In "Biophotonics and Coherent Systems in Biology " Beloussov L.V., Voeikov V.L., Martynyuk V.S., eds, Springer, 2007, pp. 89-104.

FUNDAMENTAL ROLE OF WATER IN BIOENERGETICS

Vladimir L. Voeikov[*]

Abstract: Water plays the key role in generation, transformation and utilization of energy for the realization of biological functions. Its direct involvement in hydrolytic processes in which primary "fuels" are produced, in ATP synthesis and energy gain due to ATP hydrolysis is well known, but not appreciated. Recently water became known to be one of the major sources of high grade energy – energy of electronic excitation (EEE) generated in the reactions in which active oxygen participates. Due to quazi-polymeric properties of interfacial water it may transform low grade into high grade energy. Besides, singlet oxygen may directly oxidize water in structured environment providing for the generation of EEE which may "spark" other energy donating processes. EEE may be also be used locally for the performance of different kinds of chemical and physical work; it may accumulate and pool in aqueous systems and migrate within them without dissipation on macroscopic distances. Slow combustion in water and combustion of water is capable to self-organization in space and time expressed in the development of oscillatory-wave regimes of these processes serving as time-keepers of other biochemical processes dependent on them as well as sensitive antennas for external oscillatory signals.

.

1. INTRODUCTION

A human being may survive for 40 days without food, no more than for 5 days without water and only for few minutes without breathing. Thus the role of two simple substances, water and oxygen for life support is much more important than of thousands complex molecules that food contains. Certainly full life is impossible without the latter, but still it is based on water and oxygen.

It seems clear why overwhelming majority of living organisms critically depend on oxygen: it burns food to get energy for the performance of all vital processes. It is less clear why we so depend on water taking into consideration that any organism contains

---

[*] Faculty of Biology, Lomonosov Moscow State University, Moscow, Russia, 119234.
E-mail: vvl@soil.msu.ru

plenty of it. It is usually said that water content of an adult person is around 70% by weight. But in terms of chemistry this figure is inappropriate – chemistry deals not with kilograms, but with moles. Molarity of water in water is 55.6 M. In the most "liquid" tissue – blood, sodium chloride is the next most abundant substance subsequent to water. Its molarity in plasma is only 0.15 M. Molarity of the most important low molecular weight organic compound in blood, glucose does not exceed few millimolar and of the most abundant protein, albumen is approximately 0.00005 M. Even in erythrocyte that is packed with haemoglobin concentration of the latter does not exceed 0.01 M. Thus contribution of water into chemical composition of blood exceeds 99%. If water was just a solvent for other biomolecules loss of few percent of it should not practically change its overall concentration. Nevertheless, it is well known how dangerous even mild dehydration for the state of health of a person is [1]. On the other hand concentration of the majority of specific bioorganic molecules may vary and do vary manifold in the course of normal metabolism. Crucial dependence of an organism on the constancy of water contents in it indicates that water should be no less important for all vital activities than all other biomolecules which it embraces.

Albert Szent-Gyorgyi has noted long ago "The cell is a machine driven by energy. It can thus be approached by studying matter or by studying energy" [2]. Present-day biology focuses on profound study of the tiniest details of material particles representing admixtures in the organismal water. Much less attention is paid to studying energy which drives incessant and directed transformation and renewal of all material particles of which a living matter consist. And even more sorrowful that practically no attention has been paid until recently to the basic matrix in which bioorganic and bioinorganic "solid" particles are imbedded – to water whereas water plays the primary role in metabolism (literally – "exchange of substances") – a necessary condition of living state. When metabolism comes to an end a living organism turns a dead body. Water participates in both indivisible aspects of metabolism – in exchange and transformation of substances, including water itself, and in energy supply and transduction for the persistent flow of former. Here we'll reason that both structural and dynamical aspects of life are based on unique water virtues and that studying water is a necessary condition for the better understanding a living cell, an organism, as a peculiar dynamic material organisation driven by energy.

2. WATER – A DYNAMICAL STRUCTURE

There is growing understanding that water can not be regarded as some unstructured "liquid gas". Many models of water structures are put forward [3, 4, 5, 6, for more references see 7]. In "real" water structuring is expressed much more than in ideal ultra-pure boundless water because of the contribution of multiple interfaces. They include an interface between bulk water and walls of a vessel, a water/air interface, interfaces with gas bubbles and other substances dissolved and suspended in water. Vicinal water with special properties may extend very far from the interface which it solvates [8]. For example, many layers of structured water extend beyond the initial monolayer hydrating a protein surface, and induced protein conformational change modifies the extent of non-ideally behaved water [9]. Several resilient water molecular layers close to the surface of a solid material immersed in water were detected using atomic force microscope [10]. It was shown by subfemtosecond x-ray absorption spectroscopy that liquid water in a first coordination shell of ice consists of structures with two strong hydrogen bonds of each molecule to its neighbours, resulting in water chains and rings [11]. Potentiality of water to form linear polymer-like associations has remarkable consequences for both biological shaping (not to be considered here) and for its participation in bioenergetics – to be discussed later. But in general ability of water to form bulky rather monolayer structures around the surfaces it hydrates necessitates to reconsider many long-term concepts of biochemistry based on statistical laws of classical chemistry (e.g., free 3-dimentional diffusion of substances in living cells) and to acknowledge that physical interactions between interfacial water, macromolecules and low molecular weight organic and inorganic compounds could play a primordial role in vital processes [12].

Only recently understanding has come that high concentration of macromolecules in cells and intercellular matrix which was given a special term “macromolecular crowding” may drastically influence kinetics and even specificity of biochemical reactions in comparison to that observed in diluted in vitro reaction systems [13].A significant part of water in a cell is to different extent “bound” and unavailable for classical diffusion of biomolecules due to crowding. On the other hand it is well known that the rates of biochemical reactions in a cell may exceed those in vitro hundreds and thousands fold and many of them proceed simultaneously in a tiny volume of a cell. How to resolve this contradiction between “structure” and “dynamics”?

Actually there is no such contradiction if to recall that there exist dynamical structures such as a vortex or a tornado that possess their special form and stability only due to flow of a substance, in particular of water through space. The tornado example is especially impressive, as its power, the power of a structure existsing only due to unrestricted input and output of material particles through space, is so powerful that it easily destroys seemingly robust stationary structures. This analogy could be considered artificial in relation to living structures if recently it has not been shown using molecular dynamics simulation that coherent patterns of water (nano-vortexes) may persist in locales defined as ‘‘site-dipole field’’ much longer than orientation memory of individual molecules persists [14].

Thus, when the notion of water structure is mentioned, one should consider that these structures may be extremely versatile. Rapid development of studies of water structures using computer simulation as well as sophisticated experimental techniques calls for the necessity of the emergence of classification of water structures important for the performance of particular vital processes. This classification is still lacking but intuitively it becomes clear that water of living things may exist and coexist in at least few alternative forms, for example layered and fibril water [e.g., 8, 11] vs. “globular” water [e.g., 5], “high density” water vs. “low density” water [15] and superposition of these forms. All these forms participate in metabolism including quite a few alternative and complementary vital processes: catabolism (degradation of a substance) and anabolism (synthesis of new material particles); energy gain and storage vs. energy utilization.

3. WATER PARTICIPATION IN CLASSICAL METABOLISM

Water participation in major biochemical processes is well known but until recently it has not been appreciated to its true worth. In fact different types of processes need different waters. For example food digestion and innumerous biochemical reactions in an organism represent hydrolysis of macromolecular and low molecular weight substances. For hydrolysis to be realized a water molecule should split in two parts. Thus efficient hydrolysis depends not only on chemical structures of hydrolyzed molecules, on the efficiency of enzymes that catalyze it, but on the availability of a particular water form that is appropriate for the hydrolysis.

Hydrolysis among other purposes provides building bricks for the synthesis of new molecules. New biopolymer synthesis proceeds as the reaction of polycondensation: when the new brick sticks to a growing polymer chain a water molecule is released into the surrounding. This chemical reaction is opposite to hydrolysis. The question how this process may be realized in a medium where water molecules represent the overwhelming majority of any other molecules is usually omitted by biochemical manuals though it should seem not too advantageous to push a newly appeared water molecule into surrounding water unless if most part of water in a locale where polymers are synthesized is not free but somehow bound. Alternatively water should have much higher chemical activity (be more free) in a locale where hydrolysis occur. To our knowledge this idea was initially suggested by P.M. Wiggins [16].

Energy supply for all vital processes also needs immediate participation of water. For example synthesis of generally recognized universal "energy currency" of any living cell – an ATP molecule from ADP and orthophosphate is accompanied by a water molecule release, while its splitting at which energy is gained is in fact a reaction of hydrolysis. It is logical to suppose that water organization in locales where ATP is synthesized and where it is utilized is different.

On the other hand how exactly energy value of this "currency" is yielded is still unclear, though credible models suggesting participation of water organized by specific biopolymer surfaces in this mechanism has been suggested long ago [17, see also 18]. The role of aqueous environment in both ATP synthesis and utilization with yield of energy is considered also by P.T. Wiggins who suggests that water may exist in two states – "high density" and "low density" state [19]. She experimentally proved [20] that ATP is synthesized from ADP and Pi in dense cellulose acetate membranes in the presence of KCl, but not NaCl – under the conditions favoring low density water state. Further details of this surprising mechanism and of biological significance of two states of water may be found in [21].

Thus water plays a key role in already well known metabolic processes related both to substance transformation and energy transduction. However, energy transduction process related to ATP synthesis and energy yield from it is by no means the only one significant for general bioenergetics of living organisms. There exist more deep levels of energy acquisition and processing and water actively participates in them.

4. PUTREFACTION AND COMBUSTION

According to the current concept of bioenergetics the overwhelming majority of living organisms gain energy from food burning by oxygen. In a simplified form of this concept specific dehydrogenases abstract "hot" electrons (plus protons) from "fuel" (sugars and fats) and transfer them to NAD^+ and $NADP^+$. Reduced forms of these carriers donate electrons to the respiratory chain in mitochondria, where their energy is released stepwise while they pass downhill from one red-ox center to another and is used for the synthesis of ATP. Oxygen here is the final acceptor (a "trash box") of electrons that had exhausted most part of their redox potential. As energy portions released in mitochondrial oxidation are equivalent to quanta of middle-far IR-photons (≤0,5 eV) this process is analogous to SMOLDERING COMBUSTION.

An alternative form of energy gain from oxygen-dependent oxidation is genuine COMBUSTION when direct one-electron oxygen reduction occurs, and quanta of energy equivalent to energy of visible and even UV-photons (>1 eV) are generated. One of the classical examples of combustion is direct oxygenation of hydrogen resulting in water production and at which high density energy is released. Combustion, in particular combustion of hydrogen is not commonly considered as relevant for bioenergetics. However, a lot of evidence argues that it should be taken into account as one of the most fundamental processes ensuring vital activity with high grade and well ordered energy.

Generally energy may be characterized by quantity and by qualities (forms, levels, and orderliness). Biomedical community is very much preoccupied with energy quantity rather than with its qualities while usefulness of energy for the performance of particular work is in the first place determined by its quality, in particular by the level to which it belongs. Levels of energy are subdivided into translational (energy associated with the motion of a molecule in space), rotational and vibrational energy of parts of di- and many-atomic molecules. The highest level of energy relevant to further discussion is energy of electronic excitation (EEE). As energy may be in principle transformed from one form into another, for example, mechanical energy may be transformed into electrical energy and vice versa, it is convenient to compare different levels of energy as belonging to different regions of electromagnetic spectra. Low level translational, rotational and vibrational energy belong to IR-part of electromagnetic spectrum (from ~10^{13} Hz to ~10^{14} Hz), while energy of electronic excitation covers the range of visible light – from 3,8x10^{14} (deep red) to 7,9x10^{14} Hz (violet) and transcends into UV-light region having higher frequencies. The higher is the frequency of oscillations the higher is energy density. High density energy is the energy of higher "quality" – it is easier transmitted to long distances, easier transformed into different forms of energy, more different types of work may be performed by the same quantity of high density energy that of low density energy. By the way progress of human civilization may be traced by the ability of human beings utilize energy of higher and higher density. Distinguished biologists Alexander Gurwitsch and Albert Szent-Giorgyi many decades ago already insisted that energy of electronic excitation should play a noteworthy role in bioenergetics.

It turns out that water in not less significant for realization of this form of energy generation and utilization as in classical bioenergetic processes. A. Szent-Gyorgyi was probably the first to argue that "bioenergetics is but a special aspect of water chemistry" and that "… water arranges an indivisible system with the structure elements (of a cell) making possible electronic excitations which otherwise are highly improbable… in structured water electronic excitation may be surprisingly long-living, and this may be of a paramount importance for the biological energy transfer" [22]. However, the idea of the importance of EEE for bioenergetics besides specialized biological functions such as photosynthesis and vision is not still sufficiently absorbed by biological community probably because the link between water properties, energy of electronic excitation and particular biochemical processes has not been yet clearly formulated.

The first question which needs to be answered is about the source of energy of electronic excitation, of quanta of energy equivalent to photons of visible and UV-light under mild conditions of living systems (temperature, pressure, pH, aqueous milieu). In fact, A. Gurwitsch has discovered more than 70 years ago that all living systems are the source of such hot photons as photons of UV-light (although of ultra-weak intensity), and suggested as early as in 1930ies that this energy may be provided by free radical reactions. During the last half century thousands of papers were devoted to the studies of "chemiluminescent" reactions of oxygen free radicals under conditions characteristic for living organisms. Still, most biologists consider such reactions abnormal in comparison to "normal" enzyme catalyzed biochemical reactions. Common opinion is that free radicals arise from malfunctioning of mitochondrial electron transport chain or such derogative reactions as lipid peroxidation.

But situation is rapidly changing. It becomes more and more clear that oxygen free radicals and other reactive oxygen species are regular participants of normal metabolism; moreover, they turn out to be key participants of multiple bioregulatory processes. A lot of enzymes that specifically catalyze ROS generation are ubiquitously present in living cells, there are quite a few biochemical reactions in course of which ROS are inevitably generated (see below). However, the role of water in ROS production and generation of energy of electronic excitation in the course of their reactions has been considered only very recently.

5. ROS GENERATION IS AN INTRINSIC PROPERTY OF WATER

5.1. Water – a Transformer of Low Density Energy into High Density Energy

Above it has been argued that significant part of organismal water is interfacial and that such form of water has features of polymeric substance, in particular of linear polymers. Therefore mechanochemical phenomena are expected to take place in it. Polymers can undergo chemical transformations under the action of mechanical impacts, freezing-thawing and fast temperature variations, action of audible sound and ultrasound, and other low density energy forces that are too weak to induce chemical reactions in monomers or short oligomers. If macromolecules in polymers or their solutions are reluctant to shift along each other due to weak but multiple intermolecular bonds they may accumulate and concentrate mechanical energy to densities that comprise energy quanta enough to excite and break down internal covalent bonds in polymers. That means unpairing of electrons and appearance of a pair of free radicals followed with multiple chemical and physical consequences [23].

Basing on the presumption that liquid water contains quazi-polymeric structures the team of Russian physicists headed by G.A. Domrachev started more then 15 years ago to investigate the effects of low density energy physical factors on homolytic water dissociation ($H—O—H \rightarrow HO\bullet + \bullet H$, cf. ionic water dissociation: $H—O—H \rightarrow H^+ + OH^-$). They estimated augmentation of hydrogen peroxide concentration in water because the most probable explanation for appearance *de novo* of H_2O_2 is recombination of $HO\bullet$ radicals arising in homolytic water dissociation ($HO\bullet + \bullet OH \rightarrow H_2O_2$). It was shown that water freezing-thawing, evaporation-condensation, sonication even with audible sound, filtration through narrow capillaries resulted in an increase of H_2O_2 even in ultra-pure and carefully degassed water. Efficiency of water splitting resulting from evaporation/condensation and freezing/thawing is ~10 times as effective, sonolysis ~70 times and water filtration through narrow capillaries – more than 100 times as effective as its photodissociation with far UV-light [24, 25]. Yield of H_2O_2 in water containing common ions and dissolved oxygen was much higher. What is notable, H_2O_2 concentration continued to grow for some time after resumption of any treatment. About 3% of all energy used for viscous flow of water through capillaries with diameter of 0,2 mkm was used for water splitting.

Japanese authors who were looking for the new way to produce hydrogen by water splitting have shown that powders of NiO, Cu_2O, Fe_3O_4 suspended in distilled water by magnetic stirring, catalytically decompose it into H_2 and O_2. Efficiency of the mechanical-to-chemical energy conversion under these very mild conditions exceeded 4% [26]. Here water splits to the final products because presumably metal oxides instantaneously decompose intermediate peroxides.

In case if a water molecule has dissociated as a mechanically excited polymeric entity:

$$(H_2O)_n(H_2O...H\text{-}|\text{-}OH)\ (H_2O)_m \rightarrow (H_2O)_{n+1}(H\downarrow) + (\uparrow OH)\ (H_2O)_m, \quad [1],$$

the initial products of water splitting are free radicals $H\downarrow$ and $\uparrow OH$ (here we symbolize a given electron as$\uparrow$ or $\downarrow$ to stress their alternative spin states). Indeed, if water is in an apparent rest this singlet pair of radicals readily recombines back to water:

$$H\downarrow + \uparrow OH \rightarrow H_2O \qquad [2]$$

However even in such a case this is not just a reverse, equilibrium reaction because water splitting has been achieved under the action of mechanical forces while back recombination of radicals gains an energy quantum of 5,2 eV. In condensed and organized media (such as water), long-range energy transfer of electronic and vibrational excitation has been demonstrated already in 1930ies-1940ies by J. Perrin, S. Vavilov, Th. Foerster, and others. This phenomenon was confirmed with new techniques recently [27].

The probability of radicals to move away of each other significantly increases in “real” water, in which dissolved gases and other molecules and particles are present, especially in cases when multiple layers of water are organized by surfaces which it hydrates and when these layers move along each other with different rates (consider a vortex as an example). This is proved by aforecited data on of the appearance of H_2O_2 in water filtered through narrow capillaries and H_2 and O_2 in water stirred in the presence of metal oxides. Here the following reactions may proceed:

$$HO\uparrow + HO\downarrow \rightarrow H_2O_2 \qquad [3]$$

$$H\uparrow + \downarrow H \rightarrow H_2; \qquad [4]$$

$$H\bullet + O_2 \rightarrow HO_2\bullet; \qquad [5]$$

$$HO_2\uparrow + HO_2\downarrow \rightarrow H_2O_2 + O_2; \qquad [6]$$

$$2H_2O_2 \rightarrow 2H_2O + O_2 \qquad [7]$$

Most important of them are the reactions #6 and #7 in which oxygen molecules are released. It should be reminded that O_2 is unique among other molecules because in its ground state its two electrons are unpaired $[O_2(\uparrow\downarrow)_2\uparrow\uparrow$ or $O_2(\uparrow\downarrow)_2\downarrow\downarrow]$ (besides, an oxygen atom also has two unpaired electrons). Thus, oxygen molecule is a bi-radical (in fact it is a tetra-radical) and it represents a vast store of energy. But it is stable because the laws of quantum physics forbid direct reactions of bi-radicals (they are called also particles in a triplet state) with molecules in which all electrons are paired (singlet state particles). That is why oxygen needs to be initially activated to release its energy reserve.

There are few ways for O_2 activation. It may be excited by an appropriate energy quantum ($\geq$ 1 eV) and turn into a highly reactive singlet oxygen ($O_2(\uparrow\downarrow)$, its another symbol, $^1O_2$). A peculiar feature of O_2 is that electronically excited singlet oxygen may relax only to triplet state because oxygen unlike other substances does not have ground single state. As soon as singlet-triplet transition is “forbidden” by quantum physics laws lifetime of excited singlet oxygen is usually much longer than that of any other molecules in an excited singlet state. Triplet O_2 may be also activated with transition metals because in their field its spin state is changed. Finally, triplet oxygen easily reacts with free radicals – atoms and molecular particles possessing an odd number of electrons on their valence orbital. In these reactions oxygen gains or loses an electron, turns into a free radical which can easily take new electrons releasing large portions of energy at each consecutive step of one-electron reduction. Another peculiar feature of free radical reactions in which oxygen participates is that they may easily turn into branching (or run-away) process [28], and concentration of free radicals in a reaction mixture grows up exponentially until the rates of their production and annihilation equalize. That is why elevation of H_2O_2 yield in water equilibrated with air under the conditions favorable for its splitting occurs faster, continues longer after initial perturbation, and reaches higher levels than in degassed water.

Thereupon it is interesting to speculate that an outcome of water splitting may be significantly influenced by external magnetic fields. There are a lot of reports on the long lasting effects of even a brief treatment of water with magnetic fields, though these effects are not easily reproduced. In principle, magnetic fields may modulate the outcome of free radical reactions. Initial radicals, as mentioned, emerge in a singlet form ($H\uparrow + \downarrow OH$) and they may easily recombine back into water. Under the action of a magnetic field singlet-triplet transition ($H\uparrow + \downarrow OH \rightarrow H\downarrow + \downarrow OH$) may occur. This prohibits recombination of the radicals favoring the development of the array of reactions 3-7 and others. Development of branching chain reactions in aqueous systems containing oxygen and some other admixtures significantly changes their properties, but as free radical reactions, especially branching chain reactions are highly non-linear, the overall effect should depend drastically upon slight variations of initial conditions.

5.2. Burning of Water and Burning in Water

As it is mentioned above, singlet oxygen belongs to the family of ROS. Recently it was discovered that besides being a source of O_2, water may be directly oxidized with it. This reaction is readily catalyzed *in vitro* by antibodies (immunoglobulins) provided that energy of activation for excitation of molecular oxygen to its singlet state was supplied by dim light illumination of an antibody solution [29]. In other words, antibodies promote water “burning”. Catalysts do not “invent” reactions that can not go without them. They organize the reactants in space (and time) so that thermodynamically favorable processes go much faster. Quantum chemical calculations has shown that if two or more water molecules are arranged in space in particular disposition in relation to singlet oxygen and to each other, energy of activation for oxidation of a water molecule with singlet oxygen diminishes to reasonable values and such exotic peroxides as HOOOH, HOOOOH, HOO-HOOO may be produced under mild conditions as intermediates on the way to a more stable H_2O_2 [30]. Water oxidation goes on very fast in a solution of antibody because its active center provides for the optimal arrangement of water molecules for the process.

It is reasonable to suggest that if water is organized in a favorable way by some other means, if singlet oxygen is supplied, for example by the reactions #6 and #7, water oxidation may proceed in aqueous solutions in which water splitting had been initiated. We observed that in the course of branching chain reaction of slow oxidation of amino acids in aqueous solutions initiated with H_2O_2, concentration of H_2O_2 increases to the levels that can be explained only by water oxidation with O_2 [31]. Recently it has been shown that in water containing carbonates and phosphates [32] or noble gases, such as argon [33] concentration of H_2O_2 spontaneously increases and its augmentation goes on faster in case of water stirring. Using chemiluminescent methods we also found that such process goes on in aerated mineral waters from natural sources [34].

Thus, water – the most abundant substance in any living system, should regularly produce oxygen free radical and other forms of ROS under mild physiological conditions. The fact that a substantial part of organismal water is interfacial and dynamically structured increases the probability of its splitting and oxidation with all the above-listed consequences.

6. BIOLOGICAL SIGNIFICANCE OF ROS METABOLISM.

Controversy related to biological significance of reactive oxygen species including free radicals in normal and pathological physiology and biochemistry is reaching its climax. Vast literature is devoted to ROS production especially under *in vitro* or cell culture conditions and detrimental chemical properties of ROS due to which they induce multiple lesions in important bioorganic molecules.

On the other hand during the last decade more and more evidence appear that ROS play an important and probably even ubiquitous regulatory role in all living organisms – from microbes to higher animals, and the number of works devoted to the bio-regulatory role of ROS grows now exponentially. It turned out that adequate reaction of cells upon the action of hormones, neurotransmitters, cytokines, upon physical stimuli (light, temperature, mechanical stimulation) requires certain ROS levels in the environment. Some reactions, from the reversible activation or inhibition of certain enzymatic chains to genome activity regulation, which are provoked in cells by chemical signals, may be induced in them by ROS addition [35]. ROS are shown to have wholesome effects: they promote differentiation of cultured malignant cells into their benign counterparts [36], improve properties of taken out blood [37], and exercise significant therapeutic effects [38].

Besides, it turned out that common belief that under normal physiological conditions only a few percent of oxygen goes on ROS production is wrong. Due to ubiquitous presence of enzymes belonging to NADPH-oxidase family, to other means of direct oxygen reduction even under resting conditions up to 20% of all oxygen consumed is directly reduced and go on ROS production [39]; this share may increase up to 70% when physiological activity is enhanced [40].

Although a substantial part of inhaled oxygen is used for ROS generation, stationary levels of $O_2^{-}\bullet$ in cells and tissues do not exceed 10^{-10} -10^{-11} M [41], while that of H_2O_2 in a cell cytoplasm is estimated as 10^{-7} - 10^{-9} M [42]. ROS are kept at such low levels due to their nearly immediate elimination by the powerful antioxidant system. This seems to be puzzling: an organism converts a substantial share of oxygen into ROS and immediately eliminates these particles. How to explain such apparent squandering? And how can these particles, which are so short-lived and practically devoid of *chemical specificity* exercise specific bioregulatory actions?

7. BIOENERGETIC FUNCTIONS OF ELECTRON EXCITED STATES.

We suppose that difficulties in comprehension of the real role of ROS in vital activity are related to the attitude to them only as to chemical particles, while they should be considered as participants of continuous flux of oxygen reduction to water: $O_2 + 2H_2 \rightarrow 2H_2O$. This reaction consists of several steps and in order to unveil its intimate details we suggest the following notation:

$$
\begin{array}{lr}
4(O_2 + e^- + H^+) \rightarrow 4HO_2\bullet & [8] \\
2(HO_2\bullet + HO_2\bullet) \rightarrow 2H_2O_2 + 2O_2 & [9] \\
H_2O_2 + H_2O_2 \rightarrow 2H_2O + O_2 & [10] \\
\hline
4O_2 + 4e^- + 4H^+ \rightarrow 2H_2O + 3O_2 & [11]
\end{array}
$$

From such a notation of oxygen reduction several important conclusions follow. First, if oxygen excess over the electrons that reduce it is less than 4-fold, combustion does not go to a final point, and intermediate ROS accumulate, which may initiate chain reactions with bioorganic molecules. Thus, an adequate supply of oxygen is necessary for maintaining low stationary level of ROS and other free radical particles. Second, all these reactions imply recombination of unpaired electrons. This applies also to the reaction #10 where one H_2O_2 molecule may be considered an electron donor and another an electron acceptor. Third, all these reactions are sources of energy quanta equivalent to electronic excitation energy. Energy yield in the reaction of dismutation of two superoxide radicals is ~22 kcal/mol, equal to the energy gap between triplet and excited singlet states of oxygen and equivalent to a near IR-photon (λ~1269 nm). When two singlet oxygen particles transit to triplet state simultaneously, EEE may be "pooled" and a doubled quantum of energy (equivalent to λ~635 nm, red light) is released [43]. Decomposition of two molecules of H_2O_2 donates an energy equivalent of 2 eV or $\lambda<610$ nm. When dismutation of $HO_2\bullet$ (reaction #9) is catalyzed by SOD or decomposition of H_2O_2 (reaction #10) is performed by catalase, quanta of high density energy should be generated with some megahertz frequencies due to very high turnover numbers of these two enzymes. This prevents energy from its immediate dissipation into heat and is favorable for energy pooling to even higher quanta.

A key role of EEE and related photon emission in the regulation of vital processes was discovered 80 years ago by A.G. Gurwitsch in the form of the so-called "mitogenetic radiation" – ultra-weak photon emission in the UV-range of EM-spectrum responsible for triggering cell division [44]. This radiation is emitted not only by living cells and tissues, but also by enzymatic (hydrolytic and glycolytic) and chemical reactions including gel-sol transitions in aqueous media. Water splitting and accessibility of active oxygen is a prerequisite condition for the emergence of this radiation [45]. Ultra-weak photon emission in the range from UV- to near IR of electromagnetic spectrum from living cells and chemical reactions in aqueous media [46] affect activity of enzymes [47], activity and morphology of cells and tissues [48], regulate locomotion and mutual orientation of cultured cells [49]. Back reflected photons emitted during respiratory burst in human blood affect the intensity of this immune reaction by a feed-back mechanism [50].

In our opinion regulatory role of ROS is provided by the unique feature of reactions with their participation – generation of electronic excitation energy (EEE) that continuously pumps biophotonic fields of living systems. But if reactions with ROS participation play such a versatile role, they should proceed in all living things including those that are considered to be anaerobic. Indeed, even obligate anaerobic bacteria are equipped with SOD [51] indicating that ROS appear even when molecular O2 concentration in water is negligibly low. However, the intrinsic property of water to produce oxygen radicals due to its splitting makes their appearance in liquid water practically inevitable.

8. OSCILLATORY NATURE OF ACTIVE OXYGEN DEPENDENT REACTIONS IN AQUEOUS SYSTEMS

Besides serving a role of a source of the highest grade of energy, processes going on in aqueous systems in which EEE are generated may automatically acquire oscillatory character and are capable to serve as pacemakers for biochemical reactions dependent on them. Arousal of ROS in reactions going by in water and generation of EEE provides for the involving of other substances such as nitrogen and carbon dioxide into the process. They may beget amine and carbonyl compounds, and when concentrations of the latter exceed certain thresholds amino-carbonyl (Maillard) reaction develops. In this reaction biologically significant heterocyclic, aromatic, polymeric substances appear [52]. Some of them activate oxygen resulting in ROS production and generation of EEE [28]. We found that profound oscillations of photon emission [53] and redox potential [54] emerge in Maillard reaction. Oscillations last for many hours and even days and their periods extend from fractions of minutes to tens of minutes. Amplitudes of redox potential variations may reach 0,3 V (from -0,2 V to -0,5 V).

High redox potential differences between different parts of the system can not be explained only from uneven distribution of reduced and oxidized forms of organic components because of their low concentrations (few tens of millimolar). It is interesting to speculate that these differences reflect gross changes in reduction and oxidation state of aqueous medium itself.

What is the primary cause of the development of oscillations of ROS production and oscillations of EEE generation? Our experimental data indicate that generation of EEE in reactions with ROS participation is prerequisite for self-organization observed as these processes develop. Initial building up of EEE fosters oxidation and oxygenation of available substrates resulting in an exhaust of dissolved oxygen and accumulation of reducing (easily oxidizable) equivalents. Oxygen continues to diffuse into the system from the air and when its concentration and concentration of reducers reach optimal ratio, a new wave of burning appear followed with oxygen depletion until the concentration of diffusing oxygen reaches a threshold value again. Thus, oscillatory behavior naturally emerges in such systems.

It is notable, that oxygen consumption in single neutrophils and other cells that reduce it to ROS using NADPH-oxidase exhibits multimode oscillatory patterns of ROS generation [55]. Some hormones influence the amplitude of these oscillations, other affect their frequency. In other words, both deepness of respiration of single cells and its rate are related to their functional activity. Respiration rate and deepness (especially in case when oxygen consumption is realized through it one-electron reduction) define in their turn downstream regulatory processes.

Oscillatory behavior is characteristic not only of single cells, but of their populations as well. We observed pronounced oscillations of photon emission from neutrophil suspensions containing hundreds of thousands of cells and even in whole blood, indication of a collective behavior of these big groups of cells related to metabolism of ROS in them [50].

Amino-carbonyl reaction proceeding in aqueous systems in which oscillations and waves spontaneously emerge is, in our opinion, the simplest model of arousal and performance of the respiratory process. Such conditions for the emergence of oscillations of EEE are common to all cells. A steep oxygen gradient between a metabolizing cell and its environment exists. Oxygen is poorly soluble in water, and what is more important, its delivery to a cell may be regulated by interfacial water at a cell-environment boundary. Reducing equivalents (e.g., NAD(P)H) accumulate in cells due to their metabolic activity. When the ratio of these equivalents to incoming O_2 reaches threshold values energy discharge primarily in the form of EEE occurs. Oxygen is rapidly exhausted, and released energy is directed for metabolic needs. Indeed recently it has been experimentally demonstrated that oxygen is taken by single cells in an oscillatory mode [56]. Oscillations of EEE may play the role of pacemakers for the processes going on different levels of biological organization. On the other hand oscillatory nature of all these processes provides them the properties of sensible receptors for external electromagnetic and other physical fields.

9. RESPIRATION CYCLE OF WATER: A HYPOTHESIS

Respiration as we know it is a cyclic process. Though it is not so obvious that respiration at a level of a single cell should also be cyclic, experimental evidence supports this conclusion. It can be suggested that cyclic nature of respiration emerges on the one hand from the spatial relationship of oxygen consuming system and its environment and on the other from the orderliness of energy fluxes and high density of energy (EEE) that is generated in the course of oxygen-dependent processes in which ROS participate. Taking into consideration that all the aforementioned phenomena occurred in aqueous systems and that ROS generation is the intrinsic property of water we suggest a hypothesis of the existence of the “respiratory cycle of water”. Splitting of water molecules under the action of low density energy (mechano-chemical or mechano-catalytic water decomposition) results in the appearance of oxygen and hydrogen in aqueous systems:

$$8H_2O \rightarrow 4O_2 + 16H\bullet \qquad [12]$$

Four hydrogen atoms (H•) are needed for complete reduction of one oxygen molecule, the rest hydrogen atoms recombine to H_2 molecules: $12\ H\bullet \rightarrow 6H_2 \Uparrow + n(hv)$. EEE released may be used, for example, for excitation of oxygen with the appearance of singlet oxygen, for sustaining of an aqueous system in a non-equilibrium, excited state, etc. This sequence of events may be by convention defined as the “exhale” stage because water splitting is accompanied with gas (hydrogen) release.

What may follow afterwards is analogous to the “inhale” stage, as oxygen is consumed here. We remind that for the complete reduction of oxygen molecule a 4-fold excess of oxygen is needed:

$$4O_2 + 4H\bullet \rightarrow 2H_2O + 3O_2 + m(h\nu); \qquad [13]$$

Energy released in the course of the reactions #12 and #13 is enough to excite oxygen to a singlet state, and under appropriate conditions $^1O_2$ may go on water oxidation:

$$3O_2^* + 6H_2O \rightarrow 6H_2O_2 \qquad [14]$$

“Respiration cycle of water” allows to transform low density energy (freezing-thawing, evaporation-condensation, energy of sound, energy of shearing forces of water filtration or its vortexing) into a high density one; at least some part of the latter may accumulate in water in the form of metastable substances such as H_2O_2 and other peroxides as well as in long-living water excitation making it an active physical medium.

As other gases and substances that are present in “real” water should get involved in the process, respiration cycle should be considered not as a closed loop, but rather as a single convolution of an untwisting helix. Real processes proceeding in water should significantly depend upon the presence of positive and negative catalysts of particular reactions, of substances affecting water structure, upon the nature of interfaces that it solvates, upon the action of external physical factors and fields. Studies of phenomena related to water may help in solving many practical problems of medicine, agriculture, environmental problems, in providing people with healthy drinking water, in optimization of technologies in which water is important.

10. REFERENCES

1 S. Kleiner, Water: An essential but overlooked nutrient, *J. Amer. Diet. Assoc.* **99,** 200-206, (1999).

2 A. Szent-Gyorgyi, *Bioelectronics: A Study in Cellular Regulations, Defense, and Cancer* (Academic Press, New York, 1968), p. 4.

3 N. A. Bulionkov, Periodic dissipative-module structures of “bound water” – possible constructions defining biopolymer conformations in structures of their hydrates, *Krystallografia (Moscow).* **35**, 155-159, (1988).

4 S. V. Zenin, Hydrophobic model of water molecules associates, *Zhurnal Fizicheskoi Himii.* **68**, 634-641, (1994).

5 M. F. Chaplin, A proposal for the structuring of water, *Biophys. Chem.* **83**, 211-221, (2000).

6 S. Maheshwary, N. Patel, N. Sathyamurthy, A. D. Kulkarni, and S.R. Gadre, Structure and stability of water clusters $(H_2O)_n$, n = 8-20, An Ab Initio investigation, *J. Phys. Chem.-A.* **105**, 10525-10537, (2001).

7 M. Chaplin; http://www.martin.chaplin.btinternet.co.uk/index.html

8 G. N. Ling, A new theoretical foundation for the polarized-oriented multilayer theory of cell water and for inanimate systems demonstrating long-range dynamic structuring of water, *Physiol. Chem. Phys. & Med. NMR.* **35**, 91-130, (2003).

9 I. L. Cameron, K. M. Kanal, C. R. Keener, and G. D. Fullerton, A mechanistic view of the non-ideal osmotic and motional behavior of intracellular water, *Cell. Biol. Int.* **21**, 99-113, (1997).

10 S. P. Jarvis, T. Uchihashi, T. Ishida, and H. Tokumoto, Local solvation shell measurement in water using a carbon nanotube probe, *J. Phys. Chem. B.* **104**, 6091-6094, (2000).

11 P. Wernet, D. Nordlund, U. Bergmann, M. Cavalleri, M. Odelius, H. Ogasawara, L. A. Naslund, T. K. Hirsch, L. Ojamae, P. L. Glatzel, G. M. Pettersson, and A. Nilsson, The structure of the first coordination shell in liquid water, *Science.* **304**, 995-999, (2004)

12 P. Mentre, An introduction to "water in the cell": tamed hydra? *Cell. Mol. Biol.* **47**, 709-715, (2001).

13 D. Hall, and A. P. Minton, Macromolecular crowding, qualitative and semiquantitative successes, quantitative challenges. *BBA-Proteins Proteomics.* **1649**, 127–139, (2003).

14 J. Higo, M. Sasai, H. Shirai, H. Nakamura, T. Kugimiya, Large vortex-like structure of dipole field in computer models of liquid water and dipole-bridge between biomolecules, *Proc. Nat. Acad. Sci USA.* **98**, 5961–5964, (2001).

15 P. M. Wiggins, Enzyme reactions and two-state water, *J. Biol. Phys. Chem.* 2002, **2,** 25-37, (1990)

16 P. M. Wiggins, Role of water in some biological processes, *Microbiol. Rev.* **54**, 432-449.

17 G. N. Ling *A physical theory of living state: the Association-Induction Hypothesis.* (Blaisdell Publ. Co., Waitham, MA, 1962).

18 G. H. Pollack, *Cells, Gels and the Engines of Life: A New, Unifying Approach to Cell Function.* (Ebner & Sons, Seattle, WA, 20010.

19 P. M. Wiggins, Two states of water found in hydrophobic clefts: their possible contribution to mechanism of cation pumps and other enzymes. *Internat. Rev. Cytol.* **108**, 249-303, (1987) .

20 P. M. Wiggins, Water structure in polymer membranes, *Progr. Polym. Sci.* 1988, **13**, 1-35.

21 P. M. Wiggins, Life depends upon two kinds of water. 64 pp; http://www.lsbu.ac.uk/water/monograph200904pw.pdf

22 A. Szent-Gyorgyi, *Bioenergetics.* (Academic Press. New York, 1957). [Back translation from a Russian edition of the book: (GIZ Fiz-Mat. Literature, Moscow, 1960), p. 54-56].

23 N. K. Baramboim, *Mechanochemistry of High Molecular Weight Compounds.* 9Chimiya,Moscow, 1971).

24 A. Domrachev, G. A. Roldigin, and D. A. Selivanovsky, Role of sound and liquid water as dynamically unstable polymeric system in mechano-chemically activated processes of oxygen production on Earth, *J. Phys. Chem.* **66**, 851-855, *(*1992).

25 G. A. Domrachev, G. A. Roldigin, and D. A. Selivanovsky, Mechano-chemically activated water dissociation in a liquid phase, *Proc. Russ. Acad. Sci.* **329**, 258-265, (1993) .

26 S. Ikeda, T. Takata, M. Komoda, M. Hara, J. N. Kondo, K. Domen, A. Tanaka, H. Hosono, and H. Kawazoe, Mechano-catalysis -- a novel method for overall water splitting, *Phys. Chem. Chem. Phys.* **1**, 4485-4491, *(*1999).

27 S. Woutersen, and H. J. Bakker Resonant intermolecular transfer of vibrational energy in liquid water, *Nature.* **402**, 507 – 509, (1999).

28 V. L. Voeikov, and V. I. Naletov, Weak photon emission of non-linear chemical reactions of amino acids and sugars in aqueous solutions, *Biophoton,* edited by J.-J. Chang, J. Fisch, and F.-A. Popp (Kluwer Academic Publishers, Dortrecht, The Netherlands, 1998), pp. 93-108.

29 A. D. Wentworth, L. H. Jones, P. Wentworth, Jr, K. D. Janda, and R. A. Lerner, Antibodies have the intrinsic capacity to destroy antigens, *Proc. Nat. Acad. Sci. USA.* **97**, 10930–10935, (2000).

30 X. Xu, R.P. Muller, and W.A. Goddard 3rd. The gas phase reaction of singlet dioxygen with water: a water-catalyzed mechanism. *Proc. Nat. Acad. Sci. USA.* **99**, 3376-3381, (2002).

31 V. L. Voeikov, I. V. Baskakov, K. Kafkialias, and V. I. Naletov, Initiation of degenerate-branched chain reaction of glycin deamination with ultraweak UV irradiation or hydrogen peroxide, *Russ. J. Bioorg. Chem.* **22**, 35-42, (1996).

32 V. I. Bruskov, A. V. Chernikov, S. V. Gudkov, and Zh. K. Masalimov, Activation of reducing properties of anions in sea water under the action of heat, *Biofizika.* **48**, 1022-1029, (2003).

33 V. L. Voeikov, and M. V. Khimich, Amplification by argon of luminol-dependent chemiluminescence in aqueous NaCl/H2O2 solutions, *Biofizika.* **47**, 5-11, (2002).

34 V. L. Voeikov, R. Asfaramov, V. Koldunov, D. Kononov, C. Novikov, and N. Vilenskaya, Chemiluminescent analysis reveals spontaneous oxygen-dependent accumulation of high density energy in natural waters, *Clin. Lab.* **49**, 569, (2003).

35 V. L. Voeikov, Reactive oxygen species, water, photons, and life, *Rivista di Biologia/Biology Forum.* **94**, 193-214, (2001).

36 H. Sauer, M. Wartenberg, and J. Hescheler, Reactive Oxygen Species as Intracellular Messengers During Cell Growth and Differentiation, *Cell. Physiol. Biochem.* **11**, 173-186, (2001).

37 V. Bocci, Autohemotherapy after treatment of blood with ozone. A reappraisal, *J. Int. Med. Res.* **22**, 131-144, *(*1994).

38 C. F. Nathan, and Z. A. Cohn, Antitumor Effects of Hydrogen Peroxide in Vivo, *J. Exp. Med.* **154**, 1539-1550, *(*1981).

39 H. P. Souza, X. Liu, A. Samouilov, P. Kuppusamy, F. R. Laurindo, and J. L. Zweier, Quantitation of superoxide generation and substrate utilization by vascular NAD(P)H oxidase, *Am. J. Physiol. Heart Circ. Physiol.* **282**, H466-H474, (2002).

40 J. R. Trimarchi, L. Liu, D. M. Porterfield, P. J. Smith, and D. L. Keefe, Oxidative phosphorylation-dependent and -independent oxygen consumption by individual preimplantation mouse embryos, *Biol. Reprod.* **62**, 1866-1874, *(*2000).

41 V. Niviere, and M. Fontecave, Biological sources of reduced oxygen species, *Analysis of Free Radicals in Biological Systems,* edited by A. E. Favier, J. Cadet and B. Kalyanaraman (Birkhauser, Basel, Boston, Berlin, 1995), pp. 11-19.

42 D. D. Tyler, Polarographic assay and intracellular distribution of superoxide dismutase in rat liver, *Biochem. J.* **147**, 493-504, (1975)

43 E. Cadenas, and H. Sies, Low-level chemiluminescence as an indicator of singlet molecular oxygen in biological systems, *Methods Enzymol.* **105**, 221-231, (1984)

44 A. G. Gurwitsch, and L. D. Gurwitsch, Twenty years of mitogenetic radiation, emergence, development, and perspectives, *Usp. Sovrem. Biologii.* **16**, 305-334, (1943). (English translation: *21st Century Science and Technology*. Fall, 12, 41-53, (1999).)

45 V. L. Voeikov, Mitogenetic radiation, biophotons, and non-linear oxidative processes in aqueous media, *Integrative Biopysics. Biophotonics,* edited by F.-A. Popp and L. Beloussov, (Kluwer Academic Publishers, Dordrecht, 2003), pp. 331-360.

46 J. Slawinski, Luminescence research and its relation to ultraweak cell radiation*,* *Experientia.* **44**, 559-571, (1988).

47 G. Cilento, Photobiochemistry without light, *Experientia.* **44,** 572-576, (1988).

48 V. P. Galantsev, S. G. Kovalenko, A. A. Moltchanov, and V. I. Prutskov, Lipid peroxidation, low-level chemiluminescence and regulation of secretion in the mammary gland, *Experientia.* **49**, 870-875, (1993).

49 G. Albrect-Buehler, Changes of cell behavior by near-infrared signals, *Cell. Motil. Cytoskeleton.* **32**, 299-304, (1995).

50 V. L. Voeikov, R. R. Asfaramov, E. V. Bouravleva, C. N. Novikov, and N. D. Vilenskaya, Biophoton research in blood reveals its holistic properties, *Indian J. Exp. Biol.* **43**, 473-482, (2003).

51 J. Hewitt, and J. Morris, Superoxide dismutase in some obligately anaerobic bacteria*,* *FEBS Lett.* **50**, 315-318, (1975).

52 M. Namiki, T. Hayashi, S. and Kawakishi, Free radicals developed in the amino-carbonyl reaction of sugars with amino acids, *Agric. Biol. Chem.* **37**, 2935-2937, (1973).

53 V. L. Voeikov, V. V. Koldunov, and D. S. Kononov, Long-duration oscillations of chemi-luminescence during the amino-carbonyl reaction in aqueous solutions, *Russ. J. Phys. Chem.* **75**, 1443-1448, (2001).

54 V. L. Voeikov, V. V. Koldunov, and D. S. Kononov, New oscillatory process in aqueous solutions of compounds containing carbonyl and amino groups, *Kinetics and Catalysis (Moscow).* **42**, 606-609 (2001).

55 A. L. Kindzelskii, and H. R. Petty, Apparent role of traveling metabolic waves in oxidant release by living neutrophils, *Proc. Natl. Acad. Sci. USA.* **99**, 9207-9212, (2002).

56 D. M. Porterfield, R. F. Corkey, R. H. Sanger, K. Tornheim, P. J. S. Smith, and B. E. Corkey, Oxygen consumption oscillates in single clonal pancreatic - cells (HIT), *Diabetes.* **49**, 1511–1516, (2000).