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Reactive Oxygen Species, Water, Photons, and Life.

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Abstract. *Unique properties of oxygen and of the reactions with reactive oxygen species (ROS) participation are considered, the multiple ways of ROS generation and utilization are discussed in view of evidence for the absolute necessity of ROS for the normal vital activity. Many difficulties in the realization of the real role of ROS in vital activity are caused by the attitude to them only as to chemical substances, while they should be considered in the first place as the major participants of continuous flows of highly non-linear processes in which electron excited species emerge. These processes play the significant role in energy and informational flows in all the living systems. We suggest that the mechanisms of biological action of ROS are determined by the structural patterns (frequency-amplitude patterns of electron excited states generation and their relaxation) of the processes with ROS participation taking place in the aqueous environs. Energy released in such reactions is used as an activation energy for the specific biochemical processes, for the continuous “pumping” of the non-equilibrium state of inter- and intracellular structural components, while the structural patterns of ROS reactions determine biochemical and physiological rhythmic modes. Special role of water in all these phenomena is discussed. From a more broad perspective the processes with ROS participation emerging in water preceded and were the necessary condition for origination and evolution of organic living forms on Earth.*

1. INTRODUCTION: THE SCOPE OF THE PROBLEM

There are many hallmarks indicating of the critical situation in current biology. Some sagacious biologists compare it to the great crisis in physics, which occurred a century ago (Strohman [1997]). But by the end of the XIX-th century physics had the solid theory with a reliable predictive power for a lot of phenomena of the inanimate World relevant to human practice. The crisis in physics had only revealed restrains in its theoretical foundation, while quantum physics and relativity widened its boundaries. Unfortunately biology had never been based on any firm theory. The so-called “Theory of Evolution” stemming from the Darwinian principle of the “survival of the fittest” lacks the essential feature of a genuine scientific theory – the predictive power. Yes indeed, it should be acknowledged that biology gathered the immeasurable collection of empirical data during its more than bicentennial history. A lot of generalizations of these facts are certainly genuine scientific theories. However they are confined to rather narrow fields. As soon as they are extended beyond the boundaries of their application and suggested as a scientific basing for medical, agricultural, ecological practice –

unexpected consequences follow. There are a lot of examples when the global harm caused by such scientific recommendations much surpasses the initial short-term dividends. This certainly will happen to the Theory of Genetic Determinism, which claims that the code of life is simply written in DNA sequences, selected by chance in the course of evolutionary struggle for life, and which is recklessly panegyricized these days as a paradigm of life.

Lacking its own theoretical foundation current biology ultimately bases on the principles of classical physics and chemistry of the end of the XIX century. A living organism is envisaged as a collection of more or less stable material particles. Their local interactions are considered to summate into the performance of specific functions. Most biologists are so confident in that necessarily restricted physics and chemistry, which they had studied at universities, that they avoid even to discuss the existence of other natural laws that living systems can employ to fulfill the major goal of their existence – to maintain their living state.

These days, however, a lot of indications suggest that biology is approaching the turning point that may be much more impressive than the replacement of the Ptolemy view of the Solar system for the Copernicus one. The gist of the coming turn to the New Biology is prepared by the efforts of many outstanding biologists of the past who were based on in their insights on the holistic rather than reductionistic principles. I shall name only few of them, most relevant to the topic of the discussion to follow: Ervin Bauer, Alexander Gurwitsch, and Albert Szent-Gyorgyi. Bauer accentuated the essential characteristic of the living state as a system of few interconnected principles (axioms). The major is the Principle of Stable Non-equilibrium: “No living system is ever at equilibrium [at rest]. It ceaselessly performs work against equilibrium, demanded by the physical and chemical laws appropriate to the actual external conditions.” (Bauer [1935], see also: Voeikov [1999]). This is the most straightforward indication of the primacy of the perpetual movement (“process”) characteristic for the living state over the material (corporeal) constituents of living things: the latter function as disposal instruments for the performance of the symphony of life.

Gurwitsch was the first to verify experimentally the Bauer’s principles. Among many other Gurwitsch’s insights and discoveries the main was his discovery of the pivotal role of “living light” (biophotons) for the execution of major living functions. Gurwitsch designated fluxes and flashes of these photons as “mitogenetic radiation”, as he proved that the central event of life – cell multiplication – could not come about without an acquisition of a mitogenetic photon by a cell. Gurwitsch discovered also the vital role of oxygen for the emergence of “living light” (Gurwitsch & Gurwitsch [1943]).

All vital processes occur in an aqueous environment. As it was stressed more than 40 years ago by Albert Szent-Gyorgyi [1957], water – the most abundant and still the most enigmatic substance, should be considered the active participant, if not a solicitous “cradle” for vital processes, rather than just a solvent for biomolecules. He was also the first to demonstrate the importance of oxygen dissolved in water for the specific properties of the processes related to electron excitation taking place in it. Szent-Gyorgyi suggested that biology did not succeed in understanding basic living functions because it focussed its attention on a substance in the form of particles dissecting them from two matrixes: water and electromagnetic fields.

The actual pivotal role of all these entities – oxygen, water, light in life manifestations is grossly undermined in current academic biology. However, more and more scientific confirmations of the basic role of three ancient “elements”: air (oxygen), light, and water in life dynamism accumulate.

2. UNIQUE PROPERTIES OF OXYGEN

Oxygen is a vitally important substance for life on Earth and especially for a human life. Few minutes without oxygen is fatal for a human being. Why so? At first glance the answer is obvious: as other aerobic animals, we gain most of our energy from the oxidation of different substrates. Nervous tissue and brain in particular are especially yearning for oxygen: human brain not exceeding 2% of body weight utilizes more than 20% of total oxygen consumed. But here is the paradox: according to textbooks of biochemistry overwhelming expenditure of oxygen takes place in mitochondria, where figuratively speaking, oxygen is used as a trash box for electrons, which have dispensed their energy potential for the production of ATP. But brain cells contain much less mitochondria than, say, muscle or liver cells (David [1977]). Therefore, there should exist some alternative way of O_2 utilization, and brain should consume more oxygen using this way than any other tissue. What is the essence of this alternative way, which may be preferred by brain and without which it nearly immediately collapses? To answer this question we'll have to consider some physical and chemical properties of oxygen, which are often neglected in biochemical textbooks.

Oxygen molecule (O_2) is unique among other molecules in the environment. It has two electrons with parallel spins (unpaired electrons) on its valence molecular orbitals ($M\uparrow\uparrow$, where \uparrow represents an electron with a certain spin) (Mattheus [1986]). Such constitution of an outer electron shell is termed a triplet state. Particles in a triplet state are paramagnetic (attracted into the magnetic field) and possess an excess of energy over their singlet state $[M\uparrow\downarrow]$, in which all their electrons are paired. But unlike other molecules oxygen in its singlet state is even more excited than in the triplet one. Triplet O_2 is a vast energy store, able to release more than 180 kcal/mole upon its reduction to two water molecules after gaining 4 electrons (together with their carriers – protons). But it can not be spontaneously reduced, because according to Wigner spin conservation rules it can not directly interact with singlet state molecules (Salem [1982]), and that is one of the reasons of triplet oxygen stability.

There are several ways to activate oxygen, and one of them – one-electron oxygen reduction. When electrons are taken by O_2 one after another, intermediate products – reactive oxygen species (ROS) arise. Some of them are free radicals: chemical species, which unlike usual molecules possess an odd number of electrons at their valence electron shell. In the desire to get a pair for a lone electron free radicals avidly interact with the neighboring electron donors, which are normally represented by molecules. A free radical gains an electron from a molecule and turns into a molecule, while a molecule turns into a free radical and starts to look for another electron donor. Thus, free radicals may initiate chain reactions in solutions containing bioorganic molecules such as lipids, proteins, nucleic acids, carbohydrates (Figure 1):

Oxygen molecules are good starters for radical reactions. When O_2 gains 1 electron it turns into a superoxide anion radical, $O_2^-\uparrow$. Addition of a second electron (together with 2 protons) turns the latter into hydrogen peroxide, H_2O_2 . Peroxide is not a radical, but it easily gains a third electron giving rise to a hydroxyl radical, $HO\uparrow$, and hydroxyl ion, HO^- . $HO\uparrow$, a very active chemical particle, easily initiates uncontrollable chain reactions.

Radical chain reactions indeed damage important biological molecules *in vitro*, and ROS are traditionally regarded in biochemical literature as highly hazardous particles (e. g., Fridovich [1998]). They are blamed as an alleged reason of many diseases and even as the major cause of aging (Ames *et al.* [1993]). However, a lot of old and recent data urge to assume that ROS are eminently needed for normal vital activity. First, any organism uses multiple mechanisms for a *purposeful* one-electron reduction of oxygen. The enzyme, NADP-H-oxydase, which was initially considered to be specific for immune cells, which produce

ROS as a weapon for killing microbes and viruses, turned out to be ubiquitous. It is expressed in many other cells and tissues having nothing to do with the defensive functions (for example, Jones *et al.* [1996]). Univalent oxygen reduction is performed by other ubiquitous enzymes, such as NO-synthase (Xia *et al.* [1998]), and this list is growing. Superoxide and other ROS are generated in the course of some non-enzymatic reactions, which continuously go on both intra- and extracellularly (Yim *et al.* [2000]). According to some estimates, 10-15% of oxygen consumed by an animal at rest is routed to the univalent pathway of reduction, along which ROS are generated (Shoaf *et al.* [1991]). Under the stressful conditions, when activity of ROS-generating enzymes is amplified, total oxygen consumption increases by nearly 20%, and supposedly all this excess is univalently reduced (Vlessis *et al.* [1995]). Therefore, ROS should play a very important role in normal physiology.

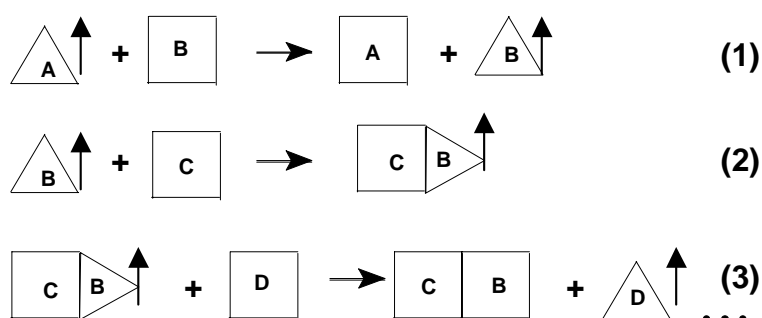


Fig. 1. -- A sketch of a chain reaction initiated with a free radical $A\uparrow$ (" \uparrow " symbolizes here an unpaired electron). Triangles symbolize radicals with an odd number of electrons, and rectangles – molecules with an even number of electrons. If odds could combine only with evens the chain would never terminate.

3. BIO-REGULATORY ROLE OF REACTIVE OXYGEN SPECIES

Living cells normally react to external stimuli in few specific ways: 1. They may be induced to perform their specialized function; 2. They may change their speciation by either differentiation or dedifferentiation; 3. They may enter into the mitotic cycle and proliferate; 4. They may proceed into apoptosis, or "programmed cell death". It was considered traditionally that particular physiological reactions in living cells are evoked by specialized molecules: hormones, cytokines, neurotransmitters. It turns out now that ROS participate in most if not all cellular reactions upon external stimuli. The particular reaction of a given cell depends not only on the interaction of the specific molecular signal with the specialized receptor, but also on the "context", -- previous history of a cell, its current state and also on the background level and composition of ROS. The latter preconditions depend both upon the mode of ROS generation by a cell itself or on their incoming flow and on the rate of their elimination (see below). ROS may themselves imitate action of many of bio-molecular signals upon cellular functions including regulation of the genome activity. Thus, ROS should be looked upon as universal information agents at the cellular level (for reviews see Khan and Wilson [1995], Saran *et al* [1998], Gamaley and Klybin [1999]) though the mechanism of their action stays puzzling in the boundaries of the conventional biochemical paradigm.

Though there are a lot of ways for ROS generation in an organism, animals for their wellbeing need to get them regularly from the environment. Alexander Chizhevsky has shown as far as in 1920s that if ordinary air is devoid of negative air ions, mice and rats die within several days with the symptoms of suffocation, and air enrichment with negative ions reverses all the pathological symptoms. These results were recently confirmed; besides it was proved

that “negative air ions” are, in fact, superoxide anion radicals, $\text{O}_2^{\cdot-}$ (Goldstein and Arshavskaya [1997]), that easily recombine to produce hydrogen peroxide and oxygen. It is important to stress, that normal “fresh” air should contain at least 500 negative air ions in 1 cm^3 , while oxygen contents in it is 10^{16} times higher. To realize this difference imagine a lake 10 meters deep and about 30 km in diameter filled with “bad” water (air devoid of superoxide). Add to it a teaspoon of an elixir (superoxide), and water turns fresh.

Air enrichment with $\text{O}_2^{\cdot-}$ up to 10^4 particles/ cm^3 , is employed for arterial pressure and blood rheology normalization; it supports tissue oxygenation, eases stress conditions, augments general adaptation of an organism to adverse factors (Kondrashova *et al* [2000]). Other ROS, ozone (O_3), and hydrogen peroxide, have been also used in the first decades of the XXth century for the treatment of multiple diseases – from tuberculosis to nervous disability (Noble [1928]). Now official medicine avoids them because of the fear of their alleged high toxicity. Still, ozone therapy begins to revitalize in the last years. For example, ozone is bubbled through a sample of taken out blood and then it is returned back to a patient. Remarkably, that this strong oxidizer, easily generating free radicals do not harm healthy components of whole blood – plasma proteins, red and white cells, which it destroys in the isolated state. Though only a small portion of patient’s blood is treated with ozone, when it is returned back to a patient erythrocytes in the whole stock much better accept and donate oxygen, and leukocytes become more active (Bocci [1994]). Similar therapeutic action is observed if taken out blood is treated with low concentrations of H_2O_2 and returned back to a patient, or when highly diluted peroxide solutions are infused intravenously (Nathan and Cohn [1981]). In the latter case therapeutic effects seem especially mysterious. Usual single dose is 200 ml of physiological saline containing 0,03% of hydrogen peroxide. Blood catalase immediately converts infused H_2O_2 into water and oxygen. The total quantity of oxygen released is equivalent to 100 ml of air (a small portion of one deep breath) and it adds practically nothing to oxygen contents of blood. Still after intravenous infusion of H_2O_2 solution oxygen saturation of blood and tissues increases, and other physiological parameters of blood and other tissues normalize.

Thus ROS in proper doses exert multiple and beneficial action upon various biological functions from the cellular level to the level of the whole organism. But how can these particles, which are practically devoid of *chemical specificity* in their reactions and are so destructive *in vitro*, exercise specific bio-regulatory action *in vivo*?

4. FREE RADICAL REACTIONS PRODUCE LIGHT

Theoretically, the only way to terminate deleterious chain reactions initiated by free radicals is recombination of a free radical with another one. But in a system, where concentration of radicals is low, and concentration of molecules is high, the probability of two radicals encounter is very low. Remarkably, O_2 , which gives birth to free radicals, is basically the only substance, which can terminate these reactions. As a bi-radical, it can provide multiplication of radicals and increase probability of their recombination. If a radical R^{\cdot} interacts with O_2 , a peroxy radical, ROO^{\cdot} , arises. It can abstract a hydrogen atom from an appropriate molecule $\text{R}'\text{H}$ making it a radical R'^{\cdot} and turning itself into a peroxide molecule ROOH . The bond $\text{O}-\text{O}$ in peroxides is relatively weak and under certain conditions it can break down giving rise to two new free radicals, RO^{\cdot} and HO^{\cdot} in addition to a radical R'^{\cdot} . This event is termed a delayed chain branching. New radicals can recombine with other radicals present in the system and terminate chain which they lead. No less important is that the reactions of radical recombination have a unique property – quanta of energy released

in such reactions may be equivalent to the energy of photons of visible or even UV-light (fig. 2).

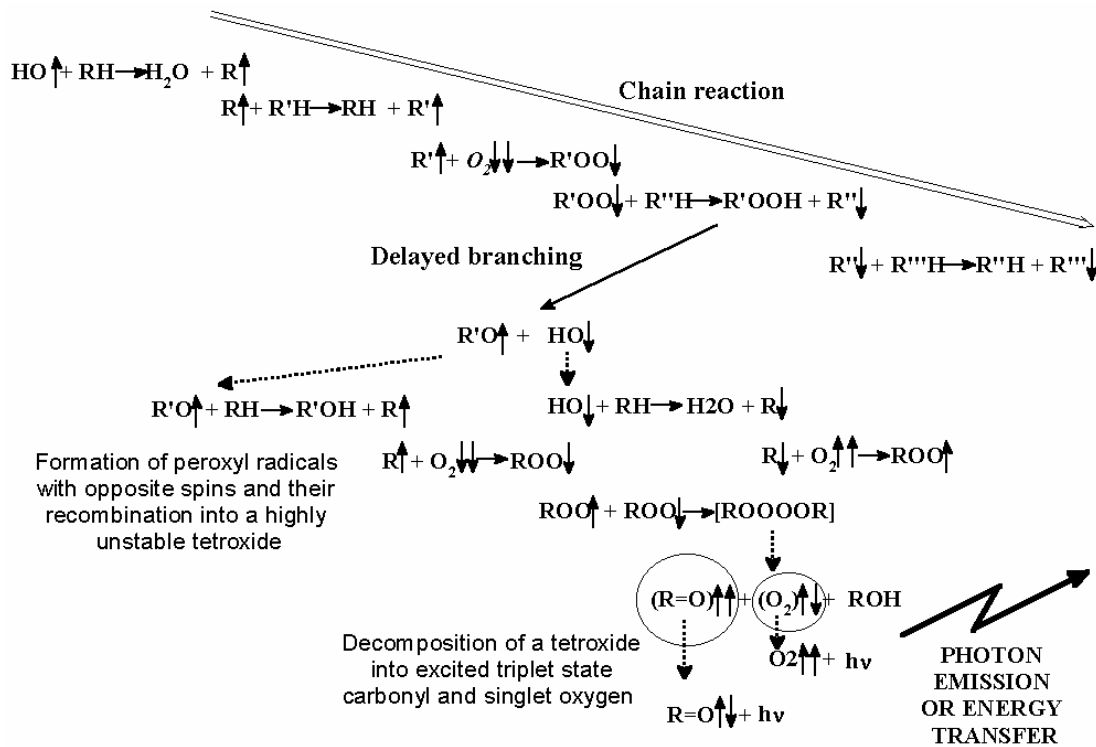


Fig. 2. -- An outline of a chain reaction with delayed branching proceeding in the presence of oxygen. Encircled are electron excited species that may emit photons at their transition to a ground state.

Gurwitsch and Gurwitsch [1938] had shown, that at sufficient oxygen content mitogenetic radiation emerges in an aqueous reaction system where radical chain reactions go on. We studied photon emission in the blue-green region of the spectrum from aqueous solutions of amino acids and amino acids together with sugars and confirmed that chain reactions with delayed branching followed with generation of electron excited states (EES) indeed take place there (Voeikov and Naletov [1998]). Even more remarkable was our recent observation of oscillatory modes of photon emission which spontaneously arise in water solutions of simple sugars or other carbonyl compounds and amino acids (Koldunov *et al.* [2000]). Figure 3 is an example of multi-modal oscillatory pattern of chemiluminescence, which accompany the reaction between methylglyoxal and glycine (similar reactions go on in cells and intercellular matrix).

Processes with the participation of ROS tend to self-organize, as it is revealed by the appearance of sustained oscillations of chemiluminescence and their other parameters, also when they are catalyzed by enzymes, such as peroxidase (Kummer *et al.* [1996]), or when they proceed at the cellular level. The latter was elegantly demonstrated by Kindzelsky, *et al.* [1998], in experiments on isolated neutrophils where sustained oscillations of superoxide production with a period of about 20 sec was demonstrated. Later the same group of authors demonstrated that both the amplitude and frequency of metabolic oscillations in these cells closely related to ROS production were finely regulated with cytokines (Adachi *et al.* [1999]). Recently we observed sustained oscillations in neutrophil suspensions where very low frequency oscillations were modulated by high frequency ones (fig. 4.). As chemiluminescence reflexes the rates of utilization and (indirectly) the rates of generation of

oxygen radicals, these oscillations indicate of collective behavior of big cell populations (in this particular case of 100 000 cells).

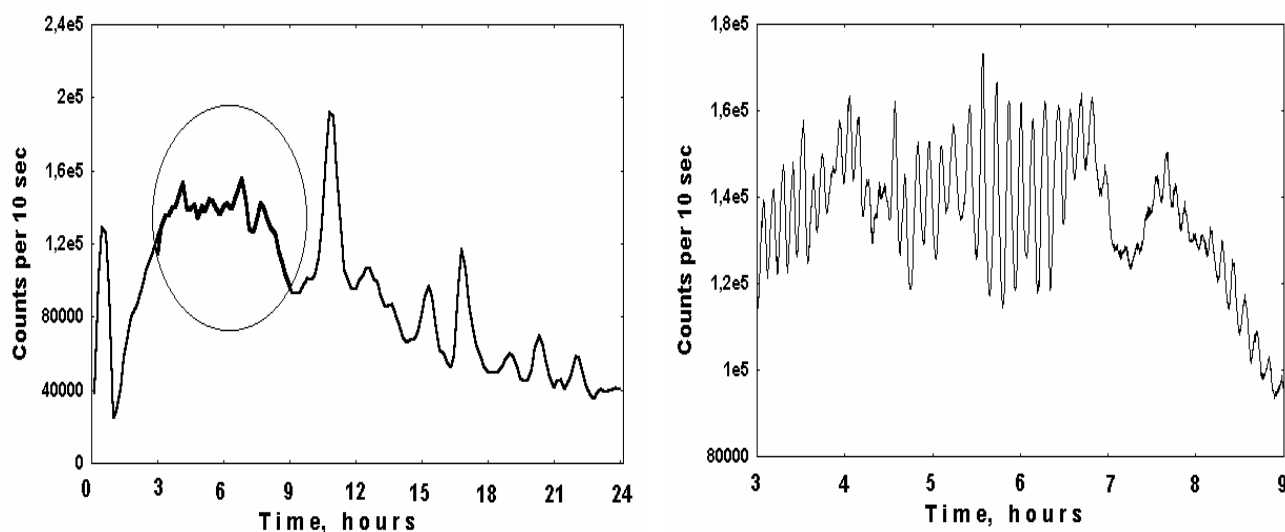


Fig. 3. -- Oscillatory pattern of photon emission from an aqueous solution of methyl glyoxal and glycine. Left panel – kinetic curve for counts averaged for 10 min sampling time; right panel – the detailed kinetics (10 sec sampling time) for a circled fragment at the left panel.

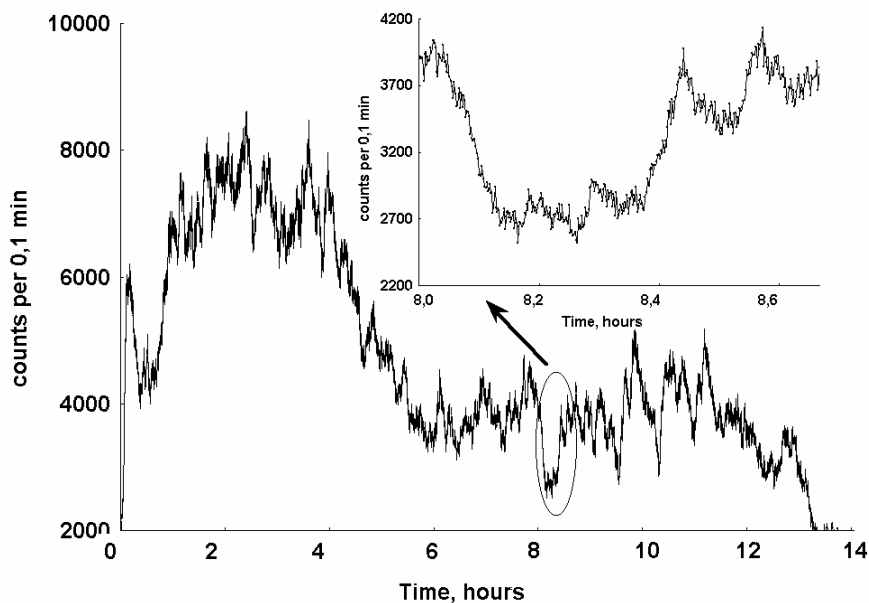
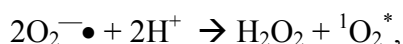


Fig. 4. – Oscillatory pattern of photon emission from a suspension of human neutrophils (100 000 cells in 0,1 ml of Henks buffer solution) without agitation and mixing observed after stimulation of respiratory burst with zymosan (0,1 mg/ml) in the presence of 0,1 mM of Luminol. Further details of experimental procedure see in Novikov *et al.* [2000].

As mentioned, a substantial part of consumed oxygen is utilized through a one-electron reduction route. Still the actual levels of free radicals and other ROS in cells and extracellular matrix are usually very low due to the presence of multiple enzymatic and non-enzymatic mechanisms known as “antioxidant defense”. Some elements of such defense act very fast. The turnover numbers of ubiquitous enzymes superoxide dismutase (SOD) and catalase

exceed 10^6 cycles/sec (Fee and Bull [1986]). SOD catalyses the recombination of two O_2^- radicals producing H_2O_2 and oxygen, and catalase reduces H_2O_2 to water and oxygen. Biochemistry currently pays attention only to detoxifying role of these enzymes and of low molecular weight antioxidants, such as ascorbic acid and tocoferol (vitamin E). But what is the sense in high rate of radical production by, for example, NADP-H-oxidase and their immediate elimination by SOD?

Let us recollect, that in all the reactions of radical recombination and peroxides decomposition whether they proceed spontaneously or under the action of catalysts electron excited states are generated. In particular, the reaction of spontaneous superoxide dismutation:



electron excited singlet oxygen (${}^1\text{O}_2^*$) arises, which converts to triplet oxygen releasing a quantum of energy of about 1 eV. In the reaction act of 2 molecules of H_2O_2 decomposition to 2 molecules of water and a molecule of oxygen 2 eV (equivalent to a quantum of yellow-red light) is released. The overall release of energy accompanying the reduction of one oxygen molecule to two water molecules by consecutive addition of four electrons to it is 8 eV (for the comparison, the energy of one UV-photon with the wavelength of 250 nm is around 5 eV).

In cases when ROS reactions are catalyzed with enzymes, such as NADPH-oxidase, superoxide dismutase, and catalase, electron excited states should be generated with frequencies reaching megaHertz range. Gurwitsch and Szent-Gyorgyi insisted that in the organized milieu of living systems the energy of electron excitation does not easily dissipate into heat, rather it may radiatively or in a radiation-less manner transfer to macromolecules, or to their ensembles. In particular, this energy may be used for enzyme activation, or modulation of their activity (see, for example, Cilento, G. and Adam, W. [1995]). It can also be suggested that this particular feature of oxygen-dependent free radical reactions with delayed branching is employed for regulation and coordination of the work of all cellular molecular “machines” (Baskakov and Voeikov [1996]; Voeikov and Naletov [1998]). Energy packages equivalent to energy of light photons released in the reactions of radical recombination may serve for biochemical reactions triggering, while their rhythmic release under certain conditions may suggest their role of pacemakers of metabolic processes.

In fact, more and more evidence appears pointing to the importance of oscillatory character of many (if not all) regulatory and executive processes in the organism. Recently it has been proved, that frequency rather than amplitude of intercellular calcium oscillations (calcium – is one of the chief intracellular messengers) is the informative factor for regulation of further cell reactions (De Koninck, and Sculman [1998]). This is a crucial observation, because up to now only the dose (amplitude) of a signal was considered to be important for the evoking a certain physiological response, and the role of frequency modulation of the concentrations of reagents or other physiological parameters was not seriously considered.

Among many different bio-regulatory substances ROS are the most likely candidates for the triggers of oscillatory processes because they are in a constant motion, more precisely, they continuously emerge and die, giving birth to EES at their elimination. Therefore *it may be suggested that the mechanisms of ROS regulatory action are specified by the structural patterns of the processes with ROS participation rather than by their average concentrations in cells and tissues. By the «structural pattern of a process» we mean the frequency-amplitude patterns of EES generation and their relaxation in the course of ROS reactions with each other or singlet molecules. Energy released in such reactions is used as an activation energy for the specific biochemical processes, for the continuous “pumping” of the*

non-equilibrium state of inter- and intracellular structural components, while the structural patterns of ROS reactions determine biochemical and physiological rhythmic modes.

This hypothesis allows to explain from the unified position many seemingly unrelated phenomena. From this position the functions of antioxidants and enzymes of ROS metabolism appear to be much more versatile than they are seen traditionally. Indeed, they prevent non-specific chemical reactions with ROS participation that may lead to cell damage. But under the normal physiological conditions their major functions are organisation of EES oscillatory patterns and ensuring their richness and flexibility. In particular, superoxide dismutase and catalase set EES oscillations up to the megaHertz range (particular frequencies may vary significantly depending on the substrate availability, as well as on other reaction conditions). Low molecule weight antioxidants supposedly set the lower frequency rhythms. The more instruments are in this “orchestra”, the richer the melody it can play. That is probably why so popular are the herbal therapy, vitamin therapy and other forms of naturopathy – all these food supplements are rich in antioxidants and coenzymes – acceptors of EES energy. Together they provide a valuable and harmonic set of rhythms of vital processes.

Regular supply of ROS from the environment with air and water is needed to “spark” internal mechanism of oxygen reduction, because oscillations in ROS metabolism tend to decay, when by-products of radical reactions proceeding in the milieu rich in organic molecules are accumulated. These by-products, in fact, may be represented by organic peroxides, cross-linked macromolecules, a variety of “Advanced Glycation End Products” including macromolecular free radicals. All of them accumulate mostly due to radical chain reactions proceeding without their proper termination under the conditions of tissue hypoxia. The best way to neutralize them – is to intensify the mechanisms of oxygen activation in the organism, which may be provided, for example, by means of ozone or hydrogen peroxide therapy. That is probably why these ROS are so effective in treatment of surprisingly wide range of diseases.

Gurwitsch was the first to suggest that “the *factor of realisation* which determines cell division is of oscillatory nature, that is, it may have something to do with a radiation process” (See Gurwitsch and Gurwitsch [1999]). His experiments with onion root in which mitogenetic radiation (UV-photons in the range of 200-280 nm triggering cell division) was discovered in 1923 were based on this idea. Recently the Georgian radiobiologist A. Kozlov [1991] wholly confirmed Gurwitsch’s discovery that absorption by any living cell of a photon with the energy around 5 eV is the necessary (though not sufficient) condition for the initiation of its mitosis. Gurwitsch also demonstrated the surprising property of this radiation: the efficiency of mitogenetic effect significantly increases if irradiation of a detector culture is performed in an intermittent (oscillatory) mode. In such experiments a rotating wheel with slits was installed between a source of emission and a detector culture. At certain frequencies of the wheel rotation the mitogenetic effect is observed at much lower exposure time or larger distance between the source and the receiver, than without intermittence. From this it follows, that bio-informational efficiency of a signal (in this particular case – of a high frequency signal, photon) increases drastically due to its very low frequency modulation.

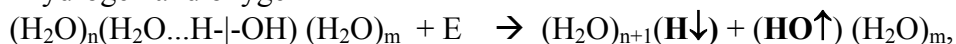
5. WATER – THE CRADLE OF ROS

If ROS implement such fundamental functions in organization of vital processes, they should do so during the whole process of life evolution. But current evolutionary teaching insists that a lengthy pre-photosynthetic period of evolution was completely anaerobic, and that free oxygen appeared on Earth as a by-product of photosynthesis. This notion is based upon the hypothesis suggested to support famous Miller’s experiments, in which he managed

to get organic substances from inorganic ones under the action of high temperature, intense UV-irradiation and electrical discharges (Calvin [1969]). It is evident that such reactions could realize only in the complete absence of free oxygen.

Contrary to this hypothesis, more and more data indicate that water, in which all the most interesting processes involving ROS proceed, plays the crucial role in their emergence and organization of the processes in which they are involved. It is known for a long time that under the action of high density energy (e.g., UV, X-ray, or gamma-irradiation) water molecules dissociate giving rise to hydrogen atoms or hydrated electron plus proton, and hydroxyl radical. However recently evidence was presented that homolytic water dissociation ($\text{H-O-H} \rightarrow \text{H}\downarrow + \text{HO}\uparrow$) may proceed under very mild conditions: in cool water having neutral pH, containing common salts in reasonable concentrations under its mechanical agitation, light illumination, sonication, filtration through narrow capillaries, or in the processes of its freezing-thawing, condensation of water vapor, etc. (Domrachev *et al.* [1993]). It should be mentioned, however, that the conclusion of homolytic water dissociation was made basing on the measurements of H_2O_2 content in water which increased due to all mentioned above treatments of water. But H_2O_2 molecules may arise in water only due to recombination of hydroxyl radicals. On the other hand, recently it was reported by Japanese scientists that in the presence of such simple catalysts as Cu_2O , NiO , Co_3O_4 , Fe_3O_4 free hydrogen and oxygen appear in water if it is illuminated with the visible light or even stirred in the darkness (Shigeru *et al.* [1998]). This work also indicates of the possibility of homolytic water splitting under the mild conditions, but in this case oxygen rather than H_2O_2 accumulate because all the mentioned salts readily catalyze the decay of hydrogen peroxide to water and oxygen. But how can low density energy released during mechanical water treatment (sonication, filtration, stirring, etc.) provide the breakdown of a covalent bond between oxygen and hydrogen in a water molecule for which application of at least 5,2 eV is needed?.

The only supposable condition, which may provide dissociation of a strong bond H-O is a polymer-like structure of water. Indeed, polymers may work as transformers of “low density” energy into “high density”, i.e. mechanical energy into the energy of electron excitation. Thus, the above described results indirectly prove that liquid water is a dynamically unstable polymer system, and that in analogy with mechanical energy induced reactions in polymers some part of mechanical energy absorbed by water is concentrated in micro scale region of liquid water structure to the level high enough to break up a covalent bond between hydrogen and oxygen



Free radicals, emerging in clear water in small quantities due to their immediate hydration with free water molecules may live for tens of seconds before they recombine with each other (Blough [1990]). Long life and high chemical reactivity of free hydrogen atom and hydroxyl radical increases the probability of their reactions with nitrogen and carbon dioxide dissolved in water. Products of these reactions -- reduced and oxidized nitrogen and carbon containing substances -- may further react to produce amino acids and other complex organic substances. Thus, as soon as liquid water appeared at the Earth surface, permanent ROS and EES generation started, so the development of the organic life on Earth from the very beginning proceeded in such an environment. In fact, even strictly anaerobic bacteria contain superoxide dismutase genes (Takao *et al.* [1991]), indicating that “anaerobicity” is a rather relative notion. Besides, as it was already pointed out, the processes with ROS participation tend to self-organize and to maintain particular temporal structures, defining the temporal structure of other processes which may develop in relation to them.

Rethinking water properties is crucial for the breakthrough in understanding the role of aqueous environment in self-organization EES-related processes and their engagement in metabolism. Besides, it should urge to reconsider all the process of evolution of life on Earth, because it becomes highly probable that the development of organic life was proceeding from the very beginning at the background of ROS generation and in the presence of molecular oxygen. Indeed, minimal estimates of oxygen production by liquid water due to abiotic factors show that in the case if the volume of oceans on the early Earth was nearly the same as now an increase of oxygen contents in the atmosphere up to the current level could occur under the action of abiogenic factors within several millions or at most several tens of million years. It is billions of years earlier, than the currently accepted evolution theories declare. As a matter of fact, of the latter indicates the recent discovery of the abundance of respiratory chains in the most ancient organisms – archaebacteria (Catresana and Moreira [1999]).

6. ROS AND LIFE

We definitely understand that the analysis of empirical data obtained in different branches of natural sciences, presented here, has brought us to rather contentious conclusions. They are in sharp contradiction to some of the currently dominating and established basics of biology and biochemistry, serving foundation for many aspects of modern biotechnology, medical and agricultural practice, for approaches to resolve ecological problems. Still we are sure, that even if the details of the above reasoning may be modified, the major conclusion: *the processes with ROS participation taking place in aqueous systems and generating electron excited states had played and currently play fundamental bio-energetic and bio-informational role in the emergence and realization of vital processes* – is correct.

As it was mentioned above, living state is the stable non-equilibrium state, sustained by the work which a living system performs against sliding down to equilibrium (E. Bauer). A living system actively extracts matter and energy from its environment, expending its own resources to obtain them, and to transform this energy-matter into the more “expensive” energy of excitation of its own structures. Basically, it is the property of all the systems, where branched chain processes based on oxygen consumption occur. The essence of a branched chain process is manifested in its dynamics, rather than in its overall balance. At the stage of multiplication of active centers and chain branching, a spontaneous growth of free energy of the system takes place. A dynamic orderliness of the system occurs, accompanied by the concentration of significant energy potentials in certain of its domains. And what is especially interesting, such system can consume oxygen *actively* from its environment. Free radical reactions are basically the reactions of an electron transfer – mini-electric currents, and oxygen molecule, as a paramagnetic particle should be attracted by an electrical field. Hence, the more active are these reactions, the more oxygen is needed for their sustaining, the more actively they attract oxygen.

In this respect it is interesting to mention the only known quantitative parameter, which defines the evolutionary progress – the total consumption of oxygen by a typical representative of a certain species during its mean life-span and divided by its mean mass. This parameter was named “Rubner constant” by Erwin Bauer [1935]. According to physiological data, Rubner constant increases several-thousand-fold in the sequence starting with primitive Coelenterates and ending with Primates (Bauer [1935], Zotin [1984]). It is notable that for *Homo sapiens*, this parameter is at least one order of magnitude higher than for Primates. As it was noted, it is human brain, which consumes oxygen most avidly than any other living system on Earth. It is the most non-equilibrium, and, if Bauer is right – the most potentially stable living system, of course only if a human being actually and effectively

uses all his energy to stay a living system. As regards for the external resources, it is really more than enough oxygen and water around to supplement his efforts.

Current molecular biology basing on the primacy of a substance (e. g. DNA) over a process overlooks the influence of the processes with ROS participation not only on the existing structures, but on the mode of their functioning and even, may be on the very fact of the emergence of at least some of them. But the building up evidence demands that biological theories should not neglect the fundamental bio-energetic and bio-informational function of electron excited states emerging due to oxygen-water interplay at the very basic level of biological organization.

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