expressed this tendency in the phrase that fact itself means nothing. Fact becomes valuable only if it supports some idea or confirms a hypothesis. This led to understanding that lack of correct and fruitful idea makes impossible summarization of enormous experimental data; moreover, interpretation of facts may be erroneous. Now not only some researchers but also historians of science believe that accumulation of huge experimental results in any field is accompanied by understanding that idea in science is very important, and non-systematic "mountains" of facts is not the principal goal in science [113]. In such periods of the development of science many specialists come to a reasonable conclusion that without an essential constructive idea even perfect methods of determination may become useless [114-116] and therefore theoretical summarizing goals become to predominate.

The need for the development of a new theory appears when numerous experimental facts exceed frameworks of existing ideas. Very often researchers solving their own scientific problems consider relative goals and problems as foreign ones. For example, researchers of the role of NO in biology and medicine often ignore metabolites of this regulatory molecule, nitrogen dioxide (NO_2) and anions NO_2^- and NO_3^-. Lack of integral understanding in interrelated goals and problems complicates subsequent progress in particular studies of NO problems. Nevertheless, progress in the accumulation of experimental data on the medico-biological aspects of NO continues. Very often this progress occurs "by inertia"; sometimes it occurs due to the absence of a generalizing idea. Sometimes this progress reflects the existence of reductionism. This direction dominating in biology and medicine over three centuries is the major reason for excessive accumulation of non-systematic data in biology and medicine and, particularly, in the NO field.

Retrospective analysis of evolution of biochemical and physiological knowledge shows that during the XVIIIth-XXth centuries biological science was oriented inside the living system. Scientists studied tissues, cells, subcellular structures, high molecular weight protein complexes and low molecular weight physiologically active compounds [117-120]. V. A. Engelhardt called this direction reductionism [101, 119]. As the result of reductionism domination in life sciences over three centuries of research are characterized by fragmental perception of scientific disciplines and the whole world. Almost all research branches of biology "suffer" with excessive accumulation of experimental material and this resulted in domination of a new tendency of scientific search called integratism. This scientific direction unites separated well-studied parts into the whole unit; it allows integration of simple structures and molecules into complex systems acquiring new properties and functions [101, 119]. Thus, actual reasons of crisis of excessive accumulation of non-systemic experimental data in biology consist in long-term "hypertrophic" attention to *analysis* of biological systems whereas *synthesis* joining various fields of this science remained underdeveloped [101, 119]. Moreover, papers containing attempts of synthetic consideration of some fields were often referred to as "philosophical" and were left out of the frameworks of this science. Thus, during analysis of literature data researchers of the NO problem meet difficulties that are typical for any intensively developing problem in biology and medicine.

F. Lipmann indicated that separating chemical continuity of processes of living organisms we must not leave out of consideration our major duty to integrate separated parts into the whole unit. In this connection, one of the main tasks of modern science consists of transition from analysis (resulting in modern crisis) to integrating synthesis. Studies of the NO problem in biological systems play an important role in this direction. The intensively developing field of NO research highlighted many important questions. How can this simple molecule be involved into the integration of structure–functional levels?

The second half of the XXth century was characterized by intensive studies of intra- and intercellular signaling, i.e., mechanisms responsible for cooperation of functional cell compartments and concerted running of metabolic processes required for cell growth and development [43, 121-123]. These studies revealed that NO together with other messenger molecules is involved in intra- and intercellular signaling. Integratism as a new research direction is focused on the study of conditions required for appearance of new connections between parts of the living, which were investigated during the reductionism epoch. So, under conditions of new orientation of the scientific search the important role of NO problem becomes evident.

However, it should be noted that modern models and also micro- and macroscopic ideas on the role of NO in biological systems represent a complex of incomplete and fragmented explanations typical for the modern level of our knowledge. Many studies on the role of NO were based on linear arrangement of processes involving NO and enzymes responsible for its formation: L-arginine $\rightarrow$ NO $\rightarrow$ NO_2^-/NO_3^-. The activity of NO synthases was evaluated by measuring L-arginine reduction or by accumulation of NO or end products of NO metabolism, nitrates and nitrites. However, researchers did not take into consideration the possibility of nitrite conversion into NO. Researchers often pooled nitrate and nitrite concentrations and evaluated either the sum of $[NO_2^-]$ and $[NO_3^-]$ or concentrations of one of these components (nitrite or nitrate). At the early stages of the NO era, such approaches demonstrated an important role of NO in intra- and intercellular regulation. However, they reduced the actual value of these experiments because they did not take into consideration reversed conversion of NO_2^- ions into NO. In other words, these studies implied linear (one-dimensional) mode of NO-linked biochemical processes terminating by formation of nitrate ions, which were mainly excreted from the body.

However, many experimental data suggest multiple regulatory functions of NO. Certain evidence exists that the use of such approach underestimates the regulatory role of NO [110, 124]. In fact, the term "*regulation*" implies the existence of negative feedback; the latter suggests that output signal may also reach the input of the system [122]. Thus, *regulatory* functions of NO suggest the necessity of closed cycles in the regulatory mechanisms involving NO. In this connection it is relevant to remember a phrase by L. A. Orbeli analyzing physiological processes in living organisms: "We often ignore the fact that all processes in the body occur in a cyclic manner and each separate process has its own cycling" [125]. Thus, many experiments have certain scientific value only within the time when they have been carried out, whereas fruitful ideas (even if they were not understood and accepted by contemporaries) may continue to live for centuries.

I do believe that our modern conceptions on the biological role of such "simple" chemical molecules as NO and products of its conversion are incomplete. Here I try to demonstrate that old concepts that were simple and natural yesterday are very incomplete today. The complexity of such simple compounds as NO becomes clearer if we take into consideration that all physico-chemical properties of NO were formed during formation of the main chemical elements (including nitrogen and oxygen) far beyond our planet. The properties of chemical elements appeared (or existed) in the primary substance determined subsequent fate of the evolution of life on the Earth and also percent ratio of all elements constituting the living system.

This paper continues a series of our publications [19, 20, 78, 105, 126-131] on the role of nitric oxide (NO) and cyclic reactions in vital activity of cells. In the present work we have analyzed some consequences related to the NO problem and mechanism of NO cyclic conversions in mammals. We have also tried to develop a general concept that would allow introducing multiple NO effects on various systems of living organisms in the form of a short and comprehensive law.

A GENERALIZING CONCEPT FOR THE NO PROBLEM

Considering the modern situation in biology, it becomes clear that the NO problem not only attracts much attention, but this problem (as well as many other biological problems) requires much more detailed analysis that would allow to overcome limitations of descriptive (accumulating) studies and to develop general concept. The latter would promote transition from simple knowledge of huge experimental material related to the NO problem to better understanding of general principles and their consequences. Such a concept may help to explain universal mode of NO action in various biological systems and to find new directions for solution of a number of very important medico-biological problems of oncology [74, 106, 132, 133] and cardiovascular diseases [28-30, 123]. However, the question arises how this generalizing concept should be formulated and what should be used as its

basis? Previous experience may help to find possible solutions of modern problems, including the NO problem.

J. Bernal indicated that during the development and generalization of any scientific problem "it is important to find and rise questions rather than to get answers to them. This is true whether we remember Newton or Lavoisier who made revolutions in physics and chemistry or Pasteur whose works gave birth to all key ideas on the biochemical basis for the origin of life" [134]. In fact, the history of science gives many examples when at certain stages it is much more important to find the right question rather than a satisfactory answer [97-99, 135, 136]. How to find which question is right? There are no algorithms or criteria for right decision. The situation like that in which researchers of the NO problem found themselves requires special approaches. These approaches consist of formulation of hypotheses and concepts possessing great generalizing power. They should be based on the analysis of numerous experimental data and deep intuitive understanding of the problem. Feynman wrote that we should extrapolate our knowledge into unknown areas; this is the only way for progress even if it is rather dangerous and unreliable [135]. Such approaches have been successfully employed in physics, molecular biology, and biochemistry [134-137].

The development of a scientific problem always begins from quantitative images, assumptions, and suggestions. Parallel ideas initially consisting from a series of separate suggestions are gradually converted into a continuously developing chain of sequential generalizations [137]. Very often scientists find themselves that the development of the concept occurs via a cyclic or helical route. Thus, an idea is the basis of all original achievements and an initial idea represents the least evident part of scientific achievements [114].

H. Selye believed that the first steps during discovery of problem and formulation of new concepts will always depend on subconscious intuitive feelings that help us to find a key to something great and completely new among a thousand things which surround us. This is a conjecture that is instinctively based on our previous experience, but, nevertheless, this is the conjecture and not a logic-controlled planned process [137]. J. Bernal expressed similar ideas [134]. According to his viewpoint, understanding of concrete mechanisms of functioning of biological structures besides knowledge of chemical properties and formulas of constituting molecules requires understanding of some general principles of their supramolecular organization. These idea and notions were also expressed by Ya. M. Varshavsky [138].

However, in the case of the NO problem even the most refined imagination cannot help to formulate any generalizing concept without knowledge of chemical properties and mechanisms of synthesis of the NO molecule. So, before the development of generalizing concepts it is necessary to consider those properties of nitric oxide and products of its metabolism which have held since the birth of the Universe (or during nuclear synthesis in the interiors of the stars before the birth of the Earth); they may help to understand some general properties of the supramolecular organization [134].

CHEMICAL ELEMENTS OF LIVING SYSTEMS

For creating living organisms, Nature selected from 109 known elements approximately 20 of them. Certain evidence exists that living organisms consist of 22 chemical elements [139-142]. However, only four of them H, C, N, and O represent the basis of life [140]. Other authors believe that the list of chemical elements constituting the basis of life also includes Ca, P, K, S, Cl, and Na [139, 141]. Hydrogen forming monovalent bonds with other elements represents ~7-10%. Carbon, nitrogen, and oxygen forming 4-, 3-, and 2-valent bonds with each other and with other elements represent more than 85% of the total elemental content in the body (Fig. 1). In living organisms, the content of each of these four elements vary from 2 to 60%; in total, they make up more 95% of mass of most organisms (Table 1) [139-142]. These facts and many other physicochemical and biological data are the basis of the concept of global evolutionism and the anthropic principle in cosmology [144, 145].

Interestingly, carbon representing the basis of living systems has only 16th position by distribution in the Earth's crust [140-143]. This suggests that geochemical factors did not play the decisive role in the selection of chemical elements for construction of the simplest biochemical structures. A unique feature of the carbon atom consists in its ability to form complex compounds with coupled bonds with alternation of single and double bonds. This bond alternation explains special properties of these molecules: high chemical activity and increased stability. This was noted by the founders of quantum biochemistry, A. Pullman and B. Pullman. They indicated that the main manifestations of life are directly linked to the existence of compounds with highly coupled chemical bonds, which predetermine the motion of electron clouds in the coupled molecules and flow of living processes [146]. This suggests that the selection of carbon as the main chemical element constituting living organ-

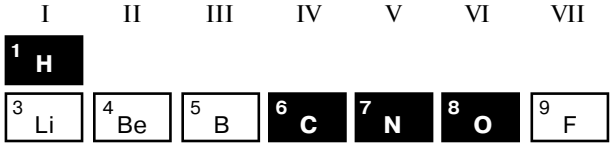

Fig. 1. Chemical elements of the periodic table of elements constituting life on the Earth.

Table 1. Elemental composition of animals and plants (% of total mass [142])

Chemical element	Mean value for mammals	Mean value for plants
Carbon	**21.15**	**53.96**
Hydrogen	**9.86**	**7.13**
Oxygen	**62.43**	**38.65**
Nitrogen	**3.10**	**0.03**
Calcium	1.90	0.007
Phosphorus	0.95	0.005
Potassium	0.23	0.006
Sulfur	0.16	0.052
Chlorine	0.08	0.001
Magnesium	0.027	0.003
Iodine	0.002	0.002
Iron	0.005	0.03
Fluorine	0.003	0.001
Aluminum	0.001	0.065
Silicone	0.001	0.057
Manganese	0.0005	0.001

Table 2. Valence of the nitrogen atom in some nitrogenous compounds [20, 127, 149]

No.	Valence of nitrogen atom	Compound
1	−3	NH_3
2	−2	NH_2NH_2
3	−1	NH_2OH
4	0	N_2
5	+1	N_2O
6	+1	$H_2N_2O_2$
7	+2	NO
8	+3	N_2O_3
9	+3	HNO_2
10	+4	NO_2
11	+4	N_2O_4
12	+5	N_2O_5
13	+5	HNO_3

isms was not accidental; it was the result of evolution of carbon compounds.

The nitrogen atom possesses a unique feature to form numerous compounds due to its physicochemical properties and the ability to change valence over a wide range (Table 2).

Thus, the huge variety of living organisms does not imply variety of chemical units constituting them. Variety of constituents of the living organisms is mainly determined by various combinations of the same chemical atoms, functional groups, and compounds [147, 148].

NUCLEAR SYNTHESIS OF ELEMENTS IN THE INTERIORS OF THE STARS AND THE NITROGEN–CARBON CYCLE

According to current concepts, the living matter of the Earth appeared from the same elements that were synthesized during sequential radio-chemical conversions in the interiors of the stars [148]. What are the main stages of synthesis of chemical elements? It is suggested that the matter initially forming the Galaxy consisted of hydrogen and helium. Sequential radio-chemical conversions that occurred in interiors of the stars resulted in formation of all known chemical elements of the periodic system. Sequential evolution of chemical elements approached the moment for transition of the star into the super new state which resulted in the big explosion accompanied by dissipation of heavy element enriched compound over the Galaxy. It is suggested that the life and the Universe cannot originate until at least one generation of stars finishes its "living cycle" and dissipates carbon containing fragments of the galaxy. When a new generation of stars and planets is formed, it utilizes this material of dead stars. Thus, it is possible that circuit of compounds exists not only in the Earth the biosphere but also in space, and some scientists even suggest that our bodies consist of ashes of stars that extinguished a long time ago [145, 148].

The carbon–nitrogen cycle attracts special attention because in this cycle carbon and nitrogen atoms are formed. According to the modern concept, atoms of the main chemical elements (carbon, nitrogen, oxygen, and hydrogen) of living organisms were elements of star matter. These atoms are involved in nuclear reactions of the Bethe carbon–nitrogen cycle, which is the main energy source in stars (Fig. 2) [148]. This cycle is rather complex and represents a chain of six reactions. In this process the carbon nucleus functions as a multiple-use catalyst. According to data of astrophysics and cosmochemistry the sequence of these nuclear reactions include carbon-mediated conversion of four hydrogen atoms (protons) into the helium nucleus. Initially unstable nitrogen isotope (^{13}N) is formed; the latter is converted into a carbon nucleus by emission of a positron. The carbon nucleus (^{13}C) accepts one proton and forms another nitrogen nucleus (^{14}N) which in its turn accepts one proton. The latter results in the formation of unstable oxygen nucleus (^{15}O), which emits a positron and is converted into nitro-

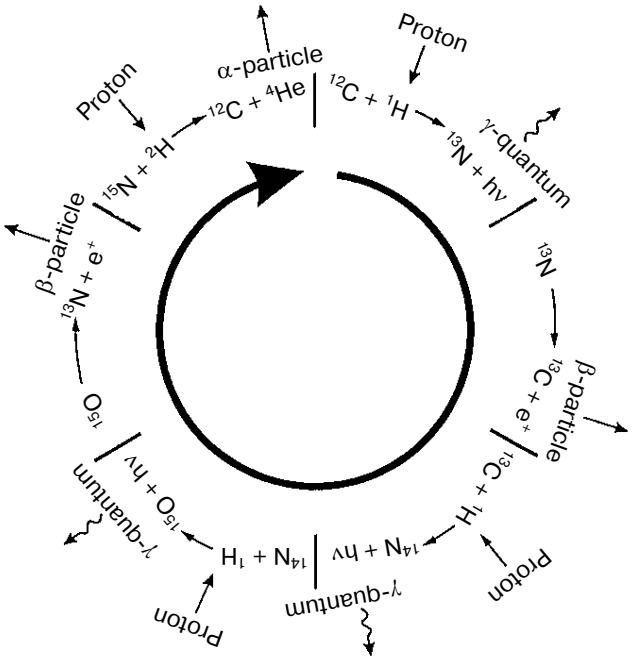

Fig. 2. Bethe carbon–nitrogen cycle.

gen. The nitrogen nucleus (^{15}N) accepts a proton and this results in formation of carbon nucleus (^{12}C) and helium nucleus (^{1}He) [148]. Thus, synthesis of the main elements of the living organisms occurs in interiors of the stars during cyclic radiochemical reactions. Moreover, each period of the Mendeleev periodic table of elements may be considered as a separate cycle depending on charge and mass of atomic nucleus. So, physicochemical properties of the main elements constituting the living organisms were determined before the appearance of the planet Earth. Some authors believe that selection of the main chemical elements of living organisms was closely related to those processes that occur in star matter [141, 145, 146, 148].

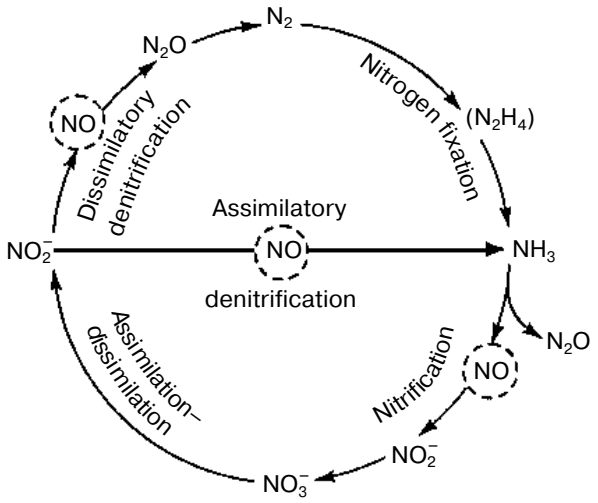

Fig. 3. Nitrogen cycle (nitrogen turnover in nature) [20, 154].

Physicochemical characteristics of the nitrogen atom and the nitrogen cycle in the biosphere. Nitrogen is one of the most widespread chemical elements. Its valence changes from –3 to +5 (Table 2). Redox transitions between these valence states occur readily. Compounds containing nitrogen in valence states from –3 to +2 react with biological nitrogen-fixing systems, whereas nitrogenous compounds containing nitrogen in valence states from +2 to +4 and from +4 to +5 represent products of metabolism by nitrite- and nitrate-reducing systems, respectively [149].

A property of living organisms to retain chemical elements in the biological circuit was noted by K. Ber while studying turnover of carbon and nitrogen atoms. He called this property of living organisms as a "salvage law". V. I. Vernadsky considered this law as very important property of the biosphere to retain carbon and nitrogen atoms and to pass them continuously from one organism to another without interruption of the biological circuit [150-152]. Subsequently, these notions were developed by Russian scientific schools of L. S. Berg, V. N. Sukachev, B. B. Polynov, L. A. Zenkevich, and V. V. Dokuchaev [150-153].

The nitrogen circuit or *nitrogen cycle* represents conversion of inorganic nitrogen into organic followed by its subsequent reversed decay [154, 155]. This cycle consists of three components: a) nitrification (oxidation of ammonia to nitrates: $NH_4^+ \rightarrow NO_2^- \rightarrow NO_3^-$); b) assimilatory ($NO_3^- \rightarrow NO_2^- \rightarrow NH_4^+$) and dissimilatory ($NO_3^- \rightarrow NO_2^- \rightarrow NO \rightarrow N_2O \rightarrow N_2$) denitrifications; c) nitrogen fixation ($N_2 \rightarrow (N_2H_4) \rightarrow NH_4^+$) (Fig. 3). During this cycle nitrifying bacteria convert soil ammonia into nitrites and nitrates. During assimilatory denitrification plants assimilate nitrites and nitrates and insert them into organic molecules. Animals consuming plants process these compounds into other complex organic molecules. Subsequent death and decay of living matter and organic compounds resulted in formation of reduced forms of nitrogen (ammonia). During dissimilatory denitrification nitrates are reduced to nitrogen (N_2); one part of nitrogen is lost into the atmosphere whereas another one is converted into ammonia by nitrogen-fixing bacteria [154-157]. Thus, almost all living matter of the Earth (i.e., all the biosphere) is involved in the nitrogen cycle [150, 155-157].

Comparison of the carbon–nitrogen cycle in the interiors of the stars and the nitrogen cycle in the biosphere shows that they have one important similarity, which consists in *cyclic* organization of both processes. However, at this stage this cyclic organization raises more

questions than gives answers. In this paper we shall try to find meanings of the cyclic organization of carbon–nitrogen cycle of interiors of the stars, the nitrogen cycle in the biosphere, and nitric oxide in living organisms.

Physicochemical characteristics of nitric oxide. NO is a neutral molecule that consists of two atoms. It readily crosses cellular and subcellular membranes due to small sizes and lack of charge. At 37°C the diffusion coefficient for NO is 1.4 times higher than for oxygen [75-77]. NO is a paramagnetic molecule containing an uneven number of electrons one of which has unpaired spin. The presence of one unpaired electron explains high reactivity of the NO molecule. Interacting with other free radicals, NO can form covalent bonds [47, 51, 75] and therefore activate [158-160] or inhibit [161, 162] free radical reactions. NO can also interact with proteins and low molecular mass compounds containing transition metal ions in the active site [20, 39-41, 75, 78, 105, 112, 163]. High reactivity of NO and permeability of cellular and subcellular membranes for this molecule lay serious limitations on mechanisms which would be used for regulation of NO concentration.

Below we consider data on physicochemical properties of NO, cellular effects, and physiological role of this compound. These data suggest that above considered features of NO molecules are used by cells for intra and intercellular signaling and for regulation of NO content.

SYNTHESIS OF NITRIC OXIDE IN THE PRESENCE OF OXYGEN

Hypothetical model of NO synthases. NO synthases (NOS) catalyze the reaction of NO synthesis from L-arginine [164-170]. For explanation of structure and function of NO synthases, a hypothetical model was proposed [164, 169-171]. According to this model, NO synthase is a dimer. The formation of this dimer occurs via intermolecular head to tail contact of calmodulin-binding domains (Fig. 4): the head of one monomer binds to the tail of another monomer. L-arginine and tetrahydrobiopterin (BH_4) are suggested to bind near the NH_2 terminus and BH_4 may be either involved into the reaction process or regulate NOS allosterically [164, 169, 170, 172]. Formation of one mole of NO requires utilization of 1.5 moles of NADPH. Flavin cofactors FAD, FMN, and cytochrome *P*-450-domain of NOS function as electron carriers from NADPH to molecular oxygen (oxidase domain). It is suggested that the hydroxylated form of L-arginine in the presence of O_2^- and heme of cytochrome *P*-450-domain is cleaved into L-citrulline and NO [164-166, 169-171].

How is NO synthesized in the presence of oxygen and its active forms? It should be noted that molecular oxygen is used for formation of both NO and L-citrulline (Fig. 5). Thus, data available indicate that the *NO synthase*

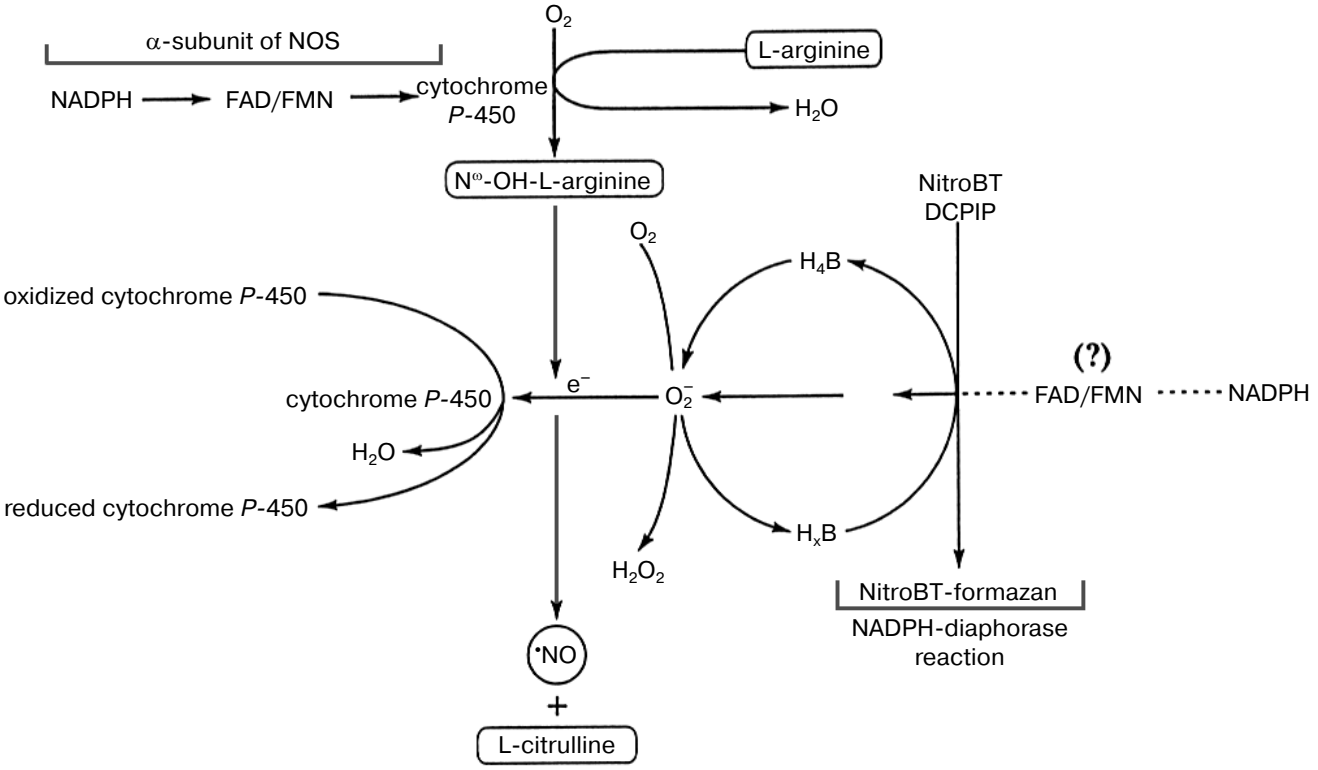

Fig. 4. A hypothetical model of NOS structure. Artificial electron acceptors: DCPIP (dichlorophenol-indophenol) and nitroBT (Blue Tetrazolium) [20, 169].

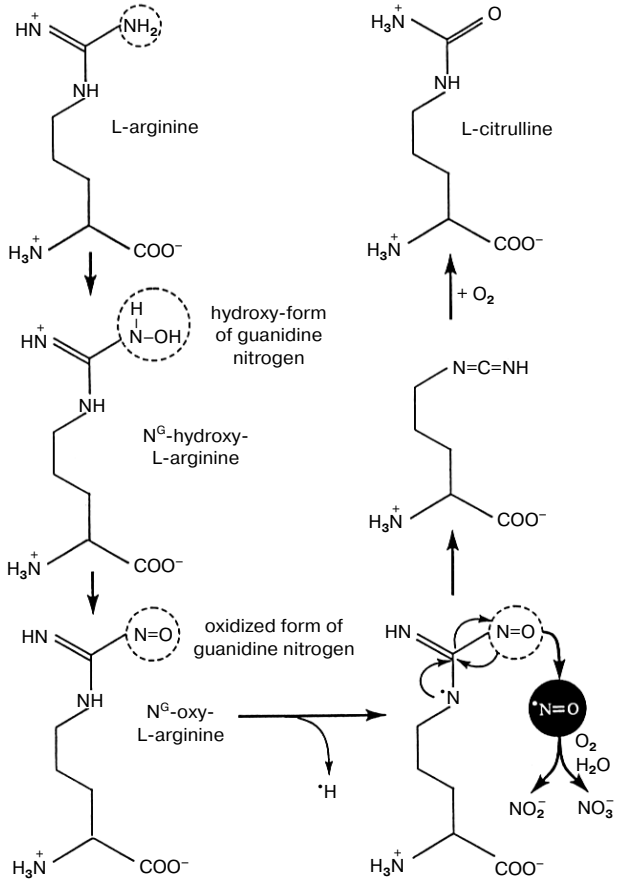

Fig. 5. Formation of NO catalyzed by NOSs.

mechanism is NO synthesis in the presence of oxygen [20, 129]. In this context it is important to stress that NOSs represent bi-functional enzymes: they activate oxygen and catalyzing binding of this oxygen to the guanidine nitrogen of L-arginine. NOSs release NO from this amino acid. These enzymes act like a sculptor who creates a statue by cutting "useless" parts of a "boulder". Some evidence exists that two functions of NOSs are distributed between the two subunits forming the enzyme dimer.

The steady state concentrations of NO produced by NOSs *in vivo* vary from 10 to 100 nM. This range of NO concentrations required for intra- and intercellular communication are not dangerous. At these NO concentrations systems producing O_2^- and superoxide dismutase may effectively regulate NO content in cells. During brain ischemia NO concentration may be increased up to 2-4 μM and activation of inducible NOS in macrophages NO concentration around these cells may be as high as 10 μM [20, 129, 164-171]. Thus, it is reasonable to propose that under physiological conditions the main principle of control of NO content in the body and cells is remarkably simple: NO is synthesized only when it is needed and in quantities which are required for a given time interval.

NO synthesis catalyzed by NOSs requires the presence of oxygen and its activated forms (Fig. 5). Deficit of oxygen may reduce the role of NO synthase mechanism and activate a nitrite reductase component (Fig. 6) that is about three orders of magnitude higher than NOS [20]. Good evidence exists that activation of this potent component under conditions of ischemia/hypoxia may represent an additional pathophysiological factor responsible for neuron damage during a reperfusion period. However, under physiological conditions the potency of this "weak" NOS component consists in limitation of feeding of "potent" nitrite reductase component with its substrate, NO_2^-. In other words, potency of the "weak" component consists in the weakness of the "potent" one.

Thus, universal integrity of the nitric oxide cycle is achieved by the interrelationship of two components involved in NO formation in the presence of oxygen and under hypoxic conditions. Perhaps, this is a paradox of the NO cycle: each component fully realizes one of the natural features typical mainly to this component. Let us consider now the mechanisms involved in NO synthesis under hypoxic conditions.

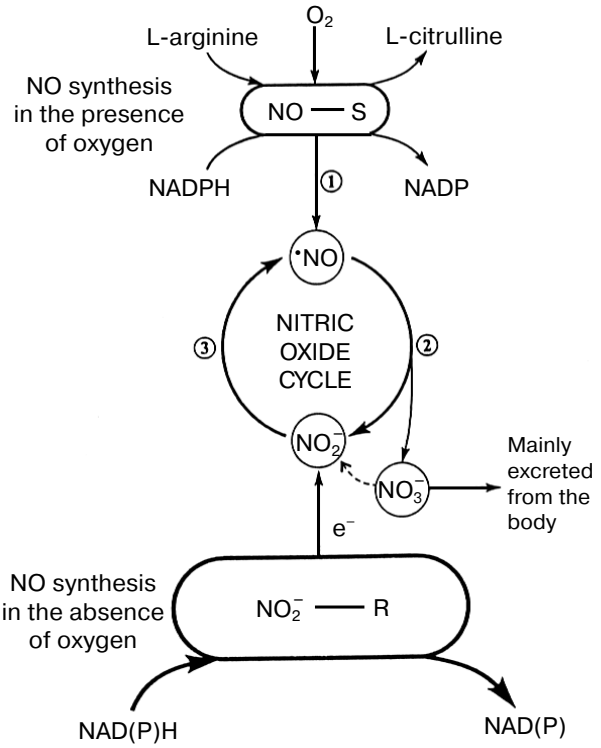

Fig. 6. NOS and nitrite reductase components involved in NO formation: 1) NO formation in NO synthase reaction; 2) NO oxidation into NO_2^- and NO_3^-; 3) NO_2^- reduction into NO with participation of nitrite-reductase systems.

NITRIC OXIDE SYNTHESIS UNDER CONDITIONS OF OXYGEN DEFICIT

We have previously demonstrated that under conditions of oxygen deficit the product of NO conversion, NO_2^- ions, may be effectively concerted into NO [126-131]. Thus, existence of NO synthase mechanism provides endogenous formation of NO, NO_2^-, and NO_3^-:

$$\text{L-arginine} \rightarrow \text{NO} \rightarrow NO_2^-/NO_3^-, \qquad (1)$$

whereas high activity of nitrite reductase systems "create" conditions for closing the chain (1) into a functional cycle, which we denominated as the NO cycle (Fig. 7). Since enzymatic oxidation of L-arginine catalyzed by NOSs requires oxygen, this mechanism should be inhibited under conditions of ischemia/hypoxia. Deficit of oxygen is a factor promoting active functioning of nitrite reductase systems linked to heme proteins, such as Hb, Mb, cytochrome oxidase, and cytochrome *P*-450 (Fig. 8). In fact, reduction of NO_2^- to NO has been detected in blood, cells, and tissues [20, 126-131]. Hb plays an important role in NO_2^- reduction in blood, but only deoxyHb is active [20, 73, 94, 126]. Hb-bound oxygen prevents NO_2^- conversion to NO [126]. In myocardium and skeletal muscles deoxyMb may play a similar role [20]. In tissues (containing and lacking Mb) NO_2^- reduction to NO may occur in mitochondria and microsomes [20, 73, 173-175]. Mitochondrial cytochrome oxidase [20, 73, 127, 173] and microsomal cytochrome *P*-450 [20, 73, 127, 174, 175] exhibit nitrite reductase activity. Thus, heme proteins, Hb, Mb, cytochrome oxidase and cytochrome *P*-450 usually interacting with oxygen may also (in deoxy form) reduce NO_2^- into NO and therefore close the chain of conversions (1) into the NO cycle (Fig. 8). Some authors consider the possibility of cyclic reactions involving NO and products of its conversion [110, 124].

Why are heme proteins able to reduce nitrite ions to NO? Electron transfer in proteins is typical for almost all biochemical reactions. This process determines the rate and direction of these reactions [176-180]. In biological systems the duration of electron transfer varies from picoseconds to milliseconds; the rate depends on the state of the active site, protein globule and its (micro)environment and also on temperature [120, 148, 155, 177]. Heme proteins possess a number of highly specific functions that are determined by the presence of the porphyrin ring in these proteins. Due to the presence of the porphyrin ring possessing a system of coupled bonds and mobile delocalized π-electrons these proteins can easily donate and accept electrons and therefore they are effective components of redox reactions [147, 178-182]. Thus, the ability of heme proteins to reduce nitrite ions to NO is determined by the presence of delocalized π-electrons in the porphyrin ring. Besides the porphyrin ring, bivalent iron

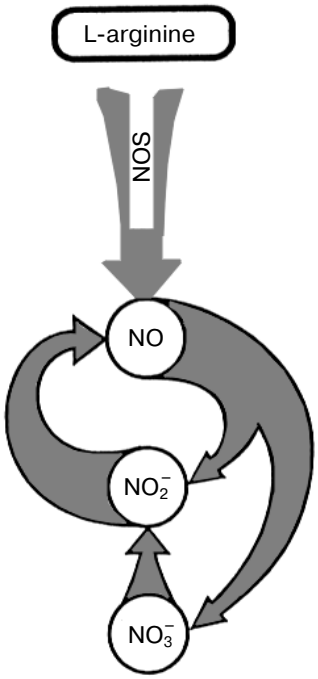

Fig. 7. Simplified scheme of nitric oxide cycle.

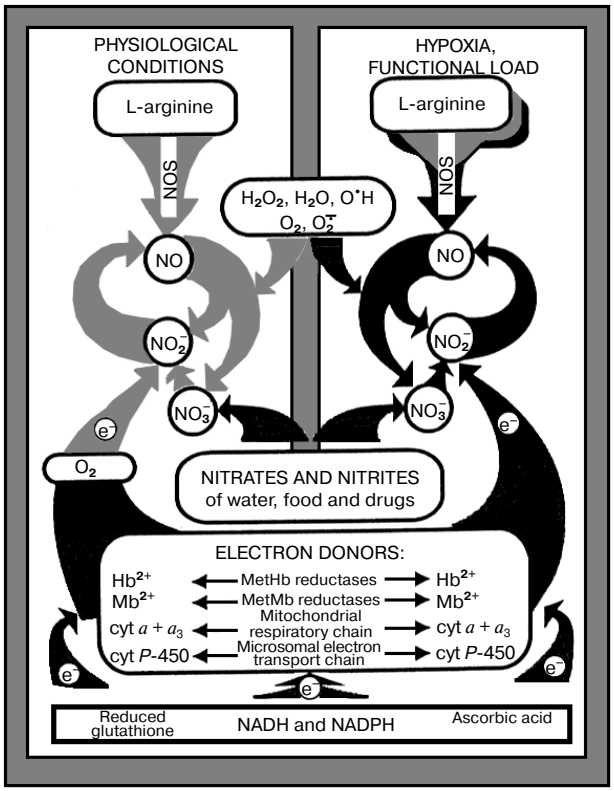

Fig. 8. Nitric oxide cycle in mammals.

Table 3. Redox potentials (E'_0) of some chemical compounds and biochemical components [20, 182, 183, 176, 188]

Redox couple	E'_0, V
$H^+/{}^1/_2\,H_2$	−0.42
NAD^+ /(NADH + H^+)	−0.32
$NADP^+$ /(NADPH + H^+)	−0.32
Cytochrome *P*-450/cytochrome *P*-420	−(0.400-0.200)
MetMb/Mb	0.05
Cytochrome (Fe^{3+}) *b*/cytochrome (Fe^{2+}) *b*	0.05
$CoQ/CoQH_2$	0.05
Dehydroascorbate/ascorbate	0.08
MetHb/Hb	0.17
Cytochrome c_0/cytochrome c_B	0.25
Cytochrome (Fe^{3+}) *a*/cytochrome (Fe^{2+}) *a*	0.29
NO_2^-/NO	(0.37-0.38)
NO_3^-/NO_2^-	0.42
Ferricyanide/ferrocyanide	0.43
Fe^{3+}/Fe^{2+}	0.77
H_2O_2/H_2O	0.82

ions and the redox potentials of these proteins are also important preconditions for this function.

As a rule, electron transfer in electron transport proteins occurs from more electronegative to less electronegative components of the chain [183, 184]. Thus, the redox potential values of components of electron transport processes are also crucial for this function. Table 3 shows redox potentials of some chemical compounds and biochemical components directly related to the respiratory chains and nitrite reduction as well. These data indicate that redox potential of the NO_2^-/NO couple is 0.37-0.38 V. This potential is more positive (or less negative) than redox potentials of Hb/metHb, Mb/metMb, cytochrome *a* (Fe^{2+}/Fe^{3+}), and cytochrome *P*-450 (Fe^{2+}/Fe^{3+}) and therefore redox potential values seem to support the possibility of electron transfer from Fe^{2+} to NO_2^-.

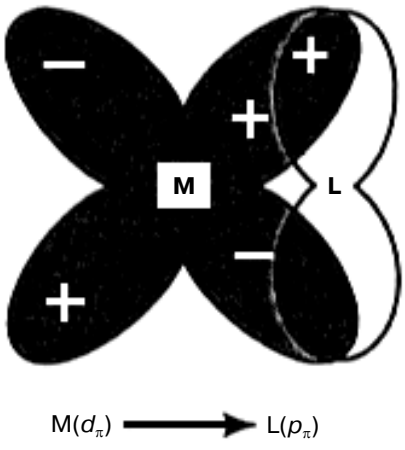

Fig. 9. Scheme of orbital overlapping showing π-bond between such ligands as NO_2^-, NO, CO, CN^- and metals (M) (which are the last in the row of transition valence elements).

However, it should be noted that redox potentials of heme proteins vary over quite a wide range (Table 3). The reason for this is in the nature of axial ligands at the fifth (or fifth and sixth) coordination positions. Different functional groups of the proteins may also contribute to these differences [177-185]. However, due to their physicochemical nature almost all Fe^{2+}-porphyrins are readily oxidized to Fe^{3+}-porphyrins [181, 185]. How does electron transfer from Fe^{2+} to oxygen or any other electron acceptor (e.g., NO_2^-) occur? Before answering this question, we should consider links between the heme protein and ligand.

How do ligands bind to heme proteins? This is an important question because bond formation between ligand and heme protein usually precedes electron transfer from Fe^{2+} to ligand. In fact, the possibility of electron transfer from heme protein to ligand requires close orientation of reactants. Only in this case effective overlapping of orbitals of protein metal and ligand followed by electron transfer to metal becomes possible [178, 181, 185].

Such ligands as CO, CN^-, and NO_2^- have free π* anti-bonding molecular orbitals possessing low energy. The interaction of vacant low energy π* anti-bonding orbitals of ligands with filled $d_{(\pi)}$-orbitals of the metal is known to shift electron charge of the metal to this ligand; this is accompanied by lowering of energy of this system [178-181].

The formation of coordination bond of heme proteins with such ligands as O_2, NO, CO, NO_2^- and some others is possible due to overlapping of a 3*d* orbital of iron with π-orbitals of ligands (Fig. 9). These bonds are designated as $(M \rightarrow L)_\pi$ or $M(d_\pi) \rightarrow L(p_\pi)$. This indicates partial transition of external $3d_\pi$ electron of Fe^{2+} on the vacant p_π orbital [178].

It should be noted that π-orbitals may be filled or vacant. In the latter case vacant π-orbitals may be involved in bond formation with *d*-orbitals of Fe^{2+} of T_{2g} symmetry (i.e., with d_{xy}-, d_{xz}-, and d_{yz}-orbitals) [178-180]. π-Orbitals may be bonding and anti-bonding orbitals. How do they appear?

The initial energy state of separate atoms may be degenerate. However, atom linking followed by molecule formation influences this energy state which "separates" into two new states with lower and higher energies. In terms of theory of molecular orbital (MO) the orbital with lower energy is called the bonding orbital whereas the orbital with higher energy is called the anti-bonding orbital [178, 181, 185]. Here we consider reaction of electron transfer from 3*d*-orbital of iron to vacant π* anti-bonding orbital of NO_2^- using MO theory.

How does the reaction of electron transfer from external heme iron 3*d*-orbital to ligand π-orbital occur? In accordance with the Frank–Condon principle, rapid electron transfer between two reacting systems requires close geometry of reacting complexes and reaction products [178, 182, 183]. In this case complex transition into corresponding oscillatory excitation state does not require much energy, otherwise significant additional energy contribution to activation energy would be needed. Electron transfer from external heme iron 3*d*-orbital to vacant π bonding or π* anti-bonding orbital results in complex formation with partial electron transfer or irreversible ligand reduction is also possible. The degree of ligand reduction depends on the degree of dative 3*d* electron transfer. It should be noted that in some cases gentle equilibrium between partial electron transfer from heme iron to ligand and irreversible ligand reduction exists. This is typical for oxygenated hemoglobin complexes, where partial electron transfer from heme iron on oxygen occurs [181, 185]. A distal histidine residue protects hemoglobin against autooxidation. Oxygenated Hb and Mb are more resistant to oxidation (e.g., by NO_2^- ions) [181-185]. The latter may be explained by localization of O_2 molecule in the heme pocket that is closed from the external side by the distal histidine residue, which blocks effective interaction of heme iron 3*d* electron with other ligands. Thus, oxygen may be considered as inhibitor of Hb nitrite reductase capacity [126]. During the interaction of Hb, Mb, cytochrome oxidase, and cytochrome *P*-450 with NO_2^- in the absence of oxygen, electron transfer from heme iron on ligands results in Fe^{2+} oxidation to Fe^{3+} and irreversible reduction of NO_2^- to NO. Reduction of NO_2^- ion occurs via filling of π* levels of the NO_2^- molecule. This leads to charge shift from metal to ligand, to a decrease of complex energy, and to general destabilization of the complex which disappears only after NO_2^- reduction to NO followed by subsequent release from the complex. Since the geometry of π* anti-bonding orbitals of NO_2^- is optimal for the interaction with heme iron 3*d*-orbitals, there are no limitations for redox reaction with this ligand [178, 181-185].

Role of enzymatic and nonenzymatic systems involved in electron transfer on heme proteins. Since only reduced forms of heme proteins can reduce NO_2^- to NO, the role of enzymatic and nonenzymatic systems involved in electron transfer on heme proteins must not be ignored. These systems include metHb- and metMb-reductases and the electron transport chains of mitochondria and endoplasmic reticulum. These enzymatic systems transfer electrons from NAD(P)H to heme proteins via a flavoprotein. Ascorbic acid and reduced glutathione can also be involved in the reduction of heme proteins [20].

When the property of deoxy forms of heme proteins to catalyze NO_2^- reduction to NO had been recognized it became clear that oxygen deficit may represent primary metabolic signal initiating subsequent changes in the activity of enzymes involved in NO_2^- reduction. Consequently, *almost all pathological processes characterized by hypoxia and/or ischemia will activate the nitrite reductase component of the NO cycle* [129]. The same component closes the chain of metabolic conversions in the NO cycle (Fig. 7). However, scientists still ignore this component. Considering NOS and nitrite reductase systems involved in NO formation from various sources (L-arginine and NO_2^-, respectively) we may find some structure–functional similarity between them.

What is in common between NOS and nitrite reductase systems? There is quite huge diversity among biochemical compounds and structures of the metabolic network. Besides involvement in NO synthesis from different sources, NOS and nitrite reductase systems share some structural similarity. What are the main characteristic features of these systems underlying their functions? Comparison of NOS and nitrite reductase systems with electron transport chains of bacteria, mitochondria, or endoplasmic reticulum shows a common plan of their structural organization. In fact, NAD(P)H may be electron donors for NOS and nitrite reductase reactions. The prosthetic groups of flavoproteins, FMN or FAD, are intermediate components involved in electron transfer from NAD(P)H. Flavoproteins act as reductases of such heme proteins as cytochrome *P*-450, cytochrome oxidase, hemoglobin, or myoglobin. Thus, it is possible that evolution of NOS and nitrite reductase systems occurred on the basis of simple appropriate processes which included the "sectional" principle of organization of biochemical structures [20, 127, 131].

Some authors believe that it is typical for nature to "build" numerous biological systems using the same selection of reliable elements and structures [117, 176, 186-188]. Considering electron transport chains, E. Racker indicated that nature maintained itself by using original variations of the same basis for provision of energy currency and by using this basis for realization of various specialized functions. This new unity in biochemistry stretches over the horizontal level of studies of the living world and also the vertical levels of studies of various biological functions. In other words, according to concept of biochemical unity all enzymes with the same functions were created by using like structure(s). However, some enzymes were created more alike than others [189]. A similar viewpoint was expressed by V. P. Skulachev: "Energetics is a backbone of metabolism: its brake by an inhibitor inevitably causes metabolic paralyses. Why is it not possible to use the type of energetics as the evolutionary sign like the structure of the backbone? It would be possible, if energetic systems were not invented so long ago that they became a universal attribute of cells" [190]. Thus, "universal attributes of the cell" were used by nature for realization of various specialized functions including NOS and nitrite reductase systems, involved into nitric oxide formation. However, the histo-

ry of science demonstrates that scientists usually separated (and separate now) only one function of the studied object. Only recently a tendency of cognition of biological systems from monofunctional to polyfunctional perception appeared [191]. Moreover, polyfunctionality is a universal characteristic of not only of living but also of inanimate systems. In other words, the principle of polyfunctionality implies that objects of living and inanimate nature possess multiple functions in the whole body of nature [191]. The principle of polyfunctionality helps to understand how electron transport chains became "universal attributes of the cell". At the same time, we should keep in mind words by L. J. Piter, who said that principle indicates tendency or general direction. In this connection, we should be resistant to illusions of apparent exceptions.

GENERAL CHARACTERISTICS OF NOS AND NITRITE REDUCTASE SYSTEMS

Analysis of NOS and nitrite reductase systems using principles of symmetry. Working with certain NOS and nitrite reductase systems scientists may usually analyze physicochemical properties of these enzymes. Sometimes they may answer a question on the biological importance of a certain phenomenon. More rarely it is possibly to carry out fundamental analysis of the problem and answer four questions. What is the research object? How does it function? Why does in function this way? What did Nature create this object for? According to M. Plank's viewpoint "the most attractive task in nature consists in finding that absolute which gives to the relative its actual meaning. We may proceed from the relative. All our intentions have relative character". The thing is that we should try "to find in all data that the absolute, common, invariant which was put into them" (cited by [192]).

These words contain program for studies of any particulate problem. Discussing the common and the invariant that was put into NOS and nitrite reductase system we cannot ignore general theory of systems. From the theory of systems the objects that consist of naturally determined similar parts are symmetrical [97, 193-195]. This definition of symmetry assumes a possibility of the absence of mirror symmetry in objects that are related to symmetric ones; this definition also allows finding among multitude of objects some sub-multitude possessing similarity of its constituents [97, 194]. In the case of NOS and nitrite reductase systems initial and terminal conditions are determined by electron donor (NADPH or NADH) and electron acceptor (O_2 or NO_2^-), respectively. The electron pathway from NAD(P)H to the terminal acceptor may vary within certain limits provided that this pathway includes flavoprotein and heme protein. Thus, besides isomorphism of NOS and nitrite reductase systems it is possible to find polymorphism of these systems. The latter may be illustrated by the fact that NOS of types I, II, and III are products of various genes.

Many authors agree that the study of molecular logic of living cells is a very interesting process. Each principle of organization of living matter, each decision made by Nature and preserved over evolution is usually brilliantly simple. And the major task is to find short and exhaustive laws in huge mountains of (often unrelated) facts. General theory of systems may represent a useful tool for finding such hidden laws. Consequently, artificial at first glance analysis of NOS and nitrite reductase systems by means of general theory of systems may open new possibilities for scientists. Using principles of symmetry, they may find among many electron transport chains that general property, which was called an invariant in physics [97]. In the context of our problem we may discuss *invariance* of electron transport chains capable of carrying our NOS or nitrite reductase systems. This in turn allows the demonstration that in spite of some structural differences, NOS and nitrite reductase systems may be elements of a wider system of electron transport chains of bacteria, plants, and animals. Let us consider invariance notions in more detail.

Problems which we are going to discuss are related to one of the most fundamental properties of Nature: the relationship between the law of conservation and principles of symmetry. These remarkable features were found by physicists. They provided evidence for the existence of a natural link between properties of space and time and so-called "laws of conservation" (the law of conservation of energy or the law of conservation of inertia) [97, 193, 196]. Symmetry of the laws of nature is related not only to physical but also to biological phenomena. Thus, it represents a universal feature of Nature.

Demonstration of empirical facts of conservation and repetition of certain characteristics in biological objects on various structure–functional levels led to understanding of the need to investigate stability as one of the basic problems of cognition of life. Determination of invariant characteristics or relations became one of the most important tools for studies of the integrity of biological objects. The second half of the XXth century was characterized by the development of a research direction that was focused on investigation of stable characteristics of the object under study. Molecular biology (after physics) approached the discovery of invariant characteristics, which are conserved during any changes. The notions of invariant relation, invariant characteristics are widely used in structure–functional analysis of biological systems. In a wide sense, invariance means something opposite to unlimited diversity of natural phenomena [97, 194, 195].

Discovery of invariance in biological objects to limit diversity and therefore to approach understanding of reasons underlying some phenomenon or class of phenomena [193, 194]. Studies of biological structures in dynamic

and evolutionary aspects promoted discovery of some monotype laws, invariant biological principles (similar to that of principle of conservation in physics) such as *principle of inhibition*, *principle of dominants*, *principle of negative feedback*. Biochemical universality of the sequence of electron transport chains pyridine nucleotides (PN) → flavoproteins (FP) → heme proteins (HP) may be better understood as the determining principle of organization of molecular structures or as the principle of conservation of invariant relations for organisms adapted to nitrate-nitrite or oxygen respiration. Now we may say that NOS and nitrite reductase systems are organized on the *principle of electron transport chain (ETC) organization*. Since ETC principle is a basis for numerous biochemical systems (mitochondrial respiratory chain, hydroxylating chains of endoplasmic reticulum, metHb and metMb reductase systems, plant leghemoglobin, NOS systems, nitrification and denitrification systems, photosynthetic systems, etc.) we believe that this widespread principle may become one of basic principles of conservation of structure–functional units in biology. The complex PN → FP → HP may already be considered as one of the optimal biochemical standards in evolution of ETC.

After this consideration, a natural question arises: why such diversity of ETC appeared during evolution? Evolution proceeded on the basis and due to mutations and changes in structure organization. Now it is known that certain correlation exists between structure variability and position of the organism in systematics, its phylogeny and evolution. For example, microbial ETC are characterized by high variability, whereas ETC of higher animals have low variability. How does this correspond to optimal biochemical standard? Optimal biochemical standard in the organization of NOS and nitrite reductase systems implies the existence of common principle of organization of these systems rather than their identity; this principle permits certain (according to Anokhin) useful adaptive result [192, 197]. In the case of NOS and nitrite reductase systems, NO formation is this result. However, NO synthesis under hypoxia and normal physiological supply with oxygen occurs via different mechanisms. These mechanisms are components of an integrative system that provides cyclic reproduction of this highly reactive compound.

NITROGEN AND NITRIC OXIDE CYCLES: SIMILARITY AND DIFFERENCES

Some components typical for the nitrogen cycle can also be found in the NO cycle. *Nitrification and denitrification* components of the nitrogen cycle are present in the NO cycle as NOS and nitrite reductase components [20]. The nitrogen-fixing component of the nitrogen cycle is present only in nitrogen-fixing organisms. The NO cycle lacks this component.

It should be noted that in spite of close similarity of nitrification processes with NOS and nitrite reductase processes they are not entirely equivalent. Almost all of the biosphere is involved in the nitrogen cycle, whereas the NO cycle existing in an organism involves only deoxy forms of heme proteins and the ETC (including NAD(P)H and flavoproteins). Nevertheless, principles of organization of biochemical systems involved in the nitrogen and NO cycles are the same. This suggests that metabolism of higher plants and animals (e.g., mammals) follows phylogenetically ancient mechanisms. Here we may find manifestation of Orbeli's principle: an old function does not disappear—it is adapted to new conditions [125]. The discovery of structurally and functionally similar cyclic biochemical chains in the organization of higher animals cells which are similar to those present in microorganisms, plants, and fungi not only helped to understand mechanisms of intracellular regulatory system functioning but also provided background for theoretical analysis.

The discovery of internal symmetry in such objects as the NOS and nitrite reductase systems promotes integral perception of these systems, their unity with such processes as nitrification and denitrification, which are components of the nitrogen cycle. This also allows recognizing the unity of all these systems with electron transport and energy transforming systems of cells. The latter in turn reveals internal symmetry between separate components of the NO and nitrogen cycles. The nitrogen cycle is traditionally considered as a general biological phenomenon of the whole the biosphere. Using nitrification and denitrification and nitrogen fixation, the biosphere developed a mechanism of nitrogen regeneration during evolution. Due to this mechanism, a possibility for "economical" nitrogen utilization in metabolic processes appeared in the biosphere. The NO cycle may be considered as a mechanism of NO regeneration allowing to maintain steady state NO concentration at nanomolar level and to participate effectively in intra and intercellular signaling.

However, the principle of symmetry does not imply the presence of the whole set of enzymes required for catalysis of similar conversions in all functionally similar metabolites of these cycles. The principle of symmetry permits exclusion elimination of some compounds and reactions from the metabolic network. Moreover, elimination of some elements of the metabolic network during evolution is considered as a logical phenomenon, which is related to asymmetry elements [97, 193-195]. So, the absence of nitrogen-fixing enzymatic systems in the NO cycle does not mean that the principles of symmetry are not applicable to this particular case.

It should be noted that it is possible to find links between unrelated (at first glance) biological phenomena. Below we shall try to consider a holographic principle and the reflection of the nitrogen cycle (nitrogen turnover in

nature) as an integral functional system that is typical for the whole biosphere, and to find features of this system in the NO cycle.

NITROGEN AND NO CYCLES AND THE HOLOGRAPHIC PRINCIPLE

It is known that in functional systems properties of each element of this system reflect activity of the whole functioning system. Such principle of organization of functional systems has been called the holographic principle [198, 199]. Universality of this principle was found during analysis of many objects. Comparing NO cycles in mammals and nitrogen cycles in the biosphere, we already found many similarities in structure and functions of the enzymatic systems catalyzing reactions of these cycles. These enzymatic systems share high similarity with mitochondrial and endoplasmic reticulum ETC.

Nitrogen and NO cycle also share one common property that is fixed in their titles: they are organized as cycles. It is especially surprising that elements constituting these cycles may not contact each other (for formation of united structure). Nevertheless, well-balanced fluxes of biosynthesis and decay of substances in the biosphere is very accurate. It is especially surprising if we take into consideration well known (especially for chemists) difficulties in regulation of chemical reactions. In some cases it is nearly impossible to stop the processes (of burning and explosions, which actively involve free radicals), in other cases it is nearly impossible to start processes. Some chemical reactions spontaneously initiate numerous unpredicted branches with formation of a huge number of side products.

Moreover, there are some reasons to believe that fluxes of biosynthesis and decomposition of substances also form a system of biogeochemical cycles. The biogeochemical cycle represents circulation of chemical elements (turnover of substances in nature), which get into organisms of the environment and from organisms into the environment. Cycles of nitrogen, carbon, sulfur, phosphorus, and water are the main biogeochemical cycles. M. M. Kamshilov [200] wrote that due to ability to self-reproduction the living that adapts to new conditions leaves behind exclusive cycle limits. However, this does not result in "demolition" of cyclic structure of life but extends biotic turnover. Circle is transformed into helix. This increases organization of life, information resources and opens possibilities for accumulation of additional information. Life "learns" to use material resources of the inorganic environment in an optimal way. This is the progress of life [200]. Considering cyclic conversions of substances in nature, we should briefly discuss biotic turnover including the interaction of organisms forming and decaying organic substance [200]. According to the modern conception, biotic turnover within community of organisms belonging to different species and living in certain environment represents a basis for long-term existence of biogeocenosis. The latter consists of the living part (biocenosis or population of organisms belonging to different species) and inanimate part, mineral elements. Biocenosis involvement into biotic cycles provides existence of effective system of biogeochemical cycles of self-reproduction [201]. Thus, cyclic recurrence, which was put into chemical compounds, is recognized at various structure–functional levels from molecular to biogeochemical and biotic cycles.

BIOTIC CYCLES AND THE PROBLEM OF BIOLOGICAL EQUILIBRIUM

Our concept is related to one important problem of natural science, the problem of biological equilibrium and conditions impairing this equilibrium. A. F. Hause formulated the principle now known as the Hause principle: "stability of community is higher the greater the number of constituting species is" [98]. Russian mathematicians A. A. Lyapunov (1963) and I. A. Poletaev (1966) came to the same conclusion. They provided the mathematical basis for the Hause principle and slightly modified its interpretation: the simpler the composition of the community the lesser the stable the community is and vice versa [202]. In N. V. Timofeev-Ressovsky's conception this conclusion can be understood without complex mathematical formulas and evolutionary adaptation of organisms to each other is characterized by appearance of some "extreme resources". These extreme resources maintain equilibrium of both biocenoses and heterogenic populations [202]. This theoretical problem received further development during studies of biocenoses, using conditions of experimental ecosystems. These ecosystems were widely used from the end of 1960s and the beginning of 1970s in space biology and medicine during preparation of cosmonauts for long-term flights. The experiments revealed that the atmosphere of a closed ecosystem is characterized by cyclic interconversions of substances, which result in dynamic equilibrium of concentrations of carbon and nitric oxides, ammonia, hydrogen sulfide, etc. The absence of cyclic conversions of substances would result in accumulation of "dead-end" metabolites (which would not be involved in subsequent conversions). This is inconsistent with life. These experiments revealed the main properties of multi-species microbial biocenoses such as high degree of metabolite coupling with each other and with human beings (the main representative of the system), their biochemical polyfunctionality, plasticity, and high resistance. It was also found that microscopic flora playing a specific medium-forming role (without which circulation of substances in this system would not be possible) was in dynamic equilibrium with accompanying microscopic flora. The latter is not essential for the

existence of the system, but it is always present in it. Moreover, this accompanying microflora is an important indicator of state of various components of this system. Such a system is under condition of stable equilibrium until medium forming microscopic flora curries out its specific (medium forming) functions and reproduces itself and accompanying microscopic flora does not exceed its limits. If the latter occurs, accompanying microscopic flora becomes a dangerous antagonist for other members of the biocenosis or becomes a dangerous competitor for functional microscopic flora [203].

These data help to understand holographic interrelationship between metabolic processes in living organisms (NO cycle) and in the biosphere (nitrogen cycle). Results of these experiments also demonstrated that closed circulation of substances, which was biologically between two types of microscopic flora exists in functional interrelationships, which are similar to those found in the circuit of nitrogenous compounds, involving NOS and nitrite reductase component.

In the beginning of this paper, we compared the carbon–nitrogen cycle in the interior of a star and the nitrogen cycle in the biosphere. They are completely different. The only similarity consists in the cyclic organization of both processes. The latter raises many more questions than answers. However, we do believe that the use of the holographic principle can give us the keys to the solution of problem of cyclic organization in interiors of the star, in the biosphere, and in living organisms. According to this principle, the properties of each element (including chemical elements) of functional systems reflect the activity of the whole functioning system in general. Thus, the existence of the Bethe carbon–nitrogen cycle in the interior of stars and nitrogen and nitric oxide cycles in the biosphere and the living organisms, respectively, are reflection of the holographic principle. Applicability of this principle to these systems suggests that systems themselves are the functional systems (i.e., interrelated, interdependent, self-organized, and self-regulated) [197-199]. It is possible that the evolution of vital activity and the development of metabolic processes and physiological functions were closely linked to space, to properties of chemical elements, and evolution of the Earth as a planet of the solar system.

The cyclic organization of products of NO metabolism (which may be converted into NO again in mammals) represents the basis of our concept. In our viewpoint, the concept of the NO cycle allows understanding of negative feedback mechanisms providing effective control of content of this compound and product(s) of its metabolism. The existence of cyclic links between certain metabolites promotes an increase in concentrations of products, which may be regenerated again. This increases the rate of their cycling and this cycling does not cause toxic effect on cells due to possible accumulation of certain products [20, 105, 204].

It should be noted that problem of cyclic recurrence (cyclicity) is not limited to the NO problem. This phenomenon is also typical for Mendeleev's periodical table of chemical elements, where each period may be considered as one of the cycles. The cyclic principle represents a basis for turnover of substances (including water, carbon and nitrogen atoms) [20, 120]. The latter means that four main chemical elements (H, C, N, and O) representing more than 95% (by mass) of all chemical elements in the living organisms are also involved into this circuit. Consequently, these basic (for life) chemical elements possess cyclic organization. The latter is also typical for radiochemical nuclear reactions involving five atoms (carbon, nitrogen, helium, hydrogen, and oxygen) that were initially considered by Bethe and Weitzeker and were called the Bethe carbon–nitrogen cycle. Interestingly, four elements of the Bethe cycle are also the main chemical elements representing the basis of life. Consequently, at different structure–functional levels in the living organisms, in the biosphere, and the star substance it is possible to find cyclic organization. So, we propose the existence of a common principle which we called *the cyclicity principle* [129]. At the same time, we do not want to simplify the specificity of each event at each structure–functional level. Nevertheless, analyzing the NO problem we have come to very simple cyclic structures that may be very important because they are related not only to living and inanimate objects but also to the whole world. Consequently, it is necessary to emphasize the universal and, possibly, global cycling, as a new cyclic principle. Attractiveness of this principle consists in its applicability to new research objects. This will allow recognizing cyclic structure–functional organization in the living systems and to find new regulatory systems with positive and negative feedback.

It was already stated above that if properties of each element of functioning system reflect activity of the whole functioning system, such principle of functional system organization is called the holographic principle. Taking into consideration two principles, cyclic principle and holographic principle, it is possible to understand the interrelationship between cycles at various structure–functional levels in living organisms, the biosphere, and in star substance. We consider this problem in more details in subsequent papers. Nevertheless, here we may stress that analysis of the interrelationship between these cycles allows to propose a new hypothesis. We do believe that *the existence of cyclic interrelationship at various structure–functional levels reflects the fact that evolution of living organisms, processes of their vital activity, and the development of physiological functions and metabolism were closely linked to space and evolution of the Earth as a planet of the solar system*. We do believe that this hypothesis may explain the effect of numerous cycles (including circadian cycles, caused by the Earth's revolution with respect to the Sun; Moon cycles, caused by the Moon's

revolution round the Earth; annual cycles, which depend on the Earth's revolution round the Sun, and 11-12 year cycles of the Sun's activity) on living organisms. Thus, the study and analysis of properties of one of the smallest regulatory molecule of the living organisms, NO, allowed us to substantiate the concept of nitric oxide cycle and to propose the existence of the common principle reflecting the existence of connections at various structure–functional levels, which we call the cyclicity principle.

This work was partially supported by the Russian Foundation for Basic Research and Belorussian-Russian Foundation for Basic Research (grants No. 98-04-48679, 00-04-48412, and 00-04-81096 Bel 2000a).

REFERENCES

1. Furchgott, R. F., and Zawadzki, I. V. (1980) *Nature*, **288**, 373-376.
2. Ignarro, L. J., Lippton, H., Edwards, J. C., Baricos, W. H., Hyman, A. L., Kadowitz, P. J., and Gruetter, C. A. (1981) *J. Pharmacol. Exp. Ther.*, **218**, 739-749.
3. Ignarro, L. J. (1990) *Annu. Rev. Pharmacol. Toxicol.*, **30**, 535-560.
4. Ignarro, L. J., Buga, G. M., Wood, K. S., Byrus, R. E., and Chandhuri, G. (1987) *Proc. Natl. Acad. Sci. USA*, **84**, 9265-9269.
5. Ignarro, L. J. (1990) *Eur. J. Pharmacol.*, **183**, 1624-1625.
6. Murad, F., and Kimura, H. (1975) *Life Sci.*, **17**, 837-845.
7. Palmer, R. M. J., Ferrige, A. G., and Moncada, S. (1987) *Nature*, **327**, 524-526.
8. Moncada, S., Palmer, R. M. J., and Higgs, E. A. (1991) *Pharmacol. Rev.*, **43**,109-142.
9. Aimasheva, N. P., Manukhina, E. B., Pshennikova, M. G., Vanin, A. F., and Malyshev, I. Yu. (1998) *Byul. Eksp. Biol. Med.*, **125**, 381-384.
10. Andreeva, N. A., Shumatova, T. A., and Motavkin, P. A. (1999) *Byul. Eksp. Biol. Med.*, **128**, 238-240.
11. Bashkatova, V. G., Vytskova, G. Yu., Narkevich, V. B., Vanin, A. F., Mikoyan, V. D., Kubrina, L. N., and Raevsky, K. S. (1996) *Neurochemistry (Moscow)*, **13**, 110-115.
12. Bashkatova, V. G., Mikoyan, V. D., Kosacheva, E. S., Kubrina, L. N., Vanin, A. F., and Raevsky, K. S. (1996) *Dokl. Ros. Akad. Nauk*, **348**, 119-121.
13. Bashkatova, V. G., and Raevsky, K. S. (1998) *Biochemistry (Moscow)*, **63**, 866-873.
14. Boldyrev, A. A., and Kukley, M. L. (1996) *Neurochemistry (Moscow)*, **13**, 271-278.
15. Borisenko, G. G., Osipov, A. N., Kazarinov, K. D., and Vladimirov, Yu. A. (1997) *Biochemistry (Moscow)*, **62**, 659-664.
16. Proskuryakov, S. Ya., Konoplyannikov, A. G., Ivannikov, A. I., and Skvortsov, V. G. (1999) *Usp. Sovr. Biol.*, **119**, 380-395.
17. Raevsky, K. S. (1997) *Byul. Eksp. Biol. Med.*, **5**, 484-490.
18. Raevsky, K. S., Bashkatova B. G., and Vanin, A. F. (2000) *Vestnik RAMN*, **4**, 11-15.
19. Reutov, V. P., and Orlov, S. N. (1993) *Fiziol. Cheloveka*, **19**, 124-137.
20. Reutov, V. P., Sorokina, E. G., Okhotin, V. E., and Kositsyn, N. S. (1998) *Cyclic Conversion of Nitric Oxide in Mammals* [in Russian], Nauka, Moscow.
21. Severina, I. S. (1998) *Biochemistry (Moscow)*, **63**, 794-801.
22. Turpaev, K. T. (1998) *Mol. Biol. (Moscow)*, **32**, 1-11.
23. Sorokina, E. G., Reutov, V. P., Pinelis, V. G., and Korshunova, T. S. (1994) *Usp. Fiziol. Nauk*, **25**, 70-71.
24. Barbul, A. (1990) in *Nitric Oxide from L-Arginine: a Bioregulatory System* (Moncada, S., and Higgs, E. A., eds.) Excerpta Medica, Amsterdam, pp. 317-329.
25. Bredt, D. S., Hwang, P. M., Glatt, C. E., Lowenstein, C., Reed, R. R., and Snyder, S. H. (1991) *Nature*, **351**, 714-718.
26. Busse, R., Fleming, I., and Schini, V. B. (1995) in *The Role of Nitric Oxide in Physiology and Pathophysiology* (Koprowski, H., and Maeda, H., eds.) Springer-Verlag, Berlin-Heidelberg, pp. 37-50.
27. Stoclet, J.-C., Muller, B., Andriantsitohaina, R., and Kleschyov, A. (1998) *Biochemistry (Moscow)*, **63**, 826-832.
28. Malyshev, I. Yu., and Manukhina, E. B. (1998) *Biochemistry (Moscow)*, **63**, 840-853.
29. Manukhina, E. B., Azamatov, Z. Z., and Malyshev, I. Yu. (1996) *Byul. Eksp. Biol. Med.*, **122**, 148-151.
30. Manukhina, E. B., Malyshev, I. Yu., and Arkhipenko, Yu. V. (2000) *Vestnik RAMN*, **4**, 16-21.
31. Moncada, S. (1994) *J. Hypertens.*, **12** (Suppl. 10), S35-S39.
32. Moncada, S., and Higgs, E. A. (1995) *FASEB J.*, **9**, 1319-1330.
33. Strukova, S. M., Chistov, I. V., Umarova, B. A., Dugina, T. N., Storozhevykh, T. P., Pinelis, V. G., and Glusa, E. (1999) *Biochemistry (Moscow)*, **64**, 658-664.
34. Severina, I. S., Bussygina, O. G., and Pyatakova, N. V. (2000) *Vestnik RAMN*, **4**, 25-30.
35. Zenkov, N. K., Men'shikova, E. B., and Reutov, V. P. (2000) *Vestnik RAMN*, **4**, 30-34.
36. Men'shikova, E. B., Zenkov, N. K., and Reutov, V. P. (2000) *Biochemistry (Moscow)*, **65**, 409-426.
37. Shebeko, V. I. (1999) *Endothelium and Complement System* [in Russian], VSMU Press, Vitebsk.
38. Shebeko, V. I., and Rodionov, Yu. Ya. (1993) *Byul. Eksp. Biol. Med.*, **11**, 479-480.
39. Vanin, A. F. (1998) *Biochemistry (Moscow)*, **63**, 782-793.
40. Vanin, A. F. (1995) *Biochemistry (Moscow)*, **60**, 225-230.
41. Vanin, A. F. (1998) *Biochemistry (Moscow)*, **63**, 731-733.
42. Dvorestky, D. P., Yartsev, V. N., Karachentseva, O. V., and Granstrem, M. P. (1998) *Ros. Fiziol. Zh. im. I. M. Sechenova*, **84**, 30-38.
43. Vasil'eva, T. V., Michurina, T. V., Radionova, Yu. V., Kuzin, B. A., Enikolopov, G. N., and Khrushchov, N. G. (1997) *Ontogenes*, **28**, 458-462.
44. Viktorov, I. V. (2000) *Vestnik RAMN*, **4**, 5-10.
45. Vlasov, T. D. (1998) *Ros. Fiziol. Zh. im. I. M. Sechenova*, **85**, 379-382.
46. Volgin, D. V., Marchenko, V. A., and Seredenko, M. M. (1997) *Neirofiziologiya*, **29**, 420-430.
47. Granik, V. G., Ryabova, S. Yu., and Grigoriev, N. B. (1997) *Usp. Khim.*, **66**, 792-807.
48. Moibenko, A. A., Pavlyuchenko, V. B., Datsenko, V. V., Yuz'kov, M. Yu., and Azarov, V. I. (2000) *Fiziol. Zh. (Kiev)*, **46**, 19-32.
49. Motavkin, P. A., and Geltser, B. I. (1998) *Clinical and Experimental Pathophysiology of Lungs* [in Russian], Nauka, Moscow.

50. Nevzorova, V. A., Eliseeva, E. V., Zuga, M. V., Protopopova, M. Yu., and Geltser, B. I. (1998) *Ter. Arkh.*, **3**, 13-18.
51. Nedospasov, A. A. (1998) *Biochemistry (Moscow)*, **63**, 744-765.
52. Gurin, A. V. (1995) *The Role of Nitric Oxide in Central Mechanisms of Thermoregulation*: Abstract of Candidate's dissertation [in Russian], Anokhin Institute of Normal Physiology, Moscow.
53. Gurin, A. V. (1998) in *The Role of Nitric Oxide in Vital Activity* [in Russian], Polibig, Minsk, pp. 27-31.
54. Gurin, V. N. (1998) in *The Role of Nitric Oxide in Vital Activity* [in Russian], Polibig, Minsk, pp. 32-35.
55. D'yakonova, T. L., and Reutov, V. P. (1994) *Vopr. Med. Khim.*, **40**, 27-30.
56. D'yakonova, T. L., and Reutov, V. P. (1998) *Ros. Fiziol. Zh. im. I. M. Sechenova*, **84**, 1264-1272.
57. Dunai, V. I. (1998) in *The Role of Nitric Oxide in Vital Activity* [in Russian], Polibig, Minsk, pp. 39-40.
58. Duizen, I. V., Kalinichenko, S. G., Okhotin, V. E., and Motavkin, P. A. (1998) *Morfologiya*, **113**, 47-51.
59. Brüne, B., Sandau, K., and von Knethen, A. (1998) *Biochemistry (Moscow)*, **63**, 817-825.
60. Pinelis, V. G., Sorokina, E. G., Reutov, V. P., Vinskaya, N. P., Isaev, N. K., and Viktorov, I. V. (1997) *Dokl. Ros. Akad. Nauk*, **352**, 259-261.
61. Samosudova, N. V., Larionona, N. P., Reutov, V. P., and Chailakhyan, L. M. (1998) *Dokl. Ros. Akad. Nauk*, **361**, 704-708.
62. Larionova, N. P., Samosudova, N. V., Reutov, V. P., and Chailakhyan, L. M. (1999) *Dokl. Ros. Akad. Nauk*, **369**, 836-840.
63. Samosudova, N. V., Reutov, V. P., and Larionova, N. P. (2000) *Tsitologiya*, **42**, 72-78.
64. Maeda, H., and Akaike, T. (1998) *Biochemistry (Moscow)*, **63**, 854-865.
65. Sakharova, A. V., and Lozhnikova, S. M. (2000) *Vestnik RAMN*, **4**, 30-34.
66. Ilnitsky, A. P., Reutov, V. P., Ryzhova, N. I., Kolpakova, A. S., Deryagina, V. P., Nekrasova, E. A., and Travkin, A. G. (1997) *Exp. Oncol.*, **19**, 101-109.
67. Zweir, J. L., Wang, P., and Kuppusamy, P. (1995*) J. Biol. Chem.*, **270**, 304-307.
68. Zweir, J. L., Wang, P., Samouilov, A., and Kuppusamy, P. (1995) *Nature Med.*, **1**, 804-809.
69. Stuehr, D. J., and Marletta, M. A. (1985) *Proc. Natl. Acad. Sci. USA*, **82**, 7738-7742.
70. Soloviev, A. I., and Stefanov, A. V. (1998) *Sovrem. Probl. Toksikol.*, **1**, 35-38.
71. Sorokina, E. G., Reutov, V. P., Pinelis, V. G., Vinskaya, N. P., Vergun, O. V., and Khodorov, B. I. (1999) *Biol. Membr. (Moscow)*, **16**, 318-323.
72. Taylor, B. S., Alarcon, L. H., and Billiar, T. R. (1998) *Biochemistry (Moscow)*, **63**, 766-781.
73. Azhipa, Ya. I., Reutov, V. P., and Kayushkin, L. P. (1990) *Fiziol. Cheloveka*, **16**, 131-149.
74. Wink, D. A., Vodovotz, Y., Cook, J. A., Krishna, M. C., Kim, S., Coffin, D., DeGraff, W., Deluca, A. M., Liebmann, J., and Mitchell, J. B. (1998) *Biochemistry (Moscow)*, **63**, 802-809.
75. Vernulet, J., and van Etten, R. (1973) in *Chemistry of Nitro- and Nitroso-groups* [Russian translation], Vol. 2, Mir, Moscow, pp. 156-220.
76. Yoshimura, T. (1996*) CVD Grant Round Series*, Vol. 3, Neuron Publishing Co., Ltd., Tokyo, pp. 1-26.
77. Yoshimura, T. (1996) *Magn. Res. Med.*, **7**, 9-13.
78. Azhipa, Ya. I., Reutov, V. P., Kayushin, L. P., and Nikishkin, E. I. (1983) *Izv. Akad. Nauk SSSR. Ser. Biol.*, **2**, 240-250.
79. Zinchuk, V. V., and Borisyuk, M. V. (1999) *Byul. Eksp. Biol. Med.*, **127**, 616-619.
80. Kalinichenko, S. G., Okhotin, V. E., and Motavkin, P. A. (1997) *Tsitologiya*, **39**, 161-165.
81. Kashtanov, S. I., Zvyagintseva, M. A., Kosharskaya, I. L., Mezentseva, L. V., and Gurin, V. N. (2000) *Vestnik RAMN*, **4**, 21-25.
82. Komissarova, L. Kh., Reutov, V. P., and Komissarov, A. L. (1994) *Vopr. Med. Khim.*, **40**, 25-26.
83. Konoplyannikov, A. G., Proskuryakov, S. Ya., Shtein, L. V., Kucherenko, A. G., Skvortsov, V. G., Ivannikov, A. I., Konoplyannikov, M. A., and Verkhovsky, Yu. G. (1999) *Byul. Eksp. Biol. Med.*, **127**, 648-650.
84. Kostyuk, E. P. (1998) *Neirofiziologiya*, **30**, 151-160.
85. Kudryavtsev, M. E., Dmitrieva, O. N., and Kuropteva, Z. V. (1996) *Izv. Ros. Akad. Nauk SSSR. Ser. Biol.*, **4**, 453-459.
86. Kultchisky, S. V., and Akulich, N. V. (2000) *Vestnik RAMN*, **4**, 41-44.
87. Lavrushenko, L. F., and Tsyganenko, O. I. (1996) *Ukr. Biokhim. Zh.*, **68**, 104-106.
88. Okhotin, V. E., and Duizenm, I. V. (1998) in *The Role of Nitric Oxide in Vital Activity* [in Russian], Polibig, Minsk, pp. 57-60.
89. Cazevieille, C., Muller, A., Meynier, F., and Bonne, C. (1993) *Free Rad. Biol. Med.*, **14**, 389-395.
90. Elofsson, R., Carlberg, M., Moroz, L. L., Nezlin, L. P., and Sakharov, D. A. (1993) *NeuroReport*, **4**, 279-282.
91. Polenov, S. A. (1998) *Ros. Zh. Gastroenterol. Gepatol. Kolonoproktol.*, **1**, 53-60.
92. Sagach, V. F., Shimanskaya, T. V., and Nadtochii, S. N. (2000) *Ukr. Fiziol. Zh.*, **46**, 33-40.
93. Soltanov, V. V., and Chumak, A. G. (1998) in *The Role of Nitric Oxide in Vital Activity* [in Russian], Polibig, Minsk, pp. 78-80.
94. Stepuro, I. I., Chaikovskaya, N. A., Solodunov, A. A., and Artsukevich, A. N. (1997) *Biochemistry (Moscow)*, **62**, 960-966.
95. Chumak, A. G., and Borisov, O. L. (1998) in *The Role of Nitric Oxide in Vital Activity* [in Russian], Polibig, Minsk, pp. 91-94.
96. Shapoval, L. N., Sagach, V. F., Pobegailo, L. S., and Doloman, L. B. (1996) *Neirofiziologiya*, **28**, 111-120.
97. Vigner, E. (1971) *Etudes on Symmetry* [Russian translation], Mir, Moscow.
98. Vorontsov, N. N. (1999) *The Development of Evolutional Ideas in Biology* [in Russian], Progress-Traditsiya, ABF, Moscow.
99. Karpinskaya, R. S. (1971) *Philosophical Problems of Molecular Biology* [in Russian], Mysl, Moscow.
100. Maidanov, A. S. (1993) *Art of Discovery: Methodology and Logics of Scientific Creative Work* [in Russian], Repro, Moscow.
101. Engelhardt, V. A. (1973) *Philosophical Problems of Biology* [in Russian], Nauka, Moscow, pp. 7-44.
102. Pulatova, M. K., Rikhireva, G. T., and Kuropteva, Z. V. (1989) *Electron Paramagnetic Resonance in Molecular Radiobiology* [in Russian], Energoatomizdat, Moscow.

103. Sharygin, V. L., Shishkina, L. N., Rozhdestvensky, L. N., Kormer, Z. S., and Pulatova, M. K. (1994) *Izv. Ros. Akad. Nauk. Ser. Biol.*, **2**, 254-270.
104. Shekhter, A. B., Kabisov, R. K., Pekshev, A. V., Kozlov, N. P., and Perov, Yu. L. (1998) *Byul. Eksp. Biol. Med.*, **126**, 210-215.
105. Reutov, V. P. (1995) *Usp. Biol. Khim.*, **35**, 189-228.
106. Ilnitsky, A. P., Reutov, V. P., Ryzhova, N. I., Kolpakova, A. S., Deryagina, V. P., Nekrasova, E. A., Savluchinskaya, L. A., and Travkin, A. G. (2000) *Vestnik RAMN*, **7**, 11-16.
107. Archer, S. (1993) *FASEB J.*, **7**, 349-360.
108. Ischiropoulos, H., Zhu, L., and Chen, J. (1992) *Arch. Biochem. Biophys.*, **298**, 431-437.
109. Janssens, S. P., Shimouchi, A., Quertemous, T., Bloch, D. B., and Bloch, K. D. (1992) *J. Biol. Chem.*, **267**, 14519-14522.
110. Maeda, H., Akaike, T., Yoshida, M., Sato, K., and Noguchi, Y. (1995) in *The Role of Nitric Oxide in Physiology and Pathophysiology* (Koprowski, H., and Maeda, H., eds.) Springer-Verlag, Berlin-Heidelberg, pp. 37-50.
111. Marletta, M. A. (1989) *Trends Biochem. Sci.*, **14**, 488-492.
112. Vanin, A. F., Stukan, R. A., and Manukhina, E. B. (1996) *Biochim. Biophys. Acta*, **1295**, 5-12.
113. Efroimson, V. P. (1998) *Geniality and Genetics* [in Russian], Russkii Mir, Moscow.
114. Shnoll, S. E. (1997) *Heroes and Villains of Russian Science* [in Russian], Kron-Press, Moscow.
115. Jemmer, M. (1985) *Evolution of Notions in Quantum Mechanics* [in Russian], Nauka, Moscow.
116. Bunge, M. (1975) *Philosophy of Physics* [in Russian], Progress, Moscow.
117. Gomazkov, O. A. (1994) *Dominant* [in Russian], IPGM, Moscow.
118. Gomazkov, O. A. (1998) *Biochemistry (Moscow)*, **63**, 125-132.
119. Engelhardt, V. A. (1983) *Cognition of Life Phenomena* [in Russian], Nauka, Moscow.
120. Shnol, S. E. (1979) *Physico-Chemical Factors of Biological Evolution* [in Russian], Nauka, Moscow.
121. Tkachuk, V. A. (1999) *Biol. Membr. (Moscow)*, **16**, 212-229.
122. Apter, M. (1970) *Cybernetics and Development* [Russian translation], Mir, Moscow.
123. Wolin, M. S., Davidson, S. A., Kaminski, P. M., Fayngersh, R. P., and Mohazzab-H., K. M. (1998) *Biochemistry (Moscow)*, **63**, 810-816.
124. Millar, J. (1995) *Med. Hypotheses*, **45**, 21-26.
125. Orbeli, L. A. (1966) *Selective of Papers "Problems of General Physiology and Pathophysiology"* [in Russian], Nauka, Moscow-Leningrad, pp. 292-293.
126. Reutov, V. P., Azhipa, Ya. I., and Kayushin, L. P. (1983) *Izv. Akad. Nauk SSSR. Ser. Biol.*, **3**, 408-418.
127. Reutov, V. P. (1999) *Biochemistry (Moscow)*, **64**, 528-542.
128. Reutov, V. P. (1999) in *Role of Neurotransmitters and Regulatory Peptides in Vital Activity* [in Russian], Polibig, Minsk, pp. 185-187.
129. Reutov, V. P. (2000) *Vestnik RAMN*, **4**, 35-41.
130. Reutov, V. P., and Dyakonova, T. L. (1999) in *Role of Neurotransmitters and Regulatory Peptides in Vital Activity* [in Russian], Polibig, Minsk, pp. 188-190.
131. Reutov, V. P., Kayushin, L. P., and Sorokina, E. G. (1994) *Fiziol. Cheloveka*, **20**, 165-174.
132. Motlokh, N. N. (1991) *Cancer: Payment for Evolution* [in Russian], Prometei, Moscow.
133. Rubenchik, B. L., Glavin, A. A., Galenko, P. M., and Oleynik, I. O. (1996) *Exp. Oncol.*, **18**, 64-69.
134. Bernal, J. (1969) *Appearance of Life* [Russian translation], Mir, Moscow.
135. Feynman, R. (1968) *Character of Physical Laws* [Russian translation], Mir, Moscow.
136. Migdal, A. B. (1972) *Ways in Unknown* [in Russian], Sovetskii Pisatel, Moscow, pp. 313-322.
137. Selye, H. (1972) *At the Level of the Whole Organism* [in Russian], Nauka, Moscow.
138. Varshavsky, Ya. M. (1982) *Preface to the book by H. Brown and J. Wolken "Liquid Crystals and Biological Structures"* [in Russian], Mir, Moscow.
139. Grushevitskaya, T. G., and Sadokhin, A. P. (1998) *Modern Concepts in Life Sciences* [in Russian], Vysshaya Shkola, Moscow.
140. Yulov, V. F. (1997) *Modern Concepts in Life Sciences* [in Russian], VGPU, Kirov.
141. Rutten, M. (1973) *Origin of Life* [Russian translation], Mir, Moscow.
142. Flindt, P. (1992) *Biology in Numbers (Collection of tables including more than 10000 data)* [Russian translation], Mir, Moscow.
143. Martynov, D. Ya. (1986) in *About Modern Status of Global Evolutionism Idea* [in Russian], Nauka, Moscow, pp. 155-157.
144. Nesteruk, A. V. (1994) in *Global Evolutionism* [in Russian], Nauka, Moscow, pp. 94-107.
145. Davis, P. (1985) *Accidental Universe* [Russian translation], Mir, Moscow.
146. Pullman, B., and Pullman, A. (1965) *Quantum Biochemistry* [in Russian], Mir, Moscow.
147. Davydov, A. S. (1986) *Solitons in Bioenergetics* [in Russian], Naukova Dumka, Kiev.
148. Hollicher, B. (1966) *Nature in the Scientific Picture of the World* [Russian translation], Progress, Moscow.
149. Mortenson, L. E. (1962) in *The Bacteria*, Vol. 3, *Biosynthesis*, Academic Press, N. Y., pp. 119-166.
150. Vernadsky, V. I. (1967) *Biosphere* [in Russian], Nauka, Moscow.
151. Vernadsky, V. I. (1989) *Biosphere and Noosphere* [in Russian], Nauka, Moscow.
152. Vernadsky, V. I. (1978) *The Living Substance* [in Russian], Nauka, Moscow.
153. Tyuryukanov, A. N., and Ferodov, V. M. (1996) *N. V. Timofeev-Resovsky: Biosphere Meditation* [in Russian], Nauka, Moscow, pp. 213-236.
154. Lvov, N. P. (1989) *Molybdenum in Nitrogen Assimilation by Plants and Microorganisms* [in Russian], Nauka, Moscow.
155. Hughes, M. (1983) *Inorganic Chemistry of Biological Processes* [in Russian], Mir, Moscow.
156. Pryanishnikov, D. N. (1945) *Nitrogen in Life of Plants and Agriculture of the USSR* [in Russian], AN SSSR Publishers, Moscow-Leningrad.
157. Tokhver, V. I. (1972) in *Ecology and Physiological-Biochemical Bases of Microbiological Conversion of Nitrogen* [in Russian], Tartu University Press, Tartu, pp. 34-46.
158. Semenov, N. N. (1934) *Chain Reactions* [in Russian], Goskhimizdat, Moscow.
159. Chrikov, N. M., and Voevodsky, V. V. (1956) *Zh. Fiz. Khim.*, **30**, 722-728.

160. Shugalei, I. V., Lopatina, N. I., Celinsky, I. V., and Panasyuk, S. L. (1986) *Zh. Fiz. Khim.*, **56**, 188-192.
161. Kanner, J. (1990) *Free Rad. Biol. Med.*, **1**, 12-13.
162. Vladimirov, Yu. A., and Archakov, A. I. (1972) *Lipid Peroxidation in Biological Membranes* [in Russian], Nauka, Moscow.
163. Gorren, A. C. F., and Mayer, B. (1998) *Biochemistry (Moscow)*, **63**, 734-743.
164. Nathan, C. (1992) *FASEB J.*, **6**, 3051-3064.
165. Nathan, C. (1995) in *The Role of Nitric Oxide in Physiology and Pathophysiology* (Koprowski, H., and Maeda, H., eds.) Springer-Verlag, Berlin-Heidelberg, pp. 1-4.
166. Marletta, M. A. (1994) *J. Med. Chem.*, **37**, 1899-1907.
167. Knowles, R. G., and Moncada, S. (1994) *Biochem. J.*, **298**, 249-258.
168. Bredt, D. S., and Snyder, S. H. (1994) *Annu. Rev. Biochem.*, **63**, 175-195.
169. Schmidt, H. H. H. W., Hofmann, H., and Ogilvie, P. (1995) in *The Role of Nitric Oxide in Physiology and Pathophysiology* (Koprowski, H., and Maeda, H., eds.) Springer-Verlag, Berlin-Heidelberg, pp. 75-86.
170. Nakane, M., Schmidt, H. H. H. W., Pollock, J. S., Forstermann, U., and Murad, F. (1993) *FEBS Lett.*, **316**, 175-180.
171. Gorren, A. C. F., Schrammel, A., Schmidt, K., and Mayer, B. (1997) *Biochemistry*, **36**, 4360-4366.
172. Morse, R. H., and Chan, S. J. (1980) *J. Biol. Chem.*, **255**, 7876-7882.
173. Brudvig, G. W., Stevens, T. H., and Chan, S. J. (1980) *Biochemistry*, **19**, 5275-5285.
174. Duthu, G. S., and Shertzer, H. G. (1979) *Drug. Metab. Dispoit. Biol. Fate Chem.*, **7**, 263-269.
175. Howard, H. G., Shertzer, H. G., and Duthu, G. S. (1979) *Biochem. Pharmacol.*, **28**, 873-879.
176. Wolkenstein, M. V. (1975) *Molecular Biophysics* [in Russian], Nauka, Moscow.
177. Day, K., and Selbin, D. (1976) *Theoretical Inorganic Chemistry* [Russian translation], Khimiya, Moscow.
178. Gray, H. (1967) *Electrons and Chemical Bond* [in Russian], Mir, Moscow.
179. Langford, K., and Gray, H. (1989) *Processes of Ligand Substitution* [Russian translation], Mir, Moscow.
180. Rifkind, D. M. (1978) in *Inorganic Biochemistry* [Russian translation], Vol. 2, Mir, Moscow, pp. 256-338.
181. Shults, H., and Shirmer, R. (1982) *Principles of Structural Organization of Proteins* [Russian translation], Mir, Moscow.
182. Skulachev, V. P. (1989) *Bioenergetics: Membrane Energy Transducers* [in Russian], Vysshaya Shkola, Moscow.
183. Archakov, A. I. (1975) *Microsomal Oxidation* [in Russian], Nauka, Moscow.
184. Pratt, J. (1978) in *Methods and Achievements in Bioinorganic Chemistry* [Russian translation], Nauka, Moscow.
185. Pimentel, H., and Spratli, R. (1973) *How Quantum Mechanics Explains Chemical Bond* [Russian translation], Mir, Moscow.
186. Green, D., and Goldberg, R. (1968) *Molecular Aspects of Life* [Russian translation], Mir, Moscow.
187. Wolkenstein, M. V. (1982) *Dokl. Akad. Nauk SSSR*, **263**, 722-725.
188. Wolkenstein, M. V. (1978) *General Biophysics* [in Russian], Nauka, Moscow.
189. Racker, E. (1967) *Bioenergetic Mechanisms* [Russian translation], Mir, Moscow.
190. Skulachev, V. P. (1980) *Preface to the book by M. V. Gusev and G. B. Gokhlerner "Free Oxygen and Evolution of the Cell"* [in Russian], Moscow State University Press, Moscow, pp. 3-6.
191. Nikitin, E. D. (1999) in *Synergetics* [in Russian], Moscow State University Press, Moscow, pp. 152-160.
192. Anokhin, P. K. (1973) in *Philosophical Problems of Biology* [in Russian], Nauka, Moscow, pp. 78-104.
193. Urmantsev, Yu. A. (1974) *Symmetry in Nature and Nature of Symmetry* [in Russian], Mysl, Moscow.
194. Urmantsev, Yu. A. (1978) *Zh. Obshch. Biol.*, **39**, 699-718.
195. Urmantsev, Yu. A. (1970) *Botan. Zh.*, **55**, 166-176.
196. Mostepanenko, A. M. (1975) *Space–Time and Physical Cognition* [in Russian], Atomizdat, Moscow.
197. Anokhin, P. K. (1980) *Theory of Functional System* [in Russian], Nauka, Moscow.
198. Sudakov, K. V. (1997) *Byul. Eksp. Biol. Med.*, **123**, 124-130.
199. Sudakov, K. V. (1997) *Reflex and Functional System* [in Russian], Novgorod University Press, Nizhnii Novgorod.
200. Kamshylov, M. M. (1974) *Evolution of Biosphere* [in Russian], Nauka, Moscow.
201. Kaganov, Yu. T. (1999) in *Synergetics* [in Russian], Vol. 2, Moscow State University Press, Moscow, pp. 168-177.
202. Timofeev-Resovsky, N. V. (1974) in *Philosophy and Evolution Theory* [in Russian], Nauka, Moscow, pp. 114-120.
203. Gitelson, J. I., Kovrov, B. G., Lisovsky, G. M., Okladnikov, Yu. N., Rerberg, M. S., Sidko, F. Ya., and Terskov, I. A. (1975) in *Problems of Space Biology* [in Russian], Vol. 28, Nauka, Moscow.
204. Reutov, V. P., and Sorokina, E. G. (1998) *Biochemistry (Moscow)*, **63**, 874-885.