In: *Biophotonics-Optical Science and Engineering for the 21st Century*,
Van Wijk, Roeland; Shen, Xun (Eds.)
Springer Science+Business Media, Berlin-Heidelberg-New York
2005, pp. **141-154** Approx. 240 p. ISBN: 0-387-24995-8 (2005).

BIOPHOTONIC ANALYSIS OF SPONTANEOUS SELF-ORGANIZING OXIDATIVE PROCESSES IN AQUEOUS SYSTEMS.

Vladimir L. Voeikov[*]

1. INTRODUCTION

Current concept of low-level photon emission (LLPE[†]) of biological objects considers it as chemiluminescence resulting from relaxation of electronically excited states generated in reactions with the participation of reactive oxygen species (ROS). In their turn oxygen free radical metabolites and other ROS had been considered until recently either as by-products of "normal" biochemical processes or at best as exotic chemical products of specialized immune cells designed to destroy alien viruses and bacteria. Thus, inasmuch as processes with ROS participation are still regarded by the majority of bio-medical scientists as auxiliary to "normal" biochemistry, LLPE which accompany these processes is looked upon as irrelevant to the performing vital functions.

Another point of view on LLPE from living matter is based on the notion that it originates from a delocalized coherent electromagnetic field that is tightly coupled to metabolic processes. In this context LLPE is termed as "biophotonic emission" and coherence theory "assigns to the presumably phase locked and mode coupled photons from DNA a permanent regulatory activity within cells and also between cells"[1]. However, this theory does not specify the source of energy which continuously pumps the biophotonic field.

At the same time it should be reminded about the works of Alexander Gurwitsch, the pioneer of the field of biophoton research. LLPE discovered by him more than 80 years ago was named "Mitogenetic radiation" because it performs a major biological function – it stimulates mitoses in competent cells. Many features of this radiation indicated its coherent nature (in a wide sense of this term), and at the same time it was proved by Gurwitsch that oxygen-dependent free radical reactions were indispensable for its origination (see[2] for the details and references therein). Gurwitsch's discoveries and ideas had initially attracted much interest and initiated in 1930 wide studies of mitogenetic

[*] Vladimir L. Voeikov, Faculty of Biology, Lomonosov Moscow State University, Moscow, 119234, Russia
[†] Abbreviations: Low level photon emission – LLPE, reactive oxygen species – ROS, electron excitation energy – EEE, electronically excited states – EES, chemiluminescence – CL, Maillard reaction – MR .

radiation. Regrettably, they are nearly completely forgotten because of an unfortunate combination of complex historical and psychological factors.

In recent years the attitude to oxygen radicals as to only hazardous by-products of "normal" metabolism started to change. More and more evidence is accumulating that they play an important if not a central role in all aspects of regulation of biological functions[3]. Still, the connection of their bioregulatory role with biophotonic field is not yet recognized. Here we'll present arguments approving such a connection and illustrate them with evidence in favor of intrinsic property of the processes running in aqueous milieu of living systems with ROS participation to self-organization which by itself is a crucial property of living matter.

2. PATHWAYS OF OXYGEN CONSUMPTION IN LIVING ORGANISMS

Practically all living organisms on Earth gain energy for performing their functions from oxygen-dependent oxidation of various "fuels". It is generally accepted that in the course of aerobic respiration oxygen is used in the terminal stage of the chain of oxidative phosphorylation in mitochondria. It is reduced there by cytochrome c reductase which transfers 4 electrons (together with 4 protons) to oxygen molecule. Oxygen plays here the role of the "trash box" for electrons that had already used their redox potential for the synthesis of ATP molecules while traveling along the respiratory chain. High initial energy potential of electrons obtained from food dehydrogenation is coined in this way into multiple small portions: energy released in hydrolysis of 1 ATP molecule does not exceed 0,5 eV, equivalent to IR-photons with λ~2,5 μ. Oxidative process in which energy is received in such a form, in principle, in a form of heat, is analogous to putrefaction or smoldering. This pathway of oxygen utilization for gaining energy in the form of ATP is considered to be practically the only one in "normal" biochemistry.

But smoldering is not the only way of energy gaining from oxygen-dependent oxidative processes. Another one is burning (combustion). The difference between the two is that in the latter O_2 is directly reduced with electrons (which often go together with protons). It should be reminded that oxygen is a unique molecule, which in its ground state contains 2 unpaired electrons, and at the same time each single oxygen atom contains also two unpaired electrons. Thus a total of 4 electrons are needed to equilibrate all its unpaired electrons. "Heavy" energy quanta are released at each act of electron pairing. On the way of one-electron O_2 reduction in few discrete steps a total of 8 eV may be received as O_2 receives electrons sequentially according to the following scheme:

$$1)\ \bullet O_2\bullet + e^- (+H^+) \rightarrow HO_2\bullet; \quad 2)\ HO_2\bullet + e^- (+H^+) \rightarrow H_2O_2;$$

$$3)\ H_2O_2 + e^- (+H^+) \rightarrow H_2O + HO\bullet; \quad 4)\ HO\bullet + e^- (+H^+) \rightarrow H_2O$$

Intermediate products of its reduction are either free radicals ("•" symbolizes an unpaired electron), or metastable molecules such as H_2O_2 possessing high chemical potential. All of them are defined as Reactive Oxygen Species (ROS).

One-electron oxygen reduction leading to the emergence of ROS is still considered by many apprentice and even connoisseurs in bio-medical research, as a dark side of dioxygen biochemistry[4]. Such an attitude stems from innumerous studies on mostly *in vitro* biochemical and cell culture models, where it has been demonstrated that ROS easily damage lipids, proteins and nucleic acids. This damage may be a massive one, because a single free radical may in principle initiate chain reaction in the course of which many molecules may be affected. However, on this way not only very active particles originate, but on each step a quantum of energy is released that belongs to the

range of EEE rather than to the range of energy of vibrational and rotational excitation as in smoldering. Thus, one-electron oxygen reduction is genuine burning, which may be accompanied by release of light photons.

Nearly anonymous attitude to ROS as universal pathogens is now gradually eroding. An exponential growth of the number of works devoted to the bio-regulatory role of ROS (see refs. in[3, 5]) is observed now. Adequate reactions of cells upon hormones, neurotransmitters, cytokines, upon physical stimuli (light, temperature, mechanical stimulation) finally depend on ROS "background". ROS added to cultured cells may induce in them normal physiological reactions, from reversible activation or inhibition of certain enzymatic chains to switching genome activity. However, until now no consistent model of the mechanism of regulatory ROS action is suggested.

But how much oxygen consumed by animals goes to ROS generation? Until now in numerous papers reference is made to estimates that were obtained decades ago in *in vitro* systems, e. g. in isolated mitochondria that were for a long time considered to be the major source of ROS due to accidental "escape" of electrons from electron-transport chain directly to oxygen. According to these estimates the share does not exceed one, at most – a few percent. However, recent direct measurements had shown that this share may be remarkably high. For example, in cleavage stage chicken embryos 70% of O_2 consumed is one-electronically reduced, and this share decreases to 30% at the blastocyst stage. This is due to increase of mitochondrial O_2 consumption, while absolute quantity of directly reduced O_2 does not change[6]. In isolated rat aorta more than 27% of all O_2 taken by aorta tissue is converted to superoxide anion ($O_2\bullet^-$)[7].

Actually, these results are not unpredicted. There are plenty of ways for one-electron oxygen reduction in an organism. For example, NADPH-oxidase, the enzyme that directly reduces O_2 to $O_2\bullet^-$ that was initially considered to be specific for immune cells such as neutrophils and eosinophils, is now found in practically all types of cells of multicellular and in unicellular organisms[5]. It is one of the group of other enzymes that are able reduce O_2 while performing their normal functions. ROS are also generated in multiple non-enzymatic reactions that continuously proceed in any organism, such as the amino-carbonyl (Maillard) reaction[8].

On the other hand stationary levels of ROS in cells and tissues are very low (of the order of 10^{-6} M or less) due to the universal presence in cells and tissues of the so-called antioxidative system represented by a variety of enzymes and low-molecular weight "antioxidants". For example, the ubiquitous enzyme, superoxide dismutase, nearly immediately converts $O_2\bullet^-$ to H_2O_2 and O_2. H_2O_2 is in turn very quickly degraded by catalase. The question of why an organism directs such a huge share of valuable oxygen for ROS production and eliminates them practically immediately is not discussed in the literature.

3. BIO-REGULATORY FUNCTIONS OF PROCESSES ACCOMPANIED WITH GENERATION OF ELECTRON EXCITATION ENERGY

A flaw in the efforts to explain the mechanism of bio-regulatory action of ROS by analogy with molecular signaling mechanisms may be related to disregard of the unique high energy output of reactions with ROS participation. It is known that when unpaired electrons recombine energy quanta sufficient to induce electronically excited states (EES) of reaction products are liberated. Such reactions are usually accompanied with chemiluminescence (CL) and other forms of luminescence. A probable role of EEE, of

EES of biomolecules, of LLPE has received little attention in contemporary cell physiology. This originates possibly from underestimation of the quantity of oxygen that is directly reduced by living organisms in the course of their normal functioning. Thus, we decided to study carefully if oxidative processes with ROS participation accompanied with PE, indicating of EEE generation, may play a regulatory role in a biological system such as blood and also in aqueous model systems in which such processes develop spontaneously.

We have chosen human blood as an experimental system not only because it donates oxygen for all cells and tissues of an organism, but because it itself contains consumers of oxygen. It has been shown recently that neutrophils and eosinophils representing the majority of leucocytes, even in their resting state convert all oxygen that they consume into free radicals[9], not to mention the production of ROS in their stimulated state when it increases more than 10-fold. Besides a short time ago it was discovered that immunoglobulins catalyze direct oxygenation of water with the appearance of H_2O_2 and even ozone[10].

Blood is a complex biological system, but at the same time it is an aqueous system. ROS that are initially produced in it represent hydrophilic particles and the processes in which they participate may proceed in aqueous phase. For revealing basic properties of such processes we used a model aqueous system – solutions of amino acids in which slow oxygen-dependent oxidative processes spontaneously develop.

As the reactions with ROS participation are accompanied with LLPE, the principle method that we used was monitoring PE from experimental systems under different conditions with single photon detectors predominantly for prolonged periods of time to reveal dynamic peculiarities of these processes.

3.1. Biophoton emission from non-diluted human blood.

It can be seen from Fig. 1 that addition to whole non-dilute human blood of zymosan – a specific inducer of respiratory burst of neutrophils, results in a significant elevation of LLPE from blood that lasts for many hours (left Y-axis). In the presence of luminol, a fluorescent probe for reactions with H_2O_2 and some other ROS participation, maximal intensity of PE elevates about 100-fold (right Y-axis), while its kinetics in the first approximation remains the same. Thus very high optical density of blood does not interfere with the registration of PE from it.

We've also found that PE may be registered even in "resting" blood after addition of lucigenin (a luminescent probe for superoxide anion). However, lucigenin-dependent PE decreases with dilution of blood with physiological salt solution, while luminol-dependent PE in blood in which respiratory burst has been induced is not affected by such dilution[11]. Hence, ROS generation in blood accompanied with generation of EEE goes on persistently even under non-stimulated conditions, provided by a close interaction of oxygen donors (erythrocytes) and oxygen acceptors (neutrophils). Under such conditions lucigenin-dependent PE may be observed even from small portions of blood for many hours.

The very fact that pronounced PE may be registered from non-diluted blood -- a highly opaque liquid because of very high concentration of hemoglobin – indicates that hemoglobin packed in erythrocytes does not quench efficiently PE. However, if free hemoglobin is added to blood at a concentration of only 0,5% of the amount present, lucigenin-dependent PE practically disappears[12]. Taking into account that concentration

of hemoglobin in erythrocytes may reach a value as high as 35-40% (hemoglobin can not reach such high concentration in a free solution) one may suggest that hemoglobin in erythrocytes is present in a liquid crystalline state. In such a form it may provide transfer of excitation energy over long distances without its dissipation, unlike hemoglobin in a solution that absorbs and dissipates energy of electron excitation.

Under certain conditions PE from blood or suspensions of isolated neutrophils may gain oscillatory patterns in which amplitude of oscillations may reach up to 25% of the mean PE intensity (Fig. 2). These conditions include lack of agitation of a sample, an optimal buffered medium containing nutrients (in the case of neutrophil suspensions) and access to air.

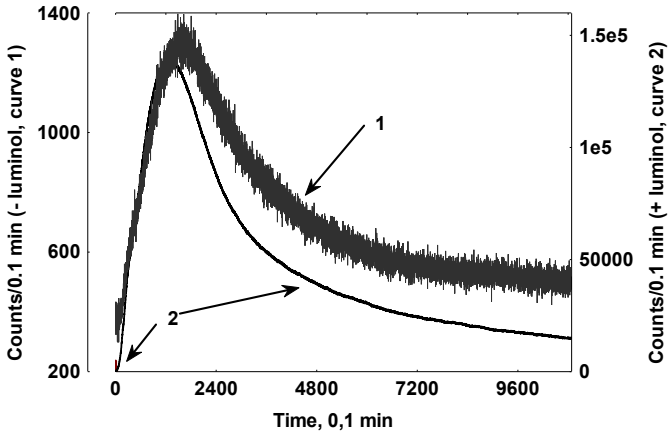

Figure 1. Photon emission from non-diluted blood (0.1 ml) without luminol (curve 1, left Y-scale) or with it (curve 2, right Y-scale) after induction of respiratory burst by zymosan (without zymosan and luminol PE from blood sample was 250-300 counts/6 sec).

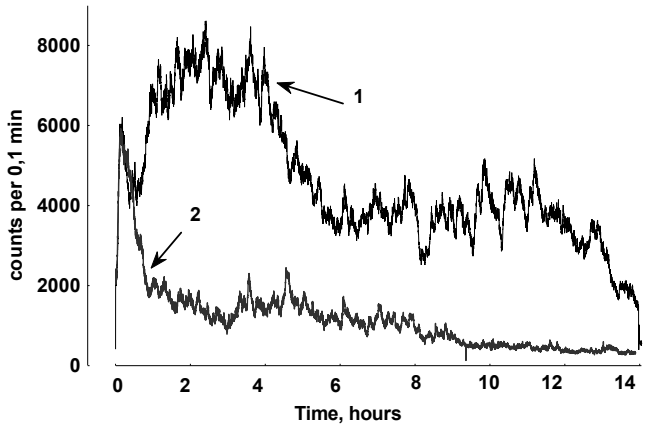

Figure 2. Sustained oscillations of luminol-dependent PE in neutrophil suspensions (20000 cells in 0.1 ml of M-PRM medium; during respiratory burst induced with zymosan. Curve 1 – aerobic conditions, Curve 2 -- suspension isolated from air

Figure 2 illustrates prominent oscillations of luminol-dependent PE from neutrophil suspensions that develop about 1 hour after the initiation of respiratory burst with zymosan. It can be seen that they are much more prolonged and prominent when the suspension is in contact with air. In the absence of air oscillatory behavior can also be seen, however, PE fades much earlier and amplitudes of oscillations are smaller.

Does EEE generated in blood in the reactions with ROS participation and expressed as LLPE have any functional role for the processes taking place or it is just a by-product of these processes? The answer to this question was obtained in experiments illustrated in Figure 3. If a test-tube with blood in placed in a glass vial, its walls reflect some part of the irradiated photon flux back into the blood. If a vial is filled with water, back reflection of photons is virtually absent.

In fact, transfer of a test tube with blood from an empty to a water-filled vial results in a sharp leap of PE resulting from increased escape of photons from the system due to the immersion effect. Besides this one can notice that self-irradiation or absence of it results in different dynamic patterns of PE from blood. At a stage when PE was approaching maximal values, blood transfer to the water-filled vial after a period of its presence in air-filled vial (period of self-irradiation) results in a significantly higher level of PE that could be expected without its self-irradiation (Fig. 3, left).

Especially prominent is the effect of self- irradiation at the stage of PE decay (Fig. 3, right). Without self-irradiation PE rapidly decays, while when this blood is again self-irradiated, respiratory burst starts to “kindle” again. It should be recognized that during self-irradiation blood receives no more than few thousands photons/sec, equivalent to heat energy gain of about $10^{-17} – 10^{-18}$ cal/sec. Thus, functional significance of ultra-weak photon fluxes can not result from gaining additional energy by blood. It is likely that EEE plays a signaling function in systems that are very far from energy equilibrium, being at the same time at thermal equilibrium with their surroundings. In a certain sense such a system as blood may be looked upon as a physical active medium.

This conclusion is supported by the results of studies of the dependence of PE from blood upon variations of its temperature. Blood sample in which respiratory burst was initiated with zymosan in the presence of luminol was sequentially heated and cooled in the range of physiological temperatures (Fig. 4). As PE is the integral result of complex chemical reactions one might expect that its intensity should change in accord with temperature variations in blood.

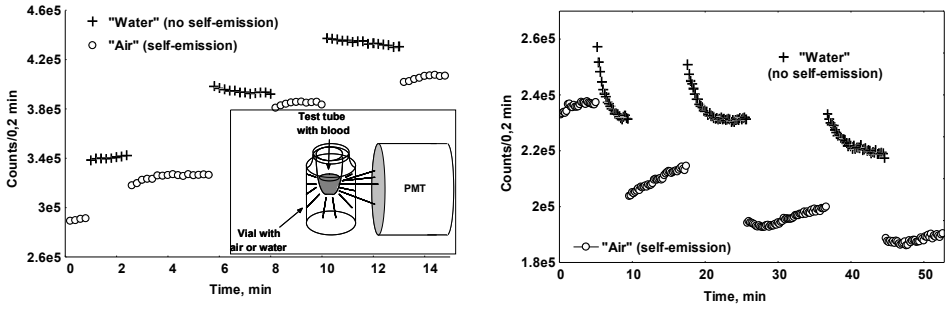

Figure 3. Effect of partial light reflection from the inner surface of the glass vial upon luminol-dependent PE at different stages of respiratory burst in whole blood. Left panel – 1 hr after zymosan addition, PE is approaching maximal intensity (insert – scheme of experimental setup). Right panel – 20 hrs after respiratory burst initiation, respiratory burst decay.

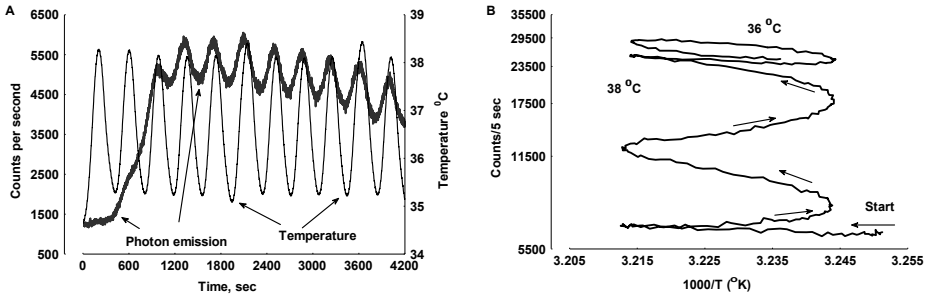

Figure 4. Temperature dependence of luminol-amplified PE from blood (3 ml) in which respiratory burst is induced with zymosan. (A) Original data. (B) Ahrrenius plot for the stage of development of respiratory burst (ca. up to 1700 sec).

Actual results indicate that this is not the case. PE rate practically does not depend on temperature at the stage of respiratory burst development. One can see even the negative slope for activation energy on the Ahrrenius plot (Fig. 4, B) for this stage. That suggests that blood is such an intense energy source by itself that it can easily overcome temperature energy losses when it is cooled. Only when PE intensity reaches maximal values temperature dependence shows itself, though a significant hysteresis is observed (upper part of the Ahrrenius plot) indicating that blood continues to oppose energy losses. Such dynamic properties of blood indicate that PE from it results from processes comprising a web of reactions with multiple positive and negative feedbacks. High level of coupling in these processes follows also from oscillatory behavior of PE from blood and neutrophil suspensions (see Fig. 2). In general, all these results argue that significant part of internal energy that provides blood non-equilibricity is represented by EEE.

3.2. Autoregulation in model aqueous systems related to ROS production and EEE generation.

Many features of the processes with ROS participation giving rise to EEE generation in blood may be considered to be related to its complex material composition. However, blood is essentially an aqueous system, so it is interesting to distinguish common features of oxygen-dependent oxidation reactions accompanied with PE going on in blood and in much more simple aqueous systems. To perform this comparison we used aqueous solutions of amino acids in which luminescent reactions emerge after addition of H_2O_2 or brief irradiation with an ultra-weak source of UV-light, and solutions containing amino acids and active reducing carbonyl compounds such as glucose or methylglyoxal[13]. Analysis of processes taking place in model systems in fact revealed some fundamental similarities between them and those observed in human blood.

We have previously shown[13], that addition of an aliquot of concentrated H_2O_2 to aqueous solutions of different amino acids to the final H_2O_2 concentrations of 0,1-0,4 M initiates in this solution the reaction of oxidative deamination of an amino acid that is accompanied by PE in the presence of different fluorescent compounds. The process is characterized with a definite lag-period after which a stage of acceleration of PE rate follows and then PE slowly declines. PE rate very well correlates with the rate of accumulation of ammonia at low (micromolar) concentrations of fluorophores, but at high concentrations of the latter both PE growth and deamination of amino acid are

retarded. Thus, PE rate reflects the rate of the major reaction going on in the system. Dependence of the rates of both parameters on concentration of fluorophores indicates that the chemical process is coupled to generation of EEE in the system[14].

One of the peculiarities of oxidative processes in model systems was the necessity to overcome some threshold conditions for these processes to develop. For example, PE does not develop in a solution of H_2O_2/asparagines if the concentration of the latter is below 25 mM, but if its concentration exceeds 30 mM the wave of emission under the same condition always develops. Thresholds (critical phenomena) are an intrinsic property of runaway chain reactions[13, 14]. Existence of critical phenomena in the studied processes indicate that they belong to a family of chain reactions with delayed branching.

Another specific feature of branching chain reactions is a significant deviation of their kinetic characteristics from the classical law of Arrhenius. In studies of temperature dependence of PE from solutions of amino acids performed in the same manner as with blood (cf. Fig. 4) similar hysteresis behavior was observed, though in blood hysteresis was more pronounced especially at the stage of PE development.

The second model system studied by us is the reaction that develops in aqueous solutions of active carbonyls (such as methylglyoxal) and amino acids, polyamines or even such simple amines as ethylamine. This model system is more complex, yet it is more physiological. The reaction that develops in it is known as amino-carbonyl or Maillard reaction (MR) and it continuously proceeds in a non-enzymatic way in the internal medium of living organisms. Disturbances in MR are considered to play a significant role in the pathogenesis of diabetes and other metabolic disorders.

Though MR is thoroughly investigated for many decades, its mechanism is far from being clear. Probably its complexity is related to the fact that already at the first stage of the reaction – the formation of Schiff bases, there appear conditions for one-electron oxygen reduction, for ROS and other free radicals generation[15], and, hence, for the emergence EEE. Owing to the appearance of fluorescent and colored products in the course of MR, propagation and modulation of EEE may occur, seriously affecting the course of the process. However, this feature of MR practically does not currently attract attention.

Monitoring PE patterns from MR of different chemical composition under non-stirring conditions for prolonged periods of time allowed to reveal interesting regularities dependent on the generation of EEE that weakly depended on particular amino- and carbonyl reagents taken initially[16, 17]. One of the major results of this study is defining the range of conditions under which regular and irregular oscillations of PE with prominent amplitude develop that last for many hours or even days. Thus for the first time it was shown that the process of self-organization may develop in such systems under conditions close to physiological. Development of oscillations depends on many factors including threshold concentrations of reagents, volume to surface ratio of the reaction system, temperature range, etc.

For example, the PE pattern of the reaction developing in a solution of methylglyoxal and ethanolamine is critically dependent on ethanolamine concentration (Fig. 5). Oscillations are practically absent at 5 mM amine concentration, they are small and early disappearing at 10 mM, but when the concentration is 30 mM very prominent oscillations are observed that last for many hours without damping. Noticeable that the integral number of photons emitted in the latter case, reflecting the intensity and yield of chemical processes, is orders of magnitude larger in comparison to the formers, though the concentration of the amine is only 3-fold larger.

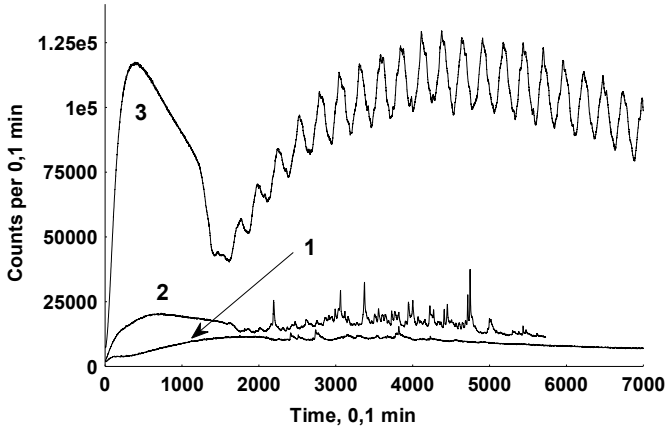

Figure 5. Dependence of PE patterns from MR developing in a solution of methylglyoxal (10 mM) on ethanolamine (EA) concentration. 1 — 5 mM EA, 2 – 10 mM EA, 3 – 30 mM EA. Other conditions: total volume – 20 ml, surface area of the reaction mixture – 400 mm^2, temperature – 20 ^oC, pH 10,3

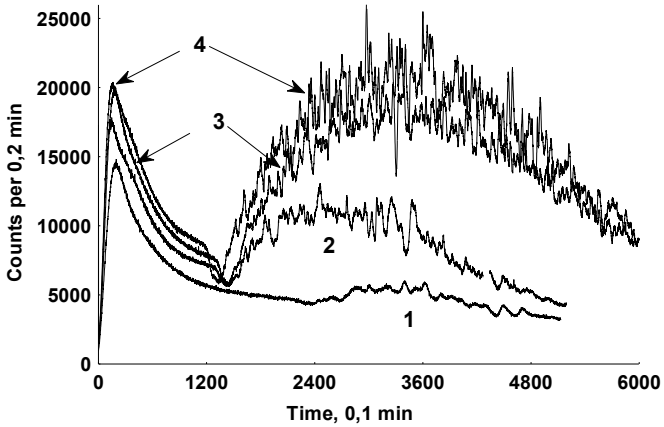

Figure 6. Dependence of PE patterns from MR developing in a solution of methylglyoxal (10 mM)/ethanolamine (30 mM) on surface area of the reaction system. 1 – 78 mm^2, 2 – 63 mm^2, 3 – 38 mm^2, 4 – 9 mm^2 at 4 ml of a solution in carbonate buffer (50 mM, pH 10,3)

Analogous threshold concentration for methylglyoxal in the presence of fixed glycin concentration was found. Under all fixed conditions oscillations practically did not develop at 2 mM of methylglyoxal and were prominent with simultaneous strong elevation of the mean PE intensity at 4 mM of methylglyoxal.

Oxygen plays a critical role in the development of PE from MR. The first wave of PE observed in Fig. 5 is related to consumption during the reaction of oxygen initially dissolved in water, and the second one is due to oxygen that diffuses to the reaction system from ambient air. If there is no contact of the reaction system with the air, the second wave of PE on which oscillations emerge does not develop for many hours. One could expect that the larger is the surface area, the better the reaction develops. Paradoxically, it turned out that excessive aeration of the reaction system prevents the

second wave development and the emergence of oscillations. Figure 6 illustrates that at constant volume the less is the surface area, the more prominent is the second wave of PE, the longer it sustains, and the larger are the amplitudes of PE intensity oscillations. Here also a strong non-linear dependence of the parameters of PE on surface area may be noted. Taking into consideration that the intensity of PE reflects intensity of oxidative processes in the course of which EEE is generated, these results indicate that optimal conditions for the development of these processes imply certain restriction of oxygen diffusion to the reaction system.

Why does it happen? Our studies have shown that the processes that are accompanied with PE modulated with oscillations proceed in the uppermost part of the reaction system, in the vicinity of the water/air boundary. Besides, the process starts to develop only when all the oxygen initially dissolved in water is consumed and the latter gains a high reducing (negative) potential[16].

This can be seen in Figure 7, where the results of simultaneous measurements of PE and redox potential changes in the uppermost part of the reaction system are presented. Note also, that after the second wave of PE starts to develop, mean value of redox potential also starts to increase and it also oscillates with exactly the same periodicity as PE (this was confirmed by the Fourier analysis of the respective time series). Thus, practically full correlation between the two processes exists.

Measurement of redox potential changes in lower parts of the reaction system did not already reveal such a correlation, but the data suggested the appearance of complex spatial patterns of energy distribution along the reaction system (from its top to the bottom). For example, the redox electrode imbedded in a middle part of the reaction vessel initially registered a very significant (down to -0,5 V) decline in the redox potential. Subsequently, very long period waves (wave periods reach several hours) of the redox potential with amplitudes reaching 0,3 V are registered. Thus intense oscillations of energy potentials apply to the whole reaction system, but their frequencies are different in different parts indicating that in the course of the whole process highly non-linear waves of energy continuously travel in the reaction system.

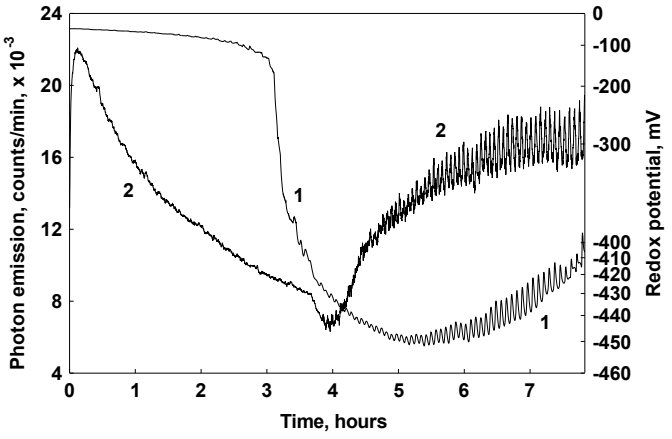

Figure 7. Simultaneous measurement of redox potential near water/air interface (1, right Y-scale) and PE (2, left Y-scale) from MR developing in a solution of methylglyoxal (10 mM)/ethanolamine (30 mM), 40 ml, surface area 440 mm^2, carbonate buffer (50 mM), pH 10,3.

Among other peculiarities revealed in the course of studies of energy processes characteristic for MR there is one more that is common to blood. It was mentioned above that self-irradiation of blood may reactivate respiratory burst in it especially at the stage of PE decline. In the case of MR we found that when two cuvettes with faded MR were placed together before a photomultiplier, integral PE was significantly higher than could be expected from simple addition. After such a contact PE from both reaction systems was also for some time higher than before.

3.3. Probable role of water.

Water is the major chemical species in the systems studied here as well as in all biological systems. Formally, its concentration is 2-5 orders of magnitude higher than that of any other individual substance present in it. It may play a significant though yet unrecognized role in the processes in which ROS are produced and EEE is generated in aqueous systems. In fact, when we studied oxidative processes induced by H_2O_2 in aqueous solutions of amino acids unexpected elevation of H_2O_2 concentration at the stage of exponential PE rate acceleration was observed. In some experiments this elevation reached 10-12% of the initial H_2O_2 concentration[14]. The only possible source for new H_2O_2 in this reaction system is direct oxidation of water with oxygen. This reaction seemed to be very improbable under mild conditions of our experiments, but it now turned out that it can indeed occur. First, it was recently discovered that all immunoglobulins irrespective of their class and specificity efficiently catalyze oxidation of water with singlet oxygen[10]. Appearance of plenty of electronically excited singlet oxygen in our reactions systems, where EEE is continuously released is inevitable. Spatial and temporal self-organization which is essential for any forms of catalysis also takes place here. Thus, the conditions for water oxidation by oxygen may exist.

On the other hand it has been recently demonstrated by several groups of authors that overall water splitting may take place under very mild conditions[18,19]. In the course of this process ROS and other free radicals such as hydrogen atoms arise. It is interesting to speculate that it is due to active involvement of water that strong oscillations of redox potential may appear reaching amplitudes of hundreds of millivolts, as we observed in the course of the development of MR. Taking into consideration that concentrations of organic reagents (carbonyl and amino compounds) do not exceed tens of millimolar, and that standard redox potentials between their reduced and oxidized forms are very modest, such strong variations of redox potential may be explained by the appearance in water of such active reducers and oxidizers such as hydrogen and oxygen in relatively high concentrations and the possibility of their spatial, though temporal separation in these seemingly homogenous reaction systems. Only new experiments and experimental approaches may refute or support these speculations.

3. GENERAL CONCLUSIONS

We demonstrated that processes in which ROS are produced and EEE is generated continuously in blood as well as in model aqueous systems. EEE arising in these processes influences by a feedback mechanism ROS production.

A lot of common features in the behavior of such processes proceeding in blood and model aqueous systems can be noted. This indicates the possible identity of the most basic mechanisms for realization of regulatory action of the ROS through generation of EEE in aqueous system, independent of the level of their complexity.

At this stage it should be pointed to the conditions for the development of oscillatory regimes in MR, because they may be important for understanding ubiquitous regulatory functions of ROS in biological systems. These conditions include:

1. The existence of a sharp gradient of oxygen between the reaction system consuming it and the gas phase which supplies it;
2. Restriction of rate of O_2 supply to the reaction system;
3. Elevation of concentrations of strong reducing compounds in the reaction system over a certain threshold value;
4. Existence of a mechanism allowing the acceleration of oxidation processes as soon as they commence, resulting in a fast decrease of O_2 concentration below the threshold level.

The same conditions of oxygen consumption are characteristic of any aerobic living cell especially under the condition of high activity when it is actively breathing to make a lot of energy. Under normal physiological conditions oxygen supply is restricted by its limited solubility in water (for free living cells) and by special regulatory mechanisms in multicellular organisms. In periods when in the nearest cell vicinity oxygen is exhausted, the concentration of reducing equivalents in a cell increases to a threshold when newly coming oxygen starts a new inflammation. In the course of this inflammation EEE is generated and it serves as energy of activation for faster burning that results in oxygen depletion. Thus spontaneous oscillatory regimes of cell breathing develop, and these oscillations may play a role of pacemakers of all vital processes in cells and their communities. In fact, such oscillatory "breathing" – oxygen consumption – has been demonstrated in single beta-cells, stimulated by the addition of glucose[20].

EEE, emerging in the considered processes in blood and model systems may be functionally significant (participate in the orderliness of the considered systems) if the following conditions apply:

1. Absorption of EEE by the components of the system shifts them into a functionally active state;
2. A system should possess own mechanisms for EEE generation;
3. A system may amplify, concentrate and prevent free dissipation of EEE;
4. EEE generation in time and space is ordered.

All these conditions, as it has been demonstrated here, more or less prominently realize in all the studied systems, including blood, that ubiquitous for living systems processes with ROS participation may regulate a wide spectrum of biochemical and physiological functions. No less important is that if such processes commence in initially disordered systems they have the intrinsic property for self-organization under rather wide boundary conditions.

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