Biochemistry (Moscow), Vol. 67, No. 2, 2002, pp. 161-170. Translated from Biokhimiya, Vol. 67, No. 2, 2002, pp. 194-204.

REVIEW

Reactive Oxygen Species as Essential Components of Ambient Air

N. Goldstein

Goldstein & Lewin Technology GmbH, Medico-Biological Department, Kastanienweg 23, 14532 Stahnsdorf, Germany; fax: + 49 30 28390251; E-mail: proinhal@mail.ru

Received June 5, 2001
Revision received July 12, 2001

Abstract—In this review evidence for the presence of the anion radical $O_2^{\cdot-}$ in atmospheric air is considered, and the biological activity of superoxide and negative air ions is compared. Various aspects of the biological effect of superoxide and other reactive oxygen species contained in air at the cell, tissue, and organism levels are discussed. The results of the therapeutic use of exogenous gaseous superoxide and low doses of H_2O_2 for the treatment of bronchial asthma, pain, and Parkinson's disease are reported. A hypothesis on the mechanism of physiological action of exogenous reactive oxygen species is discussed.

Key words: reactive oxygen species, superoxide, environment, negative air ions, adaptation, hypothalamic–pituitary complex

During evolution, the high oxidizing ability of oxygen led to selection of cells that can use the advantages of aerobic metabolism. Until recently, it was believed that cells have to pay for such energy supply by development of complex mechanisms of tolerance to the toxic intermediate products of oxygen reduction. The first of these products is the superoxide radical, $O_2^{\cdot-}$. According to the modern free radical pathology concept, the damaging effect of oxygen is associated predominantly with the reactive products of its further transformations (hydrogen peroxide H_2O_2 and hydroxyl radical $^{\bullet}OH$), singlet oxygen $^1O_2$, and the radicals produced during degradation of lipid hydroperoxides rather than with superoxide [1-4]. In terms of this insight, it is believed that "complete scavenging of the $O_2^{\cdot-}$ radical is the best possible outcome" to retain cell integrity [5]. However, after three decades of intensive studies, one of the authors of the concept of oxygen toxicity, McCord, accepted that "superoxide causes not only adverse changes" [5] and that our understanding of different aspects of $O_2^{\cdot-}$ action remains incomplete. Now another working hypothesis is becoming most common. According to this hypothesis, "in a healthy cell the optimal balance exists between superoxide production and superoxide scavenging" (ibid). If this is true, then $O_2^{\cdot-}$ production during metabolism should be considered not only as an undesirable phenomenon, but also as a reflection of an evolutionally formed requirement of the organism. Trying to determine the preconditions of occurrence of this requirement, researchers have given attention to electrically charged molecules of gases, so-called air ions, which are contained in the atmosphere [6-8].

The main insights on the biological activity of air ions were formed in the first third of the XX century [9, 10]. The attitude to this environmental factor has undergone a series of metamorphoses from determination of a broad range of biological action of air ions and the euphoria of the 1960s caused by the possibilities of their medical use to negation of their biological role and almost complete oblivion. However, convincing evidence for physiological activity of the anion radical $O_2^{\cdot-}$ of atmospheric air, which determines the biological effects of negative air ions (NAI), have been found during recent years [11]. Thus, independent at first glance directions in biology—study of the functions of superoxide $O_2^{\cdot-}$ and other reactive oxygen species (ROS) and study of the biological role of air ions—became closely related. Novel insights on the role of oxygen radicals in the vital function of organisms have been formed at the interface of these and other fields of science.

Abbreviations: ACTH) adrenocorticotropic hormone; ROS) reactive oxygen species; DABCO) 1,4-diazobicyclo{2,2,2} octane; MAO-A and MAO-B) monoamine oxidases A and B, respectively; MPTP) 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine; NBT) Nitro Blue Tetrazolium; NAI) negative air ions; LPO) lipid peroxidation; SOD) superoxide dismutase; GS) gaseous superoxide.

0006-2979/02/6702-0161$27.00 ©2002 MAIK "Nauka/Interperiodica"

SOURCES AND COMPOSITION OF NAI (BASIC INFORMATION)

The first descriptions of the biological effects of atmospheric electricity were apparently made by the Russian naturalist prince D. Golitsyn (1778, cited according to [12]) and the French abbey Bertholon [13]. More that 100 years had passed before the material carriers of the atmospheric electricity—gas ions carrying a positive or negative charge—were discovered [14, 15]. For this reason, the term "air ions" and the biological effects of the latter are usually associated with the electric properties of these particles [12].

The occurrence of atmospheric ions in nature is caused by the action of natural ionizers, mainly cosmic rays and radioactive sources in the soil and air. In the upper atmosphere, processes of photoionization also take place. However, the role of photoionization and electric discharges in the formation of air ions in ambient air is insignificant. During ionization, air ions of both signs are formed in the atmosphere. The average frequency of production of positive and negative ions is approximately 10 ions/cm^3/sec [16]. Near the Earth's surface, the steady-state concentration of air ions in 1 cm^3 of pure air is about 1,000 ion pairs. At some resorts, this parameter can reach 3,000, whereas in industrial centers the content of air ions drops to 300 and less. Owing to negative polarity of the electrical field of the atmosphere, the number of positive ions is higher than the total number of negative ions on average by 20%.

During generation of artificial air ions, air is ionized by one or several spot coronary discharges from a negatively charged point. This is the principal difference between the generation of natural and artificial air ions. In atmospheric air, which is ionized mainly by radioactive factors, the latter are distributed sufficiently regularly in the gaseous environment, whereas artificial ionization of gases during coronary discharge results in the local formation of exited and ionized products near a high-voltage electrode. The composition of produced gaseous ions also significantly differs. Is was shown that the major products of gas ionization produced by radioactive α-source involve so-called "light" ion O_2^- and its clusters $O_2^-\cdot(H_2O)_n$, where n = 1...3. The minor ionization products occurring under these conditions are ions NO_2^-, NO_3^-, HCO_3^-, and their clusters with water (Huertas and Fountain, 1983; cited according to [16]). Conversely, the main products of faint coronary discharge involve the ions NO_2^-, NO_3^-, and their clusters, whereas O_2^- ions were not found at all. Mathematical calculations are also indicative of the possibility of predominant formation of hydrated clusters $NO_3^-\cdot(H_2O)_n$ and $HCO_3^-\cdot(H_2O)_n$ [17, 18]. However, this does not mean that during coronary discharge it is principally impossible to obtain negative oxygen ions O_2^-, which are the most interesting in the context of this review and which exert some favorable effects on humans and animals. In general, the pool of these particles may contain ions and ion radicals, such as $O^{\cdot-}$, O_2^-, O_3^-, O_4^-, NO_2^-, NO_3^-, CO_4^-, and ^-OH, as well as their hydrated clusters of the type (ion)$\cdot(H_2O)_n$ [16, 19-22].

Apparently, the concentration of natural air ions in the air is very small. Given that the total content of molecules in 1 cm^3 of air is about $3\cdot10^{19}$ and that the concentration of air ions is $3\cdot10^3$, one air ion occurs for each 10^{16} neutral molecules [23]. It was shown that the damaging effect of air ions on *E. coli* is 8-fold lower than that of ultraviolet and 700-fold lower than that of X-ray irradiation [20]. In higher organisms, NAI effects are realized mainly via different physiological responses. The contradiction between the low energy potential of NAI and their effect on vital function attracts the attention of researchers. It was assumed that physiological mechanisms of amplification of air ion signaling exist and that the physicochemical properties of these particles rather than their electric charge determine their biological activity [7, 8].

NAI AND SUPEROXIDE RADICAL O_2^-

The interest generated in recent years around negative oxygen ions contained in atmospheric air is associated with the fact that one of these is the anion radical O_2^- [24], which is well known from biochemical studies of cells and tissues. Further studies showed that it is the gas-phase superoxide of atmospheric air (hereafter GS) that plays a key role in the biological activity of NAI. This finding was made possible due to the design of a GS generator that produced artificial ions of the main atmospheric gases and their mixtures, and to study them in biological experiments, and to investigate the products of ionization in condensed media, such as aqueous solutions, aprotic solvents, and tissues *in vitro* and *in vivo* [7, 11, 24, 25]. It was shown that in aprotic organic solvents, where the lifetime of O_2^- is higher than in aqueous solutions, superoxide produced in the gas phase oxidizes adrenalin to form adrenochrome, and in water solutions it reduces cytochrome *c* and NBT yielding hydrogen peroxide [24]. Later, hydrogen peroxide was found in the products of air ionization obtained with the use of a so-called Tchijevsky chandelier [26].

The major primary products of gas ionization are free electrons, which in aqueous medium form hydrated electrons e_{aq}^- and account for up to 40% of the reducing equivalents produced during mild ionization of the main air gases (nitrogen and oxygen). The processes proceeding in the course of nitrogen ionization and blowing the products into oxygen can be described by reactions (1) and (2) in gas phase and reactions (3) and (4) in aqueous phase [3]:

$$N_2 \rightarrow N_2^+ + e^-; \quad (1)$$

$$e^- + O_2 \rightarrow O_2^{\cdot-}; \quad (2)$$

$$e^- + H_2O \rightarrow e^-_{aq}; \quad (3)$$

$$e^-_{aq} + O_2 \rightarrow O_2^{\cdot-}. \quad (4)$$

The involvement of hydrated electrons is confirmed by introducing into the medium nitrous oxide (N_2O) that can react with e^-_{aq}, thus preventing NBT reduction [24]. It was shown that NBT reduction by hydrated electrons in the absence of oxygen is not inhibited by SOD [11]. By varying the conditions, it is possible to achieve prevalence of low-energy electrons with energy of ≤42 kJ/mol, which as a result of collisions "stick" to oxygen molecules, yielding GS free from other negative molecular ions and byproducts (ozone and nitrogen oxides). In atmospheric air, alternative processes with the participation of "hotter" electrons with energies of ≥360 kJ/mol also take place.

CELL AND TISSUE EFFECTS OF EXOGENOUS $O_2^{\cdot-}$

The decrease in the number of bacteria in artificially ionized air first described in the early works of Tchijevsky [12] was confirmed in numerous hygienic studies (for reviews, see [23, 27]). It was shown that suppression of growth of bacterial colonies is possible at the concentration of artificial air ions higher than $5 \cdot 10^4/cm^3$. In most cases, the bacteriostatic or lethal effect on microorganisms did not depend on the charge of air ions. The possible reasons for nonspecific effects of air ions on microorganisms involve the toxic effects of gas ionization byproducts (e.g., ozone) or the products of interaction of air ions with the minor air contaminants, as well as the effects associated with cell agglutination in strong electrostatic field or as a result of electrical interaction of cells with charged gas particles [23].

The first experimental attempt to determine the relationship between the bacteriostatic effect of NAI and superoxide chemical activity gave an unconvincing result [28]. Later studies demonstrated the enhancement of lipid peroxidation (LPO) in cells and the association between LPO enhancement and suppression of growth of bacterial colonies induced by GS and other ROS [11]. Cultures of bacteria and fungi (*Staphylococcus aureus*, *St. epidermidis*, *E. coli*, *Bacillus subtilis*, *Proteus vulgaris*, *Klebsiella pneumoneae*, *Pseudomonas aeruginosa*, *Salmonella typhimurium*, and *Candida albicans*) were used as test objects. Catalytic amounts of SOD significantly increased the suppressive effect of GS, whereas catalase almost completely abolished it. Singlet oxygen scavengers—sodium azide and DABCO—had no effect on the culture growth and accumulation of lipid peroxidation products. These data were considered as evidence for the absence of biologically significant amounts of $^1O_2$ in the pool of exited and ionized products of gas ionization and a possible association between the cytotoxic effects and formation of hydrogen peroxide (the product of spontaneous and SOD-dependent superoxide dismutation) or hydroxyl radical [11]. The cytotoxic effects of gaseous superoxide are manifested only at sufficiently high intensities of the factor (at $O_2^{\cdot-}$ generation rate reaching units of micromoles per minute). Conversely, when the intensity is 3- to 4-fold lower than cytotoxic, cytoprotective effects of superoxide can be observed (e.g., in *Saccharomyces cerevisiae* cells that underwent rehydration stress or were exposed to pure oxygen) [29].

It should be noted that the majority of studies of biological effects of NAI on the cell and tissue levels that were performed in 1960s through 1980s are descriptive, and the results of these works are not consistent with two current hypotheses of the action of air ions on living organisms (the organic electro-exchange hypothesis of Vasil'ev and Tchijevsky [10, 12] and the serotonin hypothesis of Krueger [30-33]). These effects, however, are surprisingly reminiscent of the cell effects of $O_2^{\cdot-}$ and hydrogen peroxide known from biochemical studies. For example, NAI increase the locomotor activity of trachea ciliary epithelium [33], and ROS produced in the xanthine–xanthine oxidase reaction increase the pulsation frequency of bovine trachea cilia [11]. These responses can be compared with superoxide stimulation of the evolutionally related spermatic filaments. Similarly to "biochemical" superoxide, NAI stimulate fibroblast division [34], and GS stimulates division of pancreatic insulin-producing cells *in vitro* (Goldstein, unpublished data). It was shown that the cell and tissue effects of exogenous ROS could always be modified by the addition of test enzymes SOD and catalase [11].

Study of the sensitivity of taste bud chemoreceptors showed that local applications of GS decrease the gustatory stimulus threshold [11]. We believe that these effects are associated with the local influence of GS on serotonin metabolism or with the activation of substance P-dependent system participating in the realization of trophic effects on the sensitive neurons of the gustatory apparatus. The effect of local applications of NAI on serotonin exchange in the trachea and bronchus tissues of animals was first discovered in the laboratory of Krueger [32, 33].

Another example of similarity of cell responses to "metabolic" superoxide and GS is macrophage chemotaxis. It is believed that the major chemoattractants in developing inflammation focus are inflammation mediators (e.g., leukotriene B_4), with ROS either stimulating or depressing this process [35]. It was revealed that GS alone could cause chemotactic responses of mouse peritoneal macrophages *in vitro* [11]. It was also shown that the

effect of GS on rat skin wound surface *in vivo* accelerates wound healing, especially the reparative stage (ibid). Thus, this effect of GS is similar to that of NAI, whose stimulatory effect on wound healing was described earlier [12, 36].

SYSTEMIC EFFECTS OF NAI AND GS ON ANIMALS

The effect of air ions on mammals is realized predominantly via the respiratory system. For a long time it was believed that these particles can penetrate into the lungs and transfer the electric charge of ions on the cell elements and colloid compounds of blood [10, 12, 37]. This notion, however, does not account for the known facts of predominant involvement of the CNS in the physiological effects of air ions [38-46]. Later it was found that the inhaled air ions do not penetrate into the deep parts of the respiratory tract and lose their charge in the nasopharynx area [47]. In view of these and other new data, the hypothesis of Vasil'ev and Tchijevsky is now considered incorrect [47].

The presence of superoxide ion O_2^- in atmospheric air explains various aspects of the biological activity of NAI from new points of view. The high reactivity of O_2^- and short lifetime of the radical in the aqueous medium of nasal cavity mucosa, where the majority of various receptors are located, support the hypothesis that chemical activity of inhaled O_2^- may be realized already at the level of nasal cavity mucosa receptors [11]. According to this hypothesis and with regard for the short lifetime of the radical in the aqueous medium of respiratory tract mucosa, the postulated penetration of NAI [10, 12] and O_2^- (see below) in the internal environment is excluded *a priori*. In view of this, the biological effects of NAI and GS are considered as the result of modulation of the activity of the nervous centers and structures of the brain, which are anatomically and functionally connected with nasal chemoreceptors. It is known that nasal cavity receptors are connected with the sensor entrances of the main subcortical center of neurohumoral regulation, the hypothalamus [48]. The receptor neurons of human and animal vomeronasal organ, which is now attracting increasing interest, have especially close morphological and functional connections with the hypothalamus [49-55].

The excitation of the sensor neurons of these receptor structures accounts for the structural changes occurring in the hypophysis nuclei caused by inhalation of air ions [56] and structural changes in the adeno- and neurohypophysis induced by GS inhalations [11]. It was found that after several inhalations of O_2^-, the number of hormone-producing adenocytes (especially adrenocorticotropocytes and thyrotropocytes) in the adenohypophysis increases. An interesting exception to this finding is represented by the prolactin-producing cells, whose number considerably decreases. The increase in ACTH production is reflected in the stimulation of cortisol secretion in patients with bronchial asthma who were treated with GS inhalations [57, 58]. The activation of thyrotropic function may account for the energy metabolism activation after NAI inhalation [59], whereas the depression of lactotropic function partially explains the mechanisms of activation of the dopaminergic system of the brain [11, 60]. Functional activation after NAI and O_2^- inhalations was also detected in the neurohypophysis, whose secretion elements are represented by the processes of neurons of the supraoptical and paraventricular nuclei of the hypothalamus [11, 56].

The above-listed examples show that functional changes in the organism induced by GS inhalations can account for the majority of NAI effects. The activation of the hypothalamic–pituitary complex and increase in ACTH secretion, which may result in the increase in the sensitivity of the hypothalamic neurons to the signals and shortened latent periods of responses, are considered as one of the major physiological mechanisms underlying the effect of GS on animals. A similar mechanism was proposed for NAI [47]. The validity of this hypothesis was confirmed by the finding that the temporary threshold of smell perception by volunteers decreases on combined administration of GS inhalations and smell stimuli [61].

Besides the hypothalamus and hypophysis, other morphological structures and biochemical systems of the brain are involved in the responses to endonasal applications of ROS. One of the "traces" of GS action was detected in the basal ganglia, which are functionally connected with the hypothalamus and represent the subcortical link between the association and motor areas of the brain cortex. Along with the hypothalamus, the basal ganglia are involved in the complex formation of the motor and association responses to algetic stimuli. In view of this, it is interesting to recollect that even at the end of 1950s and the beginning of the 1960s some pain relief in patients who received NAI inhalations after surgical operations was described [62]. Explanations for this phenomenon were not suggested. However, it was assumed that the observed effects could be associated with the combined physiological action of residual amounts of analgesics in blood of the patients and superoxide contained in NAI [25]. This assumption was corroborated by the finding that GS inhalations, as well as endonasal applications of diluted solutions of hydrogen peroxide enhance the analgesic effects of threshold doses of pain-relieving medicines in animals and humans [11, 25]. This phenomenon does not depend on the nature of the analgesic and may be associated with the biochemical mechanisms of pain perception and relief, especially with the modulation of activity of the serotonergic system and activation of endogenous opioid production. The evidence for the role of the free radical nature of O_2^- in the

formation of physiological responses of nasal cavity receptors was also found.

It was shown that endonasal applications of ROS suppress lipid peroxidation, decrease MAO-A and MAO-B activity in the basal ganglia and hypothalamus region, and increase dopamine content in the striatum and substantia nigra [11, 60]. This can apparently explain the reduction of oxidative stress and restoration of disturbed behavioral responses as well as neuroprotecting and trophic-like "neurorescue" effects of exogenous ROS in animals that received large doses of reserpine or the neurotoxin MPTP. It is known that these xenobiotics can cause Parkinsonism-like symptoms in animals. It was assumed that inhaled exogenous ROS activate the endogenous inhibitors of monoamine oxidases.

The adaptogenic properties of NAI and GS can be referred to the systemic effects of these exogenous compounds. Similar effects of NAI were described long before the occurrence of the terms "adaptogen" and "stress". The studies of Vasil'ev and his school corroborated the adaptogenic effect of NAI in the increase in the tolerance of organisms to hypoxia, anaphylaxigens, infectious agents, and some physical factors [63]. Reviews of these works have been published [64-66]. Later, the adaptogenic effects of GS inhalations directed against the toxic action of hyperbaric and normobaric oxygen as well as pharmacological agents were shown [11]. The adaptogenic properties of GS are characterized by nonspecificity, which is typical of natural and synthetic adaptogens [67]. With respect to adaptation to hyperbaric oxygen, the effect of GS is more pronounced than that of the well-known adaptogen eleutherococcus [11]. The development of responses to the inhalations of superoxide (animal survival, changes in behavioral responses, summation-threshold parameter, peroxidation stress, etc.) can be described in terms of general stress syndrome, which refers GS (and NAI as a whole) to natural environmental stress agents. This, in turn, may be indicative of the existence of resistance to the action of these factors. Thus, artificial NAI at concentrations close to the natural background do not affect the level of antioxidative protection of the organism, whereas GS and artificial NAI at high concentrations reduce the intensity of oxidative stress *in vivo* [11]. This is suggestive of the possibility of forming a systemic structural adaptation trace (which is characteristic of other sufficiently mild stress factors [68-70]) and using the exogenous ROS for cross adaptation to the action of adverse factors. By analogy with the mechanisms of adaptation to other stress factors, hypothalamic MAO-dependent reactions may be involved in CNS adaptation to exogenous ROS [71-73]. In terms of adaptogenic action of exogenous ROS, the induction of antioxidant protection by superoxide may be considered as a component of adaptation process. With regard for the fact that oxidative stress is a common stage in pathogenesis of different diseases, the adaptive activation of antioxidant systems may be used for increasing the resistance of an organism to the action of different damaging agents.

THERAPEUTIC EFFECTS OF NAI AND ROS

The therapeutic action of NAI has been repeatedly discussed in the literature [23, 74]. The use of NAI inhalations for treatment of respiratory organs, endocrine and vegetative nervous system disorders, rheumatism, hypertension, and stomach and duodenal ulcer, and other conditions was described in the monograph of Tchijevsky [12]. These data were confirmed by the works of Bulatov [75, 76], Finogenov [77], Jones et al. [78], Jorde and Schata [79], Graenz et al. [80], and other researchers (see reviews [65, 66, 81-83]). A good therapeutic effect is described by Yates et al., who were the first to use NAI for treating hyperactive children and those suffering from autisms [84].

Similarly to physiological effects, many therapeutic effects of NAI may be associated with the hypothalamic responses, which is especially clearly manifested in humans with elevated meteorological sensitivity [85]. Note that the expression of therapeutic effect often directly depends on the extent of dysfunction. For example, when treating patients with asthma, GS were most effective in those with higher severity of disease [11]. Successful treatment of patients with bronchial asthma is a good example of therapeutic efficacy of GS. The use of NAI for treatment of this disease has been known for a long time [12, 75, 76, 79, 86]. Importantly, chronic inflammatory process in the bronchi and lungs play a key role in the pathogenesis of bronchial asthma [87-89]. In clinical trials in adults and children it was shown that GS inhalations subjectively and objectively improve the status of the patients, which is manifested in reduction of endogenous oxidative stress, bronchial hyperreactivity and the amount of phlegm, as well as in less frequent asthma attacks [57, 58, 90, 91]. It was discovered that GS exerts higher therapeutic efficacy than NAI. For example, clinically comparable results were obtained at total exposure to GS for about 3.5 h and the recommended course of NAI inhalations of 5 to 27 h [76, 86].

Predominantly two physiological mechanisms of the therapeutic effect of GS on patients with asthma are discussed in the literature. The antioxidant system training and decrease in the oxidative stress, which are caused by local effects of hydrogen peroxide produced from GS and working at the level of deep parts of the bronchial tree, are considered as the main mechanism. If this is true, the available data do not confirm the known apprehension that hydrogen peroxide may exert spasmogenic effect on bronchus smooth muscles [92]. Probably, the absence of spasmogenic effect in the course of GS inhalations can be explained by the extremely low concentration of H_2O_2 formed during superoxide dismutation. The other mech-

anism may be associated with the activation of antiinflammatory endogenous systems (in particular, with activation of cortisol production) and can be realized via reflex response mediated by nasal cavity receptors. In both cases, the responses of the organism to GS inhalations are realized in the form of induction of adaptive mechanisms. Similar responses are observed during formation of hormesis mechanisms [93]. The latter comparison may be useful for understanding the peculiarities of the effect of GS at the whole-body level.

Another area of application of exogenous ROS is pain relief. It was shown that antinociceptive effect of analgesics can be potentiated not only by combination of NAI inhalations with injection of narcotizing analgesics (as described in animals (see above)), but also in the case of enteral administration of analgin, diclofenac, and aspirin to humans [25, 94, 95]. The effect of exogenous ROS on the algetic sensitivity system can be realized via activation of the sympathoadrenal system and stimulation of endorphin synthesis [96]. The activation of neurotransmitter systems (primarily, serotonin- and dopaminergic) also may play a certain role [11, 38, 97, 98]. The data published in the literature on the role of serotonin and dopamine in pain perception corroborate this assumption [99-102].

It was shown that GS inhalations suppress MAO-A and MAO-B activity in the hypothalamus and basal ganglia of the brain [11], whose pathological changes underlie Parkinson's disease [103]. The data on chronic pain and olfactory disorders that accompany Parkinson's disease even at the early stages of its development (often before occurrence of other clinical signs) [104-106] are indicative of certain association between the physiological structures and neuromediator processes involved in the development of this disease and in pain perception. The results of studies performed in patients with Parkinsonism confirmed the therapeutic efficacy of ROS. Clinical improvement due to administration of ROS inhalations to these patients was expressed as the decrease in Parkinsonian tremor and muscular rigidity, improvement of vegetative symptomatology (face sebum secretion and sialorrhea) and mimics, and in alleviation of depression [11].

Physiological effects of NAI are also manifested in practically healthy humans exposed to functional loading and experimental stress. For example, Baron showed that in male volunteers the inhalations of NAI at moderate concentrations increase memory volume and performance of the proof text [43]. It was also found that NAI inhalations improve the performance of psychomotor tasks and increase the productivity and quality of work [107, 108].

At the same time, some authors skeptically estimate not only the therapeutic effect of NAI, but also the very fact of their biological activity [74, 109-111]. It should be taken into account that a considerable part of the skeptical works had methodical problems associated with NAI generation and delivery of ionization products to the nasal cavity receptors. However, later studies performed with the use of GS confirmed many results obtained earlier with the use of NAI, and novel biological and therapeutic effects of ROS were discovered. At least some of these effects can be reproduced using endonasal application of hydrogen peroxide. This is considered as evidence for commonness of the factor acting in both cases [11].

GS AND HUMAN ENVIRONMENT

Air is the most general environment with which humans come into contact. In view of this, ambient air sanitation is a key problem of hygiene. Despite constant interest in air ions, their biological importance as a natural ambient air factor was often called in question. We already noted that this attitude was provoked mainly by the methodical problems in a considerable part of the published works in this field, including inadequate ways of generation of gas ions, absence of the data on their identification and dosimetry, and underestimation of the role of loss-free delivery of ionization products to the examined objects (see reviews [109, 110]). Some critical works had other experimental shortcomings, such as generation of byproducts (ozone and nitrogen oxides) by the majority of air ionizers and the absence of monitoring of the composition of gas medium, temperature, and air humidity [112]. These circumstances and extremely low content of air ions in the atmospheric air were reasons for the skeptical attitude to air ions as a biologically important environmental factor [74, 109-111].

At the same time, it was reported that after a long-term indoor stay without natural ventilation of air in compliance of the hygienic standards of the main ambient air parameters (carbon dioxide, water vapor, and oxygen concentration), humans feel worse [12]. This finding indirectly confirms the physiological need for air ions. It should be noted that one of the factors promoting the reduction of air ion content in rooms is air conditioning [113]. It was found that people working in conditioned rooms more frequently complain of unsatisfactory state of health, increased fatigue, sensation of stuffiness, and frequent headaches [114]. Similar symptoms are observed in so-called sick building syndrome [115]. It was also discovered that people who stay in conditioned rooms for a long time more frequently fall ill with influenza, acute catarrh of the upper respiratory tract, and heart diseases. The described changes are the most pronounced in the workers of special industries (for example, microchip manufacturing), where a complex of factors that promote GS elimination is most complete [116]. Some studies showed the advisability of the use of artificial NAI to increase work efficiency of people under conditions of insufficient supply with natural air ions [117, 118]. An interesting report was made by Reberg, a participant of

the Soviet Space Medico-Biological Program of the 1960s. During the ground tests of chambers for mice and rats with adequate supply with food and monitored living conditions, an unexplained mortality of the majority of animals was observed (Reberg, personal communication). Later, these phenomena and facts were explained by the deficiency of GS in the inhaled air.

Convincing evidence of the need for GS by animals was obtained under conditions of complete elimination of this factor from the inhaled air [11, 119]. It was found that GS deprivation caused 100% death of mice and rats within 16-23 days. The early pathological changes involve the suppression of behavioral responses and grooming, reduction in skeletal muscle tonus, disturbance of coordination of movements, development of conjunctivitis, refusal of food and water, loss of weight and a significant part of hair, and deterioration of enzymatic antioxidant protection of tissues. When studying the internals, marked erosive changes and ulcers in gastric mucosa and, in most cases, complete involution of the thymus were observed (ibid). Electron-microscopic study of rat hypophysis by day 10 of deprivation revealed degeneration of the majority of adenocytes and nerve termini of the neurohypophysis, which reflects the condition of pronounced functional exhaustion of the gland (hypophysial cachexia). Usually the occurrence of this syndrome is associated with such stress factors as immobilization and cold, whose long-term action may cause death of animals [120]. Complete GS deprivation was the only and controllable stressor in the above-described study. Prevention of animal death resulting from short-term interruption of deprivation by adding GS to the experimental camera confirms the vital role of GS. These results allow the interpretation of the known facts of pathological action of so-called "air ion dearth" [12, 121, 122] from modern positions.

With regard for the above-listed data, the question on expediency of compensation of NAI and/or GS shortage by artificial ionization of indoor air has been repeatedly discussed [12, 113, 114, 123, 124]. In view of this, domestic coronary air ionizers have received wide acceptance. In Russia, the so-called Tchijevsky chandelier is most popular now. It is usually emphasized that this chandelier does not generate "anything except for negative oxygen ions" [125]. It is known, however, that the coronary aeroionizers equipped with antennas "account for the occurrence of high concentrations of hazardous "heavy" air ions in the rooms" [126, 127]. They are formed as a result of precipitation in a very strong electrical field of light ions onto dust particles and/or microorganisms weighed in the air. The formed electrically charged dust can precipitate in the lungs more readily than uncharged dust [27, 64, 123, 128]. For this reason, artificial increase in NAI concentration in the air of public buildings has long been recognized as inexpedient [129]. It was also shown that coronary ionizers cannot generate only light air ions (such as O_2^-) [128]. Huertas and Fountain showed that in the air ionized with the use of a coronary ionizer the negative oxygen ion O_2^- is not registered at all (1983, cited according to [16]). In addition, the concentration of all air ions rapidly decreases with the distance from the ionizer oscillators [128]. Obviously, this also pertains to the electro-effluvial Tchijevsky chandelier. Experimental data confirming the presence of superoxide radical at the level of the patient's head during the use of devices such as the Tchijevsky chandelier are absent from the available literature. At the same time, the majority of studies report the presence of side biologically active factors (such as ozone and nitrogen oxides) [74, 124, 128] and strong electrical field with high-voltage pulses of 30-70 kV [125]. For this reason, the exposure of patients directly under the chandelier is forbidden [130]. With regard for these disadvantages, it was directly intimated [131] that "...the design of these chandeliers (e.g., the Tchijevsky chandelier, N. G.) cannot be recognized as rational".

Another way to compensate for insufficient GS content in ambient air is to use an individual generator of gaseous superoxide as proposed in [11, 24]. In devices of this type, GS is produced under mild conditions at a distance of only several centimeters from the patient's nasal receptors with a voltage on safe carbon-fiber electrodes 10- to 20-fold lower than that used in known room air ionizers. Importantly, the byproducts (such as ozone, nitrogen oxides, and emission products of metallic electrodes) are not produced in this devise. The results of GS study and the evidence for O_2^- production and delivery to the inhalation area that are reported in this review were obtained using the individual generators of superoxide [25, 58, 90]. Further studies are required to determine the expediency of practical application of GS inhalators in hygiene, preventive health care of different contingents of working people (for example, in especially clean rooms and in hermetically sealed volumes), i.e., in all cases of significant disbalance in air ion composition of ambient air.

The pool of negatively charged atmospheric ions contains superoxide anion radical O_2^-. The effect of this radical on cells and its biochemical properties are identical to those described for NAI, on one hand, and "metabolic" superoxide, on the other. It is beyond question that GS contained in NAI can affect different aspects of vital activity of terrestrial organisms. It is known, for example, that not only animals and microorganisms listed in this review, but also plants are sensitive to NAI and GS [12, 23]. The considered examples illustrate only some of the studied properties and biological functions of exogenous ROS. All these findings confirm the idea of McCord [5] that our understanding of the role of superoxide remains incomplete. The evidence for biological multipotency of ROS and the needs of organisms for GS may reflect the regularities formed during evolution and enable our

understanding of a role of free radicals of both exogenous and endogenous origin in vital function.

REFERENCES

1. Vladimirov, Yu. A., Olenev, V. I., Suslova, T. B., and Potapenko, A. Ya. (1975) in *Biophysics*, Vol. 5, *Molecular Pathology of Membrane Structures* (Vladimirov, Yu. A., ed.) [in Russian], VINITI, Moscow, pp. 56-117.
2. Halliwell, B. (1994) *Lancet*, **344**, 721-724.
3. Fridovich, I. (1979) in *Free Radicals in Biology* (Prior, U., ed.) [Russian translation], Mir, Moscow, pp. 272-311.
4. Skulachev, V. P. (2000) *Oxygen and Phenomena of Programmed Death. The First Severin Reading* [in Russian], Russian Biochemical Society, Moscow.
5. McCord, J. M. (1995) *PSEBM*, **209**, 112-117.
6. Portnov, F. G., and Goldstein, N. I. (1977) in *Problems of Clinical Biophysics* (Portnov, F. G., ed.) [in Russian], Zinatne, Riga, pp. 10-17.
7. Goldstein, N. I. (1982) in *Cellular and Subcellular Pathology of the Liver* (Blyuger, A. F., ed.) [in Russian], Zinatne, Riga, pp. 91-96.
8. Goldstein, N. I. (1988) in *Oxygen Radicals in Chemistry, Biology, and Medicine* (Goldstein, N. I., ed.) [in Russian], RMI, Riga, pp. 80-108.
9. Tchijevsky, A. L. (1922) *Report Read in the Zoological Museum of Moscow State University March 17, 1922. Lithographed at the 1st Printing House of Kaluga Gubispolkom. On the rights of a manuscript*, Kaluga, pp. 1-22.
10. Vasil'ev, L. L., and Tchijevsky, A. L. (1934) *Problems of Ionification*, in *Works of TSNILI*, **3**, 335-368.
11. Goldstein, N. I. (2000) *Biophysical Aspects of Physiological Action of Exogenous O_2^- on Animals*: Doctoral dissertation [in Russian], Moscow State University, Moscow.
12. Tchijevsky, A. L. (1999) *Air Ions and Life. Debates with Tsiolkovskii* [in Russian], Mysl', Moscow.
13. Bertholon, P. (1780) *Ouvrage couronné par l'Académie de Lyon dans lequel on traite de l'electricité de l'atmosphére, de son influence et des effets sur léconomie animal etc.*, Paris.
14. Elster, J., and Geitel, H. (1899) *Terr. Magn. Atmos. Elec.*, **4**, 213-234.
15. Wilson, C. T. R. (1900) *Proc. Camb. Phil. Soc.*, **11**, 32.
16. Dolezalek, H. (1985) *Int. J. Biometeorol.*, **29**, 211-221.
17. Portnov, F. G., and Shmidt, A. B. (1986) in *Space Biology and Airspace Medicine* [in Russian], Nauka, Moscow, pp. 449-451.
18. Livshits, A. I., Portnov, F. G., and Shmidt, A. B. (1984) *Izv. AN Latv. SSR*, **2**, 192-194.
19. Chalmers, J. A. (1957) *Atmospheric Electricity* [in Russian], Gidrometeoizdat, Leningrad.
20. Kingdon, K. H. (1960) *Phys. Med. Biol.*, **5**, 1-10.
21. Rosenthal, I., and Ben-Hur, E. (1980) *Nature*, **288**, 739.
22. Pethig, R. (1984) *Meth. Enzymol.*, **105**, 238-248.
23. Krueger, P., and Sigel, S. (1981) in *Biologic Effects of Environmental Electromagnetism* (Konig, H. L., Krueger, A. P., Lang, S., and Sonning, W., eds.) Springer, New York, pp. 144-175.
24. Goldstein, N. I., Goldstein, R. N., and Merzlyak, M. N. (1992) *Int. J. Biometeorol.*, **36**, 118-122.
25. Goldstein, N., Baumann, S., Lewin, T., Rehberg, G., Kamensky, A., Dubinin, V., and Konstantinova, O. (1996) *Inflamm. Res.*, **45**, 473-478.
26. Kosenko, E. A., Kaminsky, Yu. G., Stavrovskaya, I. G., Sirota, T. V., and Kondrashova, M. N. (1997) *FEBS Lett.*, **410**, 309-312.
27. Minkh, A. A. (1976) in *Atmospheric Electricity* [in Russian], Gidrometeoizdat, Leningrad, pp. 258-292.
28. Kellogg, E. W., Yost, M. G., Barthakur, N., and Krueger, A. P. (1979) *Nature*, **281**, 400-401.
29. Skards, I., and Goldstein, N. I. (1988) in *Oxygen Radicals in Chemistry, Biology, and Medicine* (Goldstein, N. I., ed.) [in Russian], RMI, Riga, pp. 272-277.
30. Krueger, A. P., Andriese, P. C., and Kotaka, S. (1968) *Int. J. Biometeorol.*, **12**, 225-239.
31. Krueger, A. P., and Kotaka, S. (1969) *Int. J. Biometeorol.*, **13**, 25-38.
32. Krueger, A. P., and Smith, R. F. (1960) *J. Gen. Physiol.*, **44**, 269-276.
33. Krueger, A. P., and Smith, R. F. (1959) *Nature*, **183**, 1322-1333.
34. Worden, J. L. (1961) *Proc. Int. Conf. Ioniz. Air*, **1**, 1-24.
35. Elstner, E. F. (1990) *Der Sauerstoff. Biochemie, Biologie, Medizin*, DI Wissenschaftsverlag, Mannheim.
36. Portnov, F. G., and Putse, M. A. (1972) in *Problems of Clinical Biophysics* (Portnov, F. G., ed.) [in Russian], Zinatne, Riga, pp. 177-184.
37. Vasiliev, L. L. (1960) *Am. J. Phys. Med.*, **39**, 124-128.
38. Diamond, M. C., Connor, J. R., Jr., Orenberg, E. K., Bissell, M., Yost, M., and Krueger, A. (1980) *Science*, **210**, 652-654.
39. Olivereau, J. M., and Lambert, J. F. (1981) *Int. J. Biometeorol.*, **25**, 53-62.
40. Baron, R. A., Russel, G. W., and Arms, R. L. (1985) *J. Pers. Soc. Psychol.*, **48**, 746-754.
41. Deleanu, M., and Margineanu, C. (1986) *Int. J. Biometeorol.*, **30**, 65-67.
42. Bailey, W. H., and Charry, J. M. (1987) *Bioelectromagnetics*, **8**, 173-181.
43. Baron, R. A. (1987) *J. Appl. Physiol.*, **72**, 131-137.
44. Brown, G. S., and Kirk, R. E. (1987) *Percept Mot. Skills*, **64**, 951-962.
45. Lambert, J. F., and Olivereau, J. M. (1987) *Int. J. Biometeorol.*, **31**, 323-328.
46. Beardwood, C. J., and Jordi, P. M. (1990) *Bioelectromagnetics*, **11**, 207-212.
47. Skorobogatova, A. M. (1991) *Fiziol. Zh. SSSR im. I. M. Sechenova*, **77**, 156-159.
48. But, V. I. (1966) *Fiziol. Zh. SSSR im. I. M. Sechenova*, **52**, 729-733.
49. Mendoza, A. S., and Kühnel, W. (1989) *Z. Mikr.-Anat. Forsch. Leipzig*, **5**, 801-806.
50. Liman, E. R., and Corey, D. P. (1996) *J. Neurosci.*, **16**, 4625-4637.
51. Meredith, M. (1998) *Chem. Senses*, **23**, 463-466.
52. Zanzanaro, C., Mucignat-Caretta, C., Merigo, F., and Oskulati, F. (1999) *Neuroreport*, **10**, 2033-2027.
53. Dudley, C. A., and Moss, R. L. (1999) *Neurosci.*, **91**, 1549-1556.
54. Berliner, D. L., Monti-Bloch, L., Jennings-White, C., and Diaz-Sanchez, V. (1996) *J. Steroid Biochem. Mol. Biol.*, **58**, 259-265.

55. Monti-Bloch, L., Jennings-White, C., and Berliner, D. L. (1998) *Ann. N. Y. Acad. Sci*, **855**, 373-389.
56. Olivereau, J. M. (1975) in *Problémes d'ionisation et d'aéroionisation* (Rager, R., ed.) Maloine, Paris, pp. 136-160.
57. Goldstein, N., Rehberg, G., Lewin, T., and Klefisch, F. R. (1997) *Atemwegs- und Lungenkrankheiten*, **8**, 437-438.
58. Goldstein, N., Rehberg, G., and Klefisch, F. R. (1997) *Probl. Tuberkulyoza*, **6**, 54-58.
59. Deleanu, M., and Catalin, O. (1971) *Revue Roumaine d'Endocrinologie*, **8**, 207-216.
60. Goldstein, N. (1997) Pat. DE 197 08 643.
61. Arshavsky, V. V., Goldstein, N. I., Aročnika, B., Konstantinova, O., and Raits, E. (1991) *Latv. Ārsts*, **2**, 77-80.
62. Minehart, J. R., David, T. A., McGuck, F. J., and Kornblueh, I. H. (1961) *Am. J. Phys. Med.*, **40**, 56-62.
63. Vasil'ev, L. L. (1960) in *Aeroionization and Hydroaeroionization in Medicine* [in Russian], Medgiz, Tashkent, pp. 30-34.
64. Minkh, A. A. (1963) *Ionization of Air and Its Hygienic Importance* [in Russian], Medgiz, Moscow.
65. Portnov, F. G. (1976) *Electroaerosol Therapy* [in Russian], Zinatne, Riga.
66. Minkh, A. A. (1976) in *Atmospheric Electricity* [in Russian], Gidrometeoizdat, Leningrad, pp. 258-292.
67. Kovalev, G. V., Spasov, A. A., and Bogachev, N. A. (1981) in *New Data on Eleutherococcus and Other Adaptogens* [in Russian], DVTS AN SSSR, Vladivostok, pp. 51-56.
68. Meerson, F. Z. (1981) *Adaptations*, *Stress*, *and Prophylaxis* [in Russian], Nauka, Moscow.
69. Meerson, F. Z. (1985) *Byull. Vsesoyuz. Kardiol. Nauch. Tsentra*, **1**, 34-43.
70. Meerson, F. Z., and Pshennikova, M. G. (1988) *Adaptations to Stress Situations and Physical Loadings* [in Russian], Meditsina, Moscow.
71. Masuda, M., Takeda, H., and Shibuya, T. (1993) *Nippon Yakurigaku Zasshi*, **101**, 187-196.
72. Stratakis, C. A., Gold, P. W., and Chrousos, G. P. (1995) *Horm. Res.*, **43**, 162-167.
73. Geniatullina, M. S., and Korolev, Yu. N. (1996) *Morfologiya*, **110**, 37-41.
74. Kroling, P. (1985) *Int. J. Biometeorol.*, **29**, 233-242.
75. Bulatov, P. K. (1948) *Byull. Eksp. Biol. Med.*, **25**, 288.
76. Bulatov, P. K. (1964) *Bronchial Asthma* [in Russian], Medgiz, Leningrad.
77. Finogenov, S. N. (1961) *Therapeutic Properties of Ionized Air* [in Russian], Zdorov'e, Kiev.
78. Jones, D. P., O'Konnor, S. A., Collins, J. V., and Watson, B. W. (1976) *Thorax*, **31**, 425-432.
79. Jorde, W., and Schata, M. (1979) *Atemwegs- und Lungenkrankheiten*, **6**, 443-453.
80. Graenz, A., Lischig, H., and Fischer, G. (1985) *Notal. Med.*, **1**, 490-495.
81. Bocconi, G. (1970) *Recenti Progressi in Medicina*, **47**, 451-480.
82. Rotgers, C. D. (1985) *J. Bioelectr.*, **4**, 63-74.
83. Jaskowski, J. (1985) *Wiadomosci Lekarskie*, **38**, 677-682.
84. Yates, A., Gray, F., Beutler, L. E., Sherman, D. E., and Segerstrom, E. M. (1987) *Am. J. Phys. Med.*, **66**, 264-268.
85. Sulman, F. G. (1980) *The Effects of Air Ionization*, *Electric Fields*, *Atmospherics and Other Electric Phenomena on Animals.* Monograph (Thomas, Ch. C., ed.) Springfield, III, USA.
86. Palti, Y., de Nour, E., and Abrahamov, A. (1966) *Pediatrics*, **38**, 405-411.
87. Nolte, D. (1989) *Asthma: das Krankheitsbild*, *der Asthma patient*, *die Therapie*, 4th ed., Urban und Schwarzenberg, München.
88. Jarjour, N. N., and Calhoun, W. J. (1994) *J. Lab. Clin. Med.*, **123**, 131-136.
89. Kanazawa, H., Kurichara, N., Hirata, K., and Takeda, T. (1991) *Chest*, **100**, 1319-1322.
90. Goldstein, N., Rehberg, G., Lewin, T., and Klefisch, F. R. (1997) *Physikalische Medizin*, **7**, 138-140.
91. Goldstein, N., Rehberg, G., Lewin. T., Klefisch, F. R., and Korkina, L. (1995) *Pneumologie*, **2**, 338.
92. Skulachev, V. P. (2001) *Biochemistry (Moscow)*, **66**, 1153-1156.
93. Kuzin, A. N. (1995) *Ideas of Radiation Hormesis in Nuclear Century* [in Russian], Nauka, Moscow.
94. Goldstein, N., Rehberg, G., Voskresenskaya, O., Levitskaya, N., Dubinin, V., and Kamensky, A. (1997) *Der Schmerz*, **11**, 67.
95. Goldstein, N., and Lewin, T. (1995) Pat. DE 19 514 522.
96. Wehner, A. P. (1984) *Int. J. Biometeorol.*, **28**, 47-53.
97. Aimone, L. D., and Gebhart, G. F. (1988) *Brain Res.*, **450**, 170-180.
98. Li, Y. C., Li, H. D., and Zhao, B. Y. (1990) *Chung Kuo Yao Li Hsueh Pao*, **11**, 89-92.
99. Pert, A. (1987) in *Pain Headashe*, Vol. 9, Karger, Basel, pp. 1-63.
100. Millan, M. J., and Colpaert, F. C. (1991) *J. Pharmacol. Exp. Ther.*, **256**, 983-990.
101. Taivo, Y. O., Heller, P. H., and Levine, J. D. (1992) *Neurosci.*, **48**, 479-483.
102. Murphy, Z., Murphy, R. M., and Zemlan, F. R. (1992) *Psychopharmacology*, **108**, 123-130.
103. Ebadi, M., Srinivasan, S. K., and Baxi, M. D. (1996) *Progr. Neurobiol.*, **48**, 1-19.
104. Koller, W. C. (1984) *Neurology*, **34**, 957-959.
105. Shang, A. B., and King, S. A. (1991) *Hosp. Community Psychiatry*, **42**, 1162-1163.
106. Stein, W. M., and Read, S. (1997) *J. Pain Symptom Manag.*, **14**, 255-258.
107. Tom, G., Poole, M. F., Galla, J., and Berrier, J. (1981) *Hum. Factors*, **23**, 633-636.
108. Hawkins, L. H., and Barker, T. (1978) *Ergonomics*, **21**, 273-278.
109. Charry, J. M. (1984) *Environmental Res.*, **34**, 351-389.
110. Charry, J. M. (1987) in *Air Ions: Physical and Biological Aspects* (Charry, J. M., and Kavet, R. I., eds.) CRC Press, Boca Raton, pp. 91-150.
111. Reiter, R. (1992) in *Phenomena in Atmospheric and Environmental Electricity*, Elsevier, Amsterdam, pp. 437-478.
112. Krueger, A. P. (1972) *Int. J. Biometeorol.*, **16**, 313-322.
113. Geller, I. M., Bochkareva, L. A., Takhirov, M. T., and Dolgova, N. I. (1982) *Gigiena Sanitariya*, **8**, 79-81.
114. Minkh, A. A., Gubernskii, Yu. D., Dmitriev, M. T., Serova, L. V., and Orlova, N. S. (1973) *Vestn. AMN SSSR*, **10**, 3-10.
115. Finnegan, M. J., and Pickering, A. C. (1987) in *Indoor Air 87, Proc. 4th Int. Conf. on Indoor Air Quality and Climate* (Seifert, B., et al., eds.) Berlin, pp. 542-546.
116. Wriedt, H. (1992) *Gesundheitsbelastungen in der Mikrochip Industrie*, IGM Werkstattberichte.

117. Snigur, O. I. (1966) in *Air Ionization in Occupational Hygiene* [in Russian], Medgiz, Leningrad, pp. 54-56.
118. Sapov, Sh. A., and Solodkov, A. S. (1970) *Voenno-Med. Zh.*, **10**, 66-69.
119. Goldstein, N., and Arshavskaya, T. (1997) *Z. Naturforsch. (Biosciences)*, **52**, 396-404.
120. Arshavskaya-Popovich, T. V., and Polenov, A. L. (1989) *Z. Mikrosk. Anat. Forsch., Leipzig*, **103**, 648-663.
121. Kimura, S., Masanobi, A., and Mitsushima, I. (1939) *Acta Medica Hokkaidonensia*, **17**, 505-516.
122. Skards, I. (1968) *Activation of Leukocytes* [in Russian], Zinatne, Riga.
123. Minkh, A. A. (1960) in *Aero- and Hydroaeroionization in Medicine* [in Russian], Medgiz, Tashkent, pp. 171-178.
124. Gubernskii, Yu. D., and Dmitriev, M. T. (1986) *Gigiena Sanitariya*, **3**, 10-12.
125. Norman, G. E. (2001) *Biochemistry (Moscow)*, **66**, 123-126 (Russ.).
126. Reinet, Ya. Yu. (1979) *Uchen. Zap. Tartus. Univ.*, **479**, 26-37.
127. Langus, L. E., and Reinet, Ya. Yu. (1979) *Uchen. Zap. Tartus. Univ.*, **479**, 38-56.
128. Reinet, Ya. Yu. (1984) *Gigiena Sanitariya*, **2**, 10-12.
129. Minkh, A. A., and Malysheva, I. N. (1967) *Vestn. AMN SSSR*, **8**, 31-40.
130. Gerasimova, L. I., Danilov, Yu. A., Evstigneev, A. P., Machabeli, M. S., and Tikhonov, V. P. (1996) *Aeroionotherapy. Gide Book* [in Russian], Lazer Academy of Sciences of Russian Federation, Kaluga, p. 32.
131. Tammet, Kh. V. (1960) in *Aero- and Hydroaeroionization in Medicine* [in Russian], Medgiz, Tashkent, pp. 250-256.