ISSN 0003-6838, Applied Biochemistry and Microbiology, 2019, Vol. 55, No. 2, pp. 83–98. © Pleiades Publishing, Inc., 2019.
Russian Text © O.V. Kosmachevskaya, E.I. Nasybullina, V.N. Blindar, A.F. Topunov, 2019, published in Prikladnaya Biokhimiya i Mikrobiologiya, 2019, Vol. 55, No. 2, pp. 107–123.

Binding of Erythrocyte Hemoglobin to the Membrane to Realize Signal-Regulatory Function (Review)

O. V. Kosmachevskaya[a], E. I. Nasybullina[a], V. N. Blindar[b], and A. F. Topunov[a, *]

[a] *Bach Institute of Biochemistry, Research Center of Biotechnology, Russian Academy of Sciences, Moscow, 119071 Russia*
[b] *N.N. Blokhin National Medical Research Center of Oncology, Moscow, 115478 Russia*
**e-mail: aftopunov@yandex.ru*

Received September 3, 2018; revised September 18, 2018; accepted September 25, 2018

Abstract—Reversible protein binding with membrane components and the cytoskeleton is one of mechanisms of cell metabolism control. This is a crucial mechanism for the regulation of metabolism in nuclear-free cells, mammal erythrocytes, in which it is realized via hemoglobin modification to the membrane-bound state. Hemoglobin can interact with the membrane in different ligands and redox statuses. Thus, this protein can function as a sensor of redox and oxygen conditions. Depending on the oxygen conditions, deoxyHb changes the energetic metabolism, morphology, and deformability of erytrocytes, as well as the release of vascular tone regulators, NO and ATP. This is fulfilled via interaction with the main integral protein of erythrocyte membrane, the band 3 protein. The products of hemoglobin oxidative denaturation, irreversible hemichromes, also carry out a signaling function. Accumulating over time or as result of oxidative stress, chemichromes contain information on the redox conditions and the longevity of erythrocyte functioning. It has been hypothesized that erythrocytes have a program that initiates intracellular hemolysis. The participation of hemoglobin and its membrane-bound form (MBHb) in the realization of this program is discussed. The role of NO donors in the regulation of erythrocyte stability is debated as well. The use of data on the MBHb content in erythrocytes is proposed for clinical diagnostics.

Keywords: membrane-bound hemoglobin, erythrocytes, metabolism regulation, intravascular hemolysis, nitric oxide, diagnostics

DOI: 10.1134/S0003683819020091

INTRODUCTION

The understanding of the functional properties of erythrocyte hemoglobin (Hb) has expanded significantly over the past two decades. It became clear that Hb is not only an oxygen carrier but also a regulator of its delivery to organs and tissues. Hb performs this function in several ways: (1) the regulation of the mechanical properties of the erythrocyte membrane; (2) the synthesis and deposition of NO in the erythrocyte; (3) the release of ATP from the erythrocyte. In all cases, the signal-regulatory action is mediated by changes in the quaternary Hb structure (conformational R-T transitions), which are modulated by the partial pressure of oxygen (pO_2). The tetrameric organization of Hb and its allosteric properties make possible to fine-tune both the oxygen-binding properties of Hb and its signaling functions. Note that Hb also regulates the energy metabolism, volume, deformability and life-span of the erythrocyte.

Since the mammalian erythrocyte is a highly specialized and reduced cell in which the nucleus, mitochondria, and protein synthesis apparatus are absent, its main information platform is the plasma membrane and the associated membrane cytoskeleton. The Hb signal-regulatory function is based on its binding to the membrane components.

The review summarizes modern ideas about the means of Hb interaction with the membrane, as well as the physiological significance of this process for erythrocyte functioning and its participation in maintaining homeostasis at the level of the entire organism.

Reversible binding of proteins on the membrane as a way to regulate metabolism. One of the mechanisms of cell metabolism regulation is the reversible transition of proteins from the soluble to the membrane-bound state. The so-called adsorption mechanism [1, 2] or, according to the terminology proposed by A.S. Kaprelyants, is topodynamic regulation [3]. The reversible adsorption of enzymes allows of control their catalytic activity and stability. The idea that reversible binding of enzymes to subcellular structures has regulatory significance was first expressed by A.I. Oparin in 1933 [4]. It was shown in his works that a similar means of regulating enzyme activity also works in gel-like structures (coacervates), which indicates its evolutionary antiquity.

As a rule, the binding of enzymes with membranes leads to a decrease in their activity and is directly dependent on the energy charge of the cell: a high level at a low ATP concentration and vice versa. This may allow the cell to reduce energy consumption for the constant catalytic functioning of enzymes. The proteins sorbtion–desorbtion process is highly sensitive to low-intensity influences. Due to this process, the cell can change its metabolism in seconds due to the previously bound enzymes, while the realization of the response via changes in gene expression would take much more time.

A question arises as to the origin of a factor that triggers the transformation of the proteins from the bound form to the free state. The modern hypothesis suggests that the conformational changes in membranes and proteins are such triggers. The connection between protein conformation and the redox state of its SH groups had been shown [5]. Thiol groups are the most active protein groups; in soft physiologic conditions, they can attend to the various chemical reactions (oxidation, nitrosylation, glycylation, alkylation, thiolation, etc.) [5, 6]. Many of these reactions are reversible and are thus important for the cell metabolism. Besides, the thiol-bisulfide balance is highly sensitive to the influence of redox active compounds [7].

Conformational changes in the Hb molecule can be induced by a change of the state of heme group after association with the ligands, as well as by a change of the state of the cysteine surface residues (Cysβ93). Binding (blocking) of the active thyol groups changes the electron density distribution on the heme; as a result, Hb autooxidation increases unless it is accompanied by significant conformational changes [8]. For example, the formation of superoxide anion radical $\left(O_2^{\bullet -}\right)$ occurs when low-spin oxygenated Hb (oxyHb) is modificated into low-spin oxidized met-hydroxyHb (Fe^{III}–OH) or hemichrom (His–Fe^{III}–His). Oxidized Hb, especially its low-spin forms, has an increased tendency to bind to the membrane [9]. Thus, various redox-active compounds, which are formed exogenously or endogenously during any metabolism violation, can affect the redistribution of the protein from the soluble to the membrane-bound state.

It is known that erythrocytes have a multilevel adaptation ability [10–12]. In contrast to nucleated cells, the adaptive capacities of mammalian erythrocytes are limited by cytoplasmic mechanisms. They include the reversible cooperative phase transitions of proteins, their interaction with the membrane and cytoskeleton, changes in membrane proteins and cytoskeleton proteins, and the violation of membrane permeability. Therefore, erythrocytes are a convenient model for the study of the formation of the adaptive response at the cytoplasm level. In the next chapter, we will discuss the main means of forming a regulatory signal in these cells due to Hb binding to the membrane.

Hemoglobin-membrane binding centers. The ability of erythrocyte Hb to bind to the membrane was established long ago, when researchers were trying to obtain erythrocyte shadows by hypotonic lysis. The first mention of the Hb association with erythrocyte membranes can be found in the work of Hoffman [13], in which it was called "structurally fixed hemoglobin." The term "membrane-bound hemoglobin" is well established in the scientific literature (**MBHb**). Also among the early MBHb studies are the works of Anderson and Turner [14, 15], in which it was shown that washed erythrocyte membranes contain about 3% of the total cell Hb. Fung and Eisinger [16, 17] then confirmed that Hb interacts with erythrocyte membranes under physiological conditions.

Among the means of Hb binding to the membrane are the following: deoxyHb binding on band 3 protein due to electrostatic interactions [17–21], covalent association with membrane components by disulfide bonds [22, 23], and adsorption to membrane lipids using hydrophobic interactions. Hb can also bind to cytoskeleton proteins: spectrin [24, 25], glycophorin [26–28], actin, and tubulin [29, 30].

Band 3 protein (**Band3**) is a member of the (SLC4) family of carriers (transporters) of soluble compounds [31]; it is the main integral membrane protein (25% of all membrane proteins). The monomeric band 3 consists of three domains: a membrane-penetrating domain that exchanges Cl^-/CO_2^-; the short C-terminal cytoplasmic domain, and the long N-terminal cytoplasmic domain. The C-terminal cytoplasmic domain binds carbonic anhydrase II; the N-terminal cytoplasmic domain (Cytoplasmic Domain of Band 3 protein, **CDB3**) binds glycolytic enzymes, Hb, and hemichromes, forming a complex of the band 3 protein. One of the main functions of the N-terminal domain is the binding of the cytoskeleton scaffold to the membrane, which determines the morphology and stability of the erythrocyte. The complex of band 3 protein coordinates the membrane-cytoskeletal structure with the energy state and oxygen conditions inside the cell. The structure of this protein determines the antigenic determinants of the erythrocyte membrane; Band 3 polymorphic variants determine the Diego blood group [32].

Band 3 is the center of the erythrocyte membrane organization and the regulator of ionic homeostasis; therefore, even minor modifications in its regulatory sites lead to a change in the structure and function of these cells. Phosphorylation of Band 3 tyrosines disturbs the binding of Hb and cytoskeleton proteins [33]. It can be assumed that other modifications in the binding site, e.g., tyrosine nitration, can lead to a disturbance of the oxygen-dependent regulation of metabolic fluxes in the erythrocyte. CDB3 contains reactive cysteines involved in the detection of the redox state inside the cell [34].

Table 1. MBHb content in the erythrocyte shadows of healthy donors according to the estimates of various authors

MBHb content, %*	Detection method	Reference
≤ 2	Spectrophotometric cyanmethemoglobin method (Drabkin method)	[45]
~3	Spectrophotometric cyanmethemoglobin method (Drabkin method)	[14]
7–10	Spectrophotometry at a wavelength of 536 nm	[46]
3.6	"	[47]
4.04 ± 0.54	"	[48]
4.43 ± 2.2	"	[49]
5.54 ± 0.32	"	[50]
4.53 ± 1.05	Spectrophotometric evaluation of Hb complexes with pyridine (Riggs method)	[51]
7–8	Activated chemiluminescence	[52]

***** The MBHb content is presented as a percentage of the total amount of Hb in erythrocytes.

The Hb binding to the membrane can be reversible and irreversible. It was shown with fluorescent probes that deoxyHb reversibly binds to CDB3 with high affinity (eight times of the affinity for oxyHb) [18, 35–37]. The covalent binding of Hb to the components of the membrane is irreversible due to the action of oxidizing agents that cause the formation of free radicals and ferryl and oxoferryl Hb forms (heme–Fe(IV)=O) [38–41]. The higher the level of Hb oxidation (from Fe^{III} to Fe^{V}) is, the higher is the proportion of bound Hb, and approximately 50% of Hb is bound by SH groups [41]. In addition to Band 3, Hb is also capable of forming a complex with a cytoskeletal node consisted of spectrin, ankyrin, and protein of the 4.2 band. Bound in this way, oxidized metHb retains its redox properties and ability to interact with ligands [41].

The partially oxidized Hb interacts with the membrane [42], which probably has physiological significance, since in the near-membrane region it is reduced by membrane-bound NADPH-dependent metHb-reductase [43].

It is also known that Hb can form complexes with lipids, such as cholesterol [44]. The content of Hb-cholesterol adducts is higher in winter than in summer (30 and 19% of total Hb, respectively), which reflects the level of cholesterol in high-density lipoproteins (HDL) in plasma.

Methods to determine the membrane-bound HB. The literature contains contradictory information on the MBHb content in erythrocytes, which is explained by the different physiological state of erythrocytes and the way in which shadows are prepared (pH, ion strength, number of washes).

The MBHb concentration in erythrocytes is currently most often determined spectrophotometrically, via assessment of the Hb associated with erythrocyte shadows [14, 45–51]. We developed a simple spectrophotometric method to determine the MBHb in blood samples, which makes it possible to estimate the Hb concentration in scattering media [51]. SDS-PAGE of shadow proteins, followed by densitometry of the Hb band [53, 54], measurement of the Hb quenching of the fluorescence of probes embedded in the membrane, those specifically interacting with the band 3 protein [17, 21], and luminol-activated chemiluminescence [20, 52] are also used. Among the advantages of the latter method, it is worth noting the high sensitivity (up to 0.01 pmol MBHb in the sample) and the ability to carry out measurements in whole erythrocytes. Table 1 summarizes the information available on this subject.

According to two-dimensional electrophoresis, the Hb content in the erythrocyte membranes was 1.740 ± 0.074% of the total membrane proteins and was the smallest compared to the content of the remaining membrane proteins [54]. Similar values (1.3 ± 0.3% of total membrane proteins) were obtained in the study [41].

A quick and accurate method for the quantitative determination of bound deoxyHb with CDB3 is based on the measurement of Hb quenching of the fluorescence of a green fluorescent protein (eGFP) associated with the COOH-end of CDB3 [21]. An unusual method of MBHb analysis via giant Raman spectroscopy was proposed in another study [55]. For this, erythrocytes were pre-immobilized on coatings in the form of annular silver nanostructures. This approach makes it possible to estimate the changes in the conformation and oxygen-binding properties of MBHb in intact erythrocytes.

MEMBRANE-ASSOCIATED HEMOGLOBIN AS A MEDIUM OF BIOLOGICAL SIGNALING

The reversible nature of Hb binding to the membrane indicates the physiological role of this process. Let us consider the physiological and pathophysiological effects caused by the transition of Hb to the membrane-bound state.

Allosteric regulation of CDB3. Hb binds to band 3 protein by embedding the anionic CDB3 segment in the cationic central cavity formed by β-chains, which

is also the binding site of 2.3-diphosphoglycerate (2.3-DPG), the allosteric Hb regulator. This interaction makes it possible to consider the band 3 protein to be a heterotropic allosteric effector that induces a transition to the T-conformation or stabilizes the T conformation for the deoxyHb [56]. Conversely, Hb regulates the anion-transport properties of the band 3 protein, such as Na^+/K^+ – transport and $K^+–Cl^-$ and $Na^+–K^+–2Cl^-$ – cotransport [56].

Regulation of gas exchange. The close contact of Hb and carbonic anhydrase with the participation of CDB3 makes it possible to coordinate the absorption of carbon dioxide and the release of oxygen in the capillaries [57]. The protons released during bicarbonate formation are localized in the region of the complex of band 3, where they contribute to Hb deoxygenation due to the Bohr effect.

Hb can affect gas exchange in another way, by changing the structure of the membrane-associated cytoskeleton (submembrane barrier) [58]. Increased contact with the membrane cytoskeleton leads to a reduction in the wells of the cytoskeletal frame, reducing gas diffusion. This mechanism seems quite likely when the supply of NO to erythrocytes is regulated [42, 59, 60].

Energy metabolism regulation. CDB3 is known to bind glycolytic enzymes: aldolase, phosphofructokinase, and glyceraldehyde-3-phosphate dehydrogenase [61]. These enzymes, as well as pyruvate kinase and lactate dehydrogenase, form a multienzyme complex (metabolone) [62, 63]. The organization of enzymes in the metabolone provides a more efficient regulation of the glycolytic pathway. Glycolytic enzymes usially have a low catalytic activity in the bound state [64]. Conformational changes in the Hb molecule during deoxygenation lead to an increase in its affinity for CDB3, the displacement of glycolysis enzymes from the binding site, and their transition to a soluble active state [19, 57, 65]. Thus, deoxyHb binding to CDB3 switches metabolism from the pentose phosphate pathway to the glycolytic pathway in response to changes in the cellular oxygen status [57, 64, 66, 67]. On the one hand, it contributes to an increase in the level of intracellular ATP and, simultaneously, 2.3-DPG. On the other, it reduces the flow of glucose entering the pentose-phosphate pathway. In this pathway, NADPH is formed, which is necessary for the recovery of metHb and antioxidant enzymes.

Organic phosphates (ATP and 2.3-DPG) are the link that connects the cellular energy with the functional activity of the Hb molecules contained in them. They decrease HB affinity to oxygen due to specific binding to this protein [68]. It is one of the ways of the regulation of oxygen delivery in hypoxia conditions: the lower pO_2 is, the higher is the proportion of deoxyHb, the higher the ATP and 2.3-DPG concentrations, and the higher is the O_2 release by Hb. In this case, the regulation of the oxygen-binding properties of Hb due to the allosteric mechanism can be very fast, since a part of 2.3-BFG (approximately 30%) is in the membrane-bound state [68]. Thus, deoxyHb and 2.3-DPG can compete for membrane binding sites.

The switching of the energy metabolism to the predominant synthesis of organic phosphates is of great importance for membrane stabilization and the creation of a physiologically optimal erythrocyte form [69], as well as for the regulation of local blood flow under hypoxic conditions [70–72]. A 15% or more reduction of the ATP content changes the nature of the interaction of spectrin, actin, and other integral proteins of the erythrocyte membrane. Another glycolysis metabolite, 2.3-DPG, reversibly binds to spectrin and therefore also affects the properties of the membrane cytoskeleton. Increasing its concentration increases the deformability of the membrane.

Regulation of capillary blood flow. Two complementary mechanisms of erythrocyte involvement in the regulation of capillary blood flow (local intravascular pressure) are described: a rapid decrease in blood viscosity due to an increase in the deformability of erythrocytes and a slow-but-steady increase in vessel diameter due to the release of vascular tone regulators (NO and ATP) into its lumen. The latter mechanism has three means of implementation: the allosterically controlled release of NO with the participation of SH groups of β-subunits of Hb (SNO-Hb) [73, 74]; NO formation in the reduction reaction of nitrite ions of deoxyHb [75–77]; and local purineergic signaling with ATP released from erythrocytes [72, 78, 79]. All of the above mechanisms are implemented under conditions of low pO_2 and are modulated by the interaction of Hb with the membrane and cytoskeleton components.

The involvement of HB in NO export from erythrocytes is discussed. CDB3 is the acceptor of the NO group from SNO-Hb in the membrane [80]. The interaction of Hb with CDB3 favors the transition of the protein to the T conformation, which is accompanied by the transfer of the NO group in the transnitrosylation reaction from the cysteines of the Hb β-chains to the vicinal cysteine residues (Cys201, Cys317) of CDB3 [80, 81]; therefore, the oxidation of CDB3 thiols leads to disruption of molecular mechanism of intracellular NO transfer [80]. CDB3-associated deoxyHb can also reduce nitrite ions and thus generate NO on the inner surface of the erythrocyte membrane [82].

In 1992, a work showed the participation of Hb in the hypoxic emission of ATP from erythrocytes [78]. The suggested mechanism of this process included the binding of desoxyHb with CDB3, which resulted in G-protein activation and triggered the signal pathway cascade. Its final step is accompanied with the release of ATP through specific channels in the plasma membrane [79]. This erythrocytic ATP binds to P2Y-purine-ergic receptors on the surface of endothelial cells and activates the synthesis of NO and other vaso-

dilating factors, thereby contributing to an increase in the capillary blood flow rate and the efficiency of O_2 delivery to tissues. The question that arises as to the channels involved in selective ATP transport has not yet been resolved. Presumably, these include pannexin channels (Panx1) and mechanosensitive nonselective cation channels (Piezo 1) [83]. The hypothesis of the release of ATP from lysed erythrocytes was confirmed experimentaly [70–72].

Along with hypoxia, the release of ATP from erythrocytes can be caused by their mechanical deformation during passage through capillaries, acidification of the medium, and an excessive amount of CO_2 in the blood [84]. The release of ATP from human erythrocytes is shown to increase in the presence of nitrites, penetrating cAMP analogs, activators of the cAMP-mediated signaling system (adrenaline, isoproterenol, prostacyclin PGI_2, forskolin, papaverine), and dimethyl sulfoxide [84].

An increase in the synthesis and release of ATP in the presence of nitrites is accompanied by a decrease in blood pressure, as shown in vitro and in vivo experiments [20]. This effect was observed in both normoxic and hypoxic erythrocytes, which indicates its independence from the nitrite reductase reaction. It was also shown that the MBHb content increased in erythrocytes treated with physiological concentrations of $NaNO_2$ (9.9 ± 0.2 μM as compared to 2.0 ± 0.6 μM in the control). The combination of these facts allowed the authors to propose the involvement of Hb in the nitrite-dependent release of ATP [20]. The form in which Hb binds to the membrane is still unknown. Presumably, these include oxidized low-spin forms of the protein, which are formed in the reaction of deoxy- and oxyHb with metHb–NO2 and bis-histidine–metHb [9]. The possibility of the formation, with the participation of CDB3, of an intraerythrocyte pool of membrane-bound ATP, which is released under hypoxic conditions when the signalling pathway for ATP release is activated, is discussed.

Induction of Hb redistribution from the membrane-bound to soluble state by sodium nitrite was also observed in our experiments with isolated erythrocytes [51]; moreover, in concentrations an order of magnitude higher than the concentration of nitrite ions, nitrosothiols did not lead to the formation of additional MBHb. This is consistent with the results of another study [41], in which it was shown that the incubation of erythrocytes with sodium nitrite led to a tenfold increase in MBHb.

Regulation of erythrocyte deformability. The supply of oxygen to tissues depends not only on the Hb properties but also on the rheological characteristics of the erythrocyte membrane. The high deformability of these cells plays an important role in ensuring microcirculation, since it allows cells to pass through vessels with a diameter smaller than their own size [85]. It was shown that the the involvement of theHb-CDB3 complex in cytoskeletal reorganization in the microvasculature depends on the oxygen conditions [21, 86, 87]. The binding of deoxyHb to CDB3 in the immediate vicinity of the binding sites of ankyrins connecting Band3 and the cytoskeleton leads to the displacement of ankyrin from the complex with Band3 [21, 86]. Due to this, there is a weakening of membrane-cytoskeletal interactions, which significantly increases erythrocyte deformability during the period of deoxygenation. Conversely, an increase in pO_2 enhances the interaction of ankyrins with Band3, which stabilizes the erythrocyte membrane during their movement in the turbulent flow from the lungs to the capillaries.

Stabilization of the cytoskeleton during periods of prolonged deoxygenation (ischemia, hypoxia) can lead to the intravascular formation of vesicles and hemolysis. There is a point of view that this seemingly unfavorable process may be physiologically justified. This issue will be discussed below.

Induction of intracapillary oxidative stress. Various forms of oxidized Hb (metHb, ferrylHb, oxoferrylHb) and products of its oxidative denaturation (irreversible hemichromes) and degradation (heme and divalent iron) can firmly bind with the components of the membrane and be a source of reactive oxygen species (**ROS**) that initiate lipid peroxidation (**LPO**) [39–41, 52, 88–93]. Since the near-membrane region is practically unavailable for cytosolic antioxidant enzymes, the precipitation of Hb to the erythrocyte membrane can only enhance the process of free radical oxidation of lipids and provoke hemolysis.

Under hypoxic conditions, oxyHb may also be a source of ROS. If Hb gives the tissues about one third of the bound O_2 under normal conditions, then, with lowered pO_2, Hb reaches a state of half-saturation, in which it is easily oxidized and loses stability. Deep acidosis caused by hypoxia can also intensify the process of autooxidation. Due to the high deoxyHb level in hypoxic erythrocytes, the Hb content in the near-membrane region increases, which reduces the contribution of the hexose-monophosphate shunt to the cellular energy and to the synthesis of NADPH. This may exacerbate the negative effects of ROS.

The processes of ROS generation in erythrocytes described above explain the induction of the inflammatory response under hypoxic conditions [94]. The H_2O_2 formed by erythrocytes goes into the capillary lumen, diffuses to the microvascular endothelium, where it launches Ca^{2+}-dependent leukocyte recruiting. The possibility of such an in vivo scenario has been demonstrated in rat lungs [94]. Thus, Hb triggers the development of inflammatory disorders, such as acute lung injury or cardiovascular diseases with low cardiac output.

Redox signaling. An increase in the proportion of membrane-bound oxidized forms of Hb does not always have negative consequences for the cell; in some cases, it can be a physiologically determined

process aimed at the removal of old, damaged, and infected erythrocytes from the bloodstream. [95, 96]. Functionally defective erythrocytes have altered antigenic determinants, so that they are recognized by phagocytes and macrophages and destroyed.

Several hypotheses have been proposed to explain the role of Hb in the formation of the "death signal." According to one of them, the copolymerization of hemichromes with CDB3 and/or spectrin contributes to the aggregation of Band 3 and the exposure of the aging antigen to which circulating antibodies (IgG) bind, forming opsonization sites [96–99]. It is assumed that erythrocytes are recognized by macrophages as old when the cluster size exceeds a certain threshold value. Normally, approximately 0.1% of erythrocytes contain aggregated Band 3 [100]. The combined effect of membrane lipid peroxidation and elevated levels of hemichromes is necessary for Band 3 aggregation [100].

Hemichromes bind to the cytoplasmic membrane at high affinity (on CDB3) and low affinity (on the spectrin) sites [97]. Binding to CDB3 has the following stoichiometry: one dimer of the band 3 protein account for 2.5 tetrameric hemichromes. With the help of hemichromes, erythrocytes can "feel" oxidative stress (redox state) [37, 96].

The sequence of events leading to the formation of a signal of redox conditions inside the cell is considered as follows. The initial event is the reversible oxidation of closely located cysteine residues (Cys201 and Cys317) in Band 3 to form an intermolecular disulfide bond (covalently cross-linked Band 3 dimer) [81]. The formation of disulfide bonds also occurs under the action of hemichromes. Such cross-linking causes conformational changes in Band 3, which enables a specific interaction with Syk-kinase that phosphorylates tyrosine residues in the cytoplasmic domain of CDB3 [37, 101]. The Band 3 hyperphosphorylation caused by hemochromes reduces its affinity to ankyrin and promotes dissociation from the spectrin-actin cytoskeleton [37, 101]. This leads to a violation of the membrane structural integrity and, therefore, to intravascular hemolysis. In contrast, reversible phosphorylation does not cause erythrocytes lysis.

Hemichromes also induce phosphorylation of serine residues of cytoskeleton proteins. This phenomenon apparently plays a key role in the release of membrane microparticles (extravesicles formation), which are loaded with hemichromes [102]. Such particles are contained in large quantities in the blood of thalassemia patients. [101]. In this case, the process of the formation of vesicles is physiologically justified, since it aims to remove damaged membrane fragments, thereby preserving fully viable red blood cells [102].

Before the onset of physiological aging, erythrocytes can undergo damage that violates their integrity and, thus, triggers their death program, eryptosis. Erythrocytes exhibit the following apoptotic signs: cell shrinkage, vesiculation, and redistribution of membrane lipids, which leads to the exposure of phosphatidylserine on its surface [103]. Phosphatidylserine is recognized by the macrophages that utilize such erythrocytes. Several eryptosis mechanisms that can be launched simultaneously are described. One of these mechanisms can be realized with the participation of oxidized forms of Hb, hemichromes, that specifically bind to Band 3.

Enhanced eryptosis occurs in diabetes mellitus, malignant neoplasms, heart and kidney failure, anemia of various origins, hemolytic uremic syndrome, sepsis, and mycoplasma infection [104]. Due to eryptosis induction, red blood cells infected with malaria plasmodium (*Plasmodium falciparum*) are quickly removed from the bloodstream, thereby protecting the entire body from the spread of infection [56, 104].

Even though eryptosis, in general, is a useful physiological mechanism, its excessive activation may cause extensive intravascular hemolysis. Eryptosis stimulation of in malignant tumors is further aggravated by cytostatic treatment, thus contributing to the occurrence of anemia [105]. To reduce anemia in cancer patients, it is advisable to use eryptosis inhibitors, one of which is nitric oxide [106]. The development of various NO-donor compounds for the correction of disorders in the microcirculation system is a promising direction in pharmacology. Dinitrosyl iron complexes (DNIC, see below) can act as such physiological carriers for NO groups.

Cell volume regulation. Several studies have investigated the role of Hb in the regulation of cell volume. Two mechanisms of such regulation are possible. According to the first, Hb is involved in the detection of volume via the effect of crowding (the nonspecific effect of macromolecule crowding) [107]. The fluctuations in cell volume result in the concentration or dilution of the intracellular Hb solution, which can affect the activity of protein kinases and phosphatases involved in volume regulation. An alternative mechanism is that Hb binding to the membrane activates the K^+–Cl^--cotransporter involved in the regulation of cell volume [108]. In hyper- and hypotonic environments, Hb reduces the transport of K^+ and Cl^-. Oxygenation stimulates ion transfer (via the activation of the K^+–Cl^--cotransporter) and causes swelling, while deoxygenation causes wrinkling (through the activation of the Na^+–K^+–$2Cl^-$ and Na^+/H^+ channels) [109]. Cell swelling, being an analog of shear stress, leads to the discovery of mechano-sensitive cationic channels permeable to Ca^{2+}; this triggers Ca^{2+}-dependent signaling cascades that regulate cytoskeletal rearrangement, the proteins of which have Ca^{2+} binding sites [110].

Regulation of the erythrocyte membrane charge. Several mechanisms have been proposed to explain Hb participation in the regulation of the erythrocyte membrane electric field [111]. One of them is Hb

adsorption–desorption on the membrane. Deoxyhemoglobin forms a thick continuous layer on the membrane. This thickness compensates for about half of the membrane charges, thereby halving the transmembrane potential difference. The addition of oxygen to Hb converts it to the R state, in which it is desorbed from the membrane and the electrostatic field in it increases. The second mechanism is the release of protons due to the enhanced acidic properties of Hb during oxygenation and exit from the erythrocyte, which increases the negative charge of the cytoplasm and, consequently, the electric field in the membrane. All of this leads to a decrease in membrane permeability.

STABILIZING ACTION OF HEMOGLOBIN ON ERYTHROCYTE MEMBRANES

In the 1970s researchers have already revealed the stabilizing influence of the near-membrane Hb layer on erythrocytes, which manifested at a lowering hemolysis level at mechanical impact [112, 113]. Experiments with erythrocyte shadows have shown that the destruction of the lipoprotein structure of erythrocyte membranes with a high Hb content was happening to a much lesser degree in comparison with membranes with small a Hb content. Research on the molecular mechanisms of this effect showed that oxyHb and metHb stabilize the membranes. At the same time, the products of Hb oxidation and denaturation (reversible, irreversible hemichromes, and Heinz bodies), which accumulate under oxidative stress or during erythrocyte aging, break the membrane interaction with the cytoskeleton [114]. The noticeable stabilization of the membrane cytoskeleton is caused by the oxyHb and metHb contribution to the self-organization of spectrin dimer to tetramer [114, 115]. The oxyHb/spectrin binding constant exceeds the CDB3 constant by nearly 20 times.

The destructive effect of oxidized Hb forms is caused by the appearance of hemin. Hemin has been shown to be a potent hemolytic agent that nonspecifically disrupts protein–protein and protein–lipid interactions [116, 117]. Hemin weakens intermolecular interactions within the spectrin–protein 4.1–actin complex in the erythrocyte [114]. Hemichromes are the source of hemin. As the oxidative stress increases, the hemichromes form stable aggregates, Heinz bodies. They are attached to the erythrocyte membrane and can be detected in the form of violet inclusions after intravital methyl-violet staining. This process is accompanied by an increase in permeability for potassium ions, lipid peroxidation, the cross-linking of membrane proteins, and a decrease in cell deformability [114]. Lipid peroxidation induced both by Hb itself and its decay products (iron and heme group) can also contribute to the process of erythrocyte destruction [88, 91].

Thus, Hb has a multidirectional effect on cell resistance: when the oxidation is insignificant and reversible, the influence is stabilizing, while the influence is destabilizing in case of deep oxidation (Fig. 1). As Hb oxidizes, the stabilizing effect on the cytoskeleton gradually disappears, and the negative effect increases. The curve of the dependence of erythrocyte stability on the level of oxidative stress shown in the figure is a graphic expression of the hormesis effect [118]. Hormesis is described by a U-shaped or an inverted U-shaped curve that shows the change in the direction of the biological effect with an increasing chemical dose [118, 119]. Such a two-phase curve describes the compensatory–adaptive response of the living system.

We observed a phase change in the MBHb level during the treatment of isolated human erythrocytes with increasing doses of various redox active substances (methylglyoxal, hypochlorite, tert-butyl peroxide, sodium nitrite) [51, 120]. It must be noted that the increased MBHb levels correlated with increased resistance to oxidative hemolysis. Figure 2 represents two curves characterizing the change in the MBHb level and the erythrocyte hemolytic stability depending on the hypochlorite concentration (NaOCl). There is a correlation between the two parameters. A range that corresponds to an increase in cell resistance (also called the hormesis zone) can be selected on the graph. Such dependences were obtained for other substances as well.

The stabilizing effect of low oxidant doses observed in the experiment can be associated with the formation of protein–protein cross-links, the formation of a near-membrane protein layer, and cytoskeleton reorganization. For example, the reduction of the cytoskeletal framework ("submembrane reticulum") during ATP depletion causes the repair of "defective zones" or channels of ion leakage that are formed in the membrane by oxidizers [121].

ENDOGENOUS NITROGEN OXIDE DONORS AS REGULATORS OF ERYTHROCYTES STABILITY

One of the universal regulators of various biochemical and physiological processes are NO metabolites, nitrosothiols, and DNIC, which include three components: the NO group, ferrous iron, and ligands. Various endogenous substances, such as phosphates, thiols, imidazolate anions, and carnosine dipeptides, can act as DNIC ligands [122–125]. The formation of low molecular weight DNIC with glutathione ligands (DNIC-GS) in erythrocytes is the most likely.

In a few studies, it has been shown that DNIC-GS exert a protective effect on erythrocytes in various pathological situations [106, 126, 127]. The following mechanisms underlie the cytoprotective effect of these complexes: (1) the inhibition of lipid peroxidation reactions, (2) protection against oxidation of SH

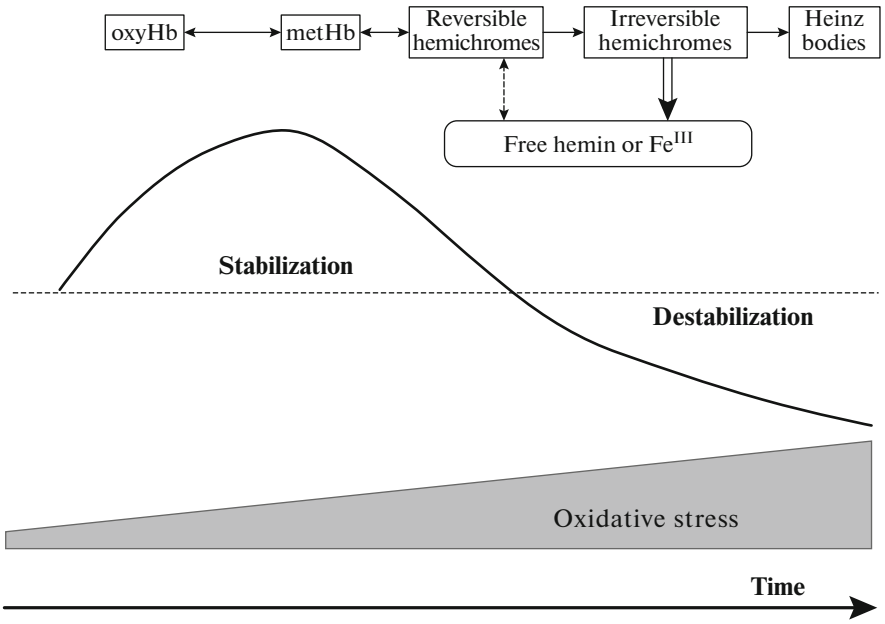

Fig. 1. Effect of Hb and its oxidation products on the mechanical stability of erythrocyte membranes.

groups of erythrocyte membrane proteins, (3) targeted transfer of NO or [Fe–NO_2]-fragment to plasma membrane and cytoskeletal proteins, and (4) the recovery of oxoferryl Hb. Earlier, we showed the formation of DNIC associated with the SH group of a cysteine residue of the Hb β-chain [124]. These complexes protected the Hb thiol groups from oxidation under conditions of induced oxidative stress. DNIC-GS also prevented the formation of oxoferrylMb [128].

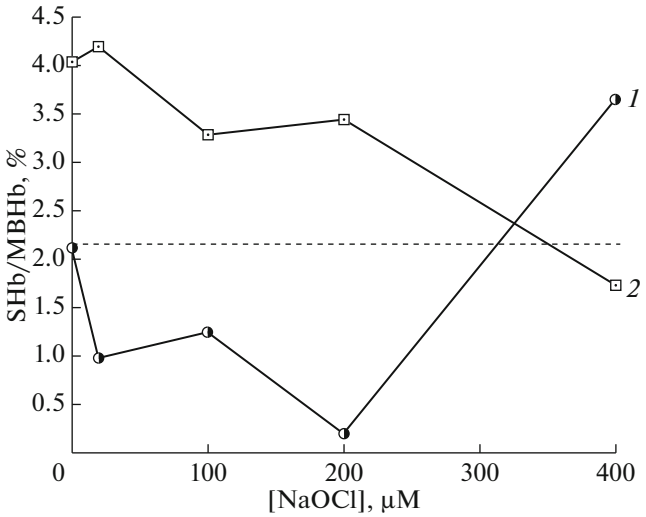

Fig. 2. Relationship between the amount of Hb released into the solution (*1*) and the MBHb level (*2*) under the action of increasing concentrations of NaOCl on a suspension of isolated erythrocytes. Each point is the mean of three experiments. SHb (Soluble Hb), Hb released into the solution as a result of hemolysis, % of the total Hb content in erythrocytes.

The effect of low physiological doses of DNIC-GS on the resistance of isolated erythrocyte suspensions to NaOCl-induced hemolysis was studied [120]. The hemolysis level in the erythrocytes pretreated with dinitrosyl complexes was lower than in the control sample for the entire range of used oxidant concentrations (Fig. 3). Figure 3 shows that DNIC-GS reduced the level of autohemolysis caused by experimental manipulations. Since old and damaged erythrocytes are first to be subjected to lysis, it is likely that DNIC-GS have a stabilizing effect on these cells. For comparison, the cytoprotective effect of the following substances formed during the dissociation of DNIC or their oxidative decomposition was studied: nitrosoglutathione (GSNO), reduced glutathione (GSH), ferrous iron, and nitrite ions. Pretreatment of the erythrocyte suspensions with the listed substances taken in concentrations equivalent to their content in DNIC resulted in the inhibition of erythrocyte oxidative hemolysis. It was reduced by 42% with GSH, by 35% with nitrite, and by 20% with GSNO. Ferrous iron had almost no effect on cell resistance. The degree of DNIC-GS inhibition under similar conditions was 55%.

The stabilizing effect of DNIC-GS can partly be explained by the transfer of the [Fe–NO_2] fragment to the SH groups of membrane receptors, which reversibly blocks their activity and thus decreases the cellular sensitivity to external influences. It is necessary to consider that protein modification can also cause structural changes in the membrane, increasing its rigidity.

The peculiarity of DNIC as bioregulators is that the difference between the biologically active dose and a damaging one for these compounds is much greater

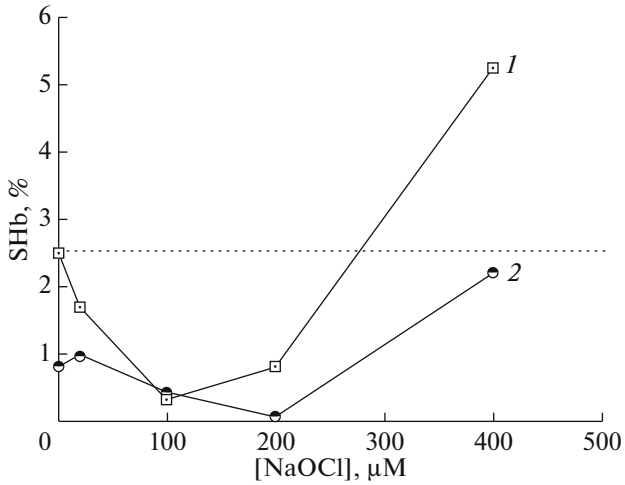

Fig. 3. Hypochlorite-induced erythrocyte hemolysis. *1*, Control points; *2*, pretreated with DNIC–GS. Each point is the mean of three experiments. The horizontal dotted line is the level of autohemolysis in the control. SHb (Soluble Hb), Hb released into the solution as a result of hemolysis, % of the total Hb content in erythrocytes.

than that of the nonphysiological stimuli. In our experiments [129], 400 μM DNIC-GS still had a stabilizing effect on the cells, while hypochlorite at the same concentration caused hemolysis (Fig. 4).

The results are consistent with the previously established facts of the positive effect of DNIC on erythrocyte metabolism [127].

HYPOTHESIS: HEMOGLOBIN AS A COMPONENT OF PROGRAMMED HEMOLYSIS

As noted above, erythrocytes exhibit hypoxia-induced reactions aimed at the regulation of blood flow. The view that considers the importance of intravascular hemolysis of single erythrocytes as an ATP source is being revised throughout the article for the regulation of local purinergic blood flow [70–72, 84]. According to this concept, the intravascular hemolysis of aging cells can be physiologically justified in conditions of hypoxia and during prolonged physical exertion. Calculations showed that even a small fraction of aging erythrocytes contains a sufficient quantity of ATP to have a regulatory effect on blood flow [72].

A positive correlation was found between the degree of erythrocytes hemolysis (with shear stress, hypoxia, and hypotonic shock) and the plasma ATP content [70, 71]. A question arises as to whether there are molecular mechanisms that destabilize the membrane in these situations. Many facts indicate that such intravascular hemolysis is not a random process [72]; there is a special program to weaken the membrane and to form the lytic micropores in it. The decrease in the ATP level, which supports the cytoskeleton structure, and/or an increase in the intracellular Ca^{2+} concentration are discussed as possible triggering mechanisms for hemolysis [72]. A possible factor in the violation of membrane stability can also be the weakening of the membrane-cytoskeletal interactions during prolonged deoxygenation, which increases the susceptibility of the membrane to rupture [72]. It should be emphasized that predominantly aging erythrocytes with multiple microdefects in the membrane are subject to hemolysis [130].

Since we are talking about hemolysis induced primarily by hypoxia, it is quite natural to assume that Hb participates in this process. It is well known that Hb is not only a pO_2 sensor but also an ROS source. Figure 5 schematically depicts a sequence of events leading to hemolysis under conditions of prolonged hypoxia. The very first stages are caused by Hb autooxidation and result in the formation of ROS and oxidized Hb

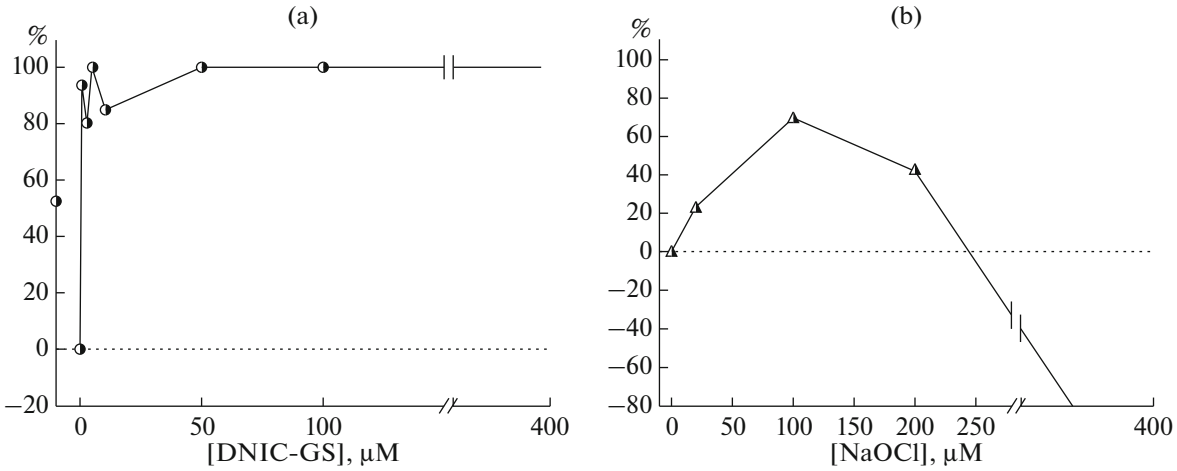

Fig. 4. Degree of erythrocyte autohemolysis inhibition (%) by increasing concentrations of DNIC–GS (a) and HOCl (b). The degree of hemolysis inhibition in the control was taken as zero. Each point is the mean of three experiments.

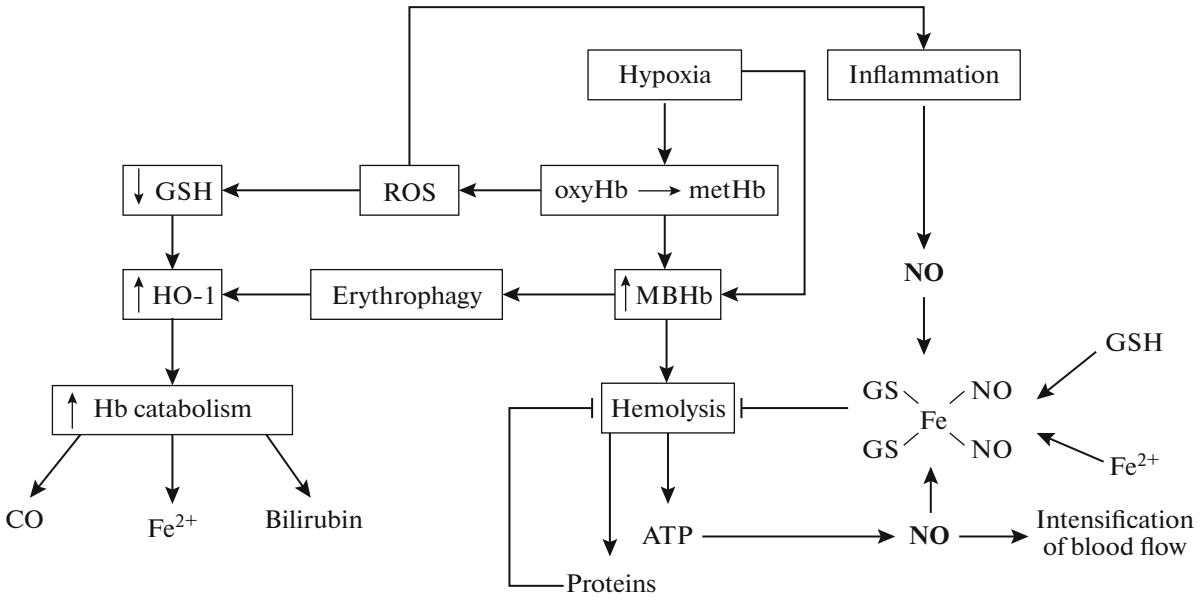

Fig. 5. Scheme of Hb participation in programmed hemolysis. HO-1, hemoxygenase; GSH, reduced glutathione. DNIC with glutathione ligands includes NO, formed as a result of stimulation of purinergic receptors of the endothelium and/or macrophage activation, e.g., during inflammation. The arrows indicate process enhancement (activation), blant ends, and inhibition.

forms [9]. As noted above, the excessive accumulation of these forms destabilizes the membrane (Fig. 1). We believe that the presence of a special hemolysis program initiated by Hb as a result of a change in its redox state due to a change in pO_2 or the redox conditions is quite likely. This is more possible, because a positive correlation is found between the hemolysis level and the MBHb content [71].

As described in the previous section, DNIC-GS have a stabilizing effect on a erythrocyte population predisposed to lysis. Therefore, it is possible that these complexes are part of the mechanism that regulates erythrocyte death in the bloodstream (Fig. 5). The ability of DNIC to participate in the regulation of programmed hemolysis is indicated by the fact that the level of these NO metabolites in the bloodstream is an integral indicator of oxygen and redox conditions. The molecular mechanism of the regulatory action of DNIC-GS is currently being studied in our laboratory.

All of the above led to the assumption that minor intravascular hemolysis can be considered a physiologically determined adaptive response of the body to moderate oxidative stress and hypoxia. The beneficial physiologic effects of Hb-decomposition products (hemin, CO, Fe^{2+}, and biliverdin) also indicate the benefits of hemolysis [131–133]. We emphasize that these products can only be beneficial at low concentrations.

It is known that hypoxia is a universal nonspecific syndrome in many pathological conditions. It may be associated with the development of anemia, which results from both a disturbance of iron homeostasis and the development of chronic inflammatory conditions. In the case of iron deficiency anemia, an increased MBHb level can be explained by the need for enhanced oxygen release by Hb (due to the release of membrane-bound 2.3-BPG) and the need for increased blood supply to the capillaries (due to the action of extracellular NO and ATP). In this case, the Hb binding is reversible. In anemia caused by chronic diseases and inflammation, increased MBHb levels are caused by the formation of oxidized and denatured Hb forms, which irreversibly bind to the membrane and cause vesicle formation, erythrocyte transformation, and destruction. Thus, the control of the oxygen-binding Hb properties, the rheological characteristics of erythrocytes, and the duration of its life is an adaptive regulatory mechanism.

USE OF MEMBRANE-BOUND HEMOGLOBIN IN CLINICAL AND BIOCHEMICAL DIAGNOSTICS

Erythrocytes are highly sensitive indicators of the metabolic state of the blood system that are capable of perceiving and accumulating damage; the reactivity of erythrocytes can be used as a criterion to assess the severity of the organism stress and the effectiveness of pharmacotherapy. It changes under the influence of physical exertion, hypoxia, endotoxins, and pharmacological drugs. There are various ways to assess erythrocyte reactivity: the suspension resistance to oxidative or osmotic hemolysis, the sorption ability, and electrophoretic mobility of erythrocytes [134], the reactivity of plasma membranes and their lipid composition, and the ability to bind alcian blue dye by gly-

cocalyx components [111]. The study [135] reported the existence of several adaptive variants of erythrocytes with different cell membrane reactivities.

The existence of a direct correlation between the oxidative stress level and the amount of MBHb allowed the use of this indicator to assess oxidative damage to erythrocytes [23, 136, 137]. An increase in MBHb was observed in metabolic syndrome [138, 139], coronary atherosclerosis [49], myocardial infarction [140], ischemia [49, 139–142], hypertension [139], psoriasis [143], chronic renal failure [144], chronic obstructive pulmonary disease [50], atypical/severe alcohol delirium [145], and diabetes mellitus [146]. Elevated MBHb levels were also detected in the erythrocytes of patients with thalassemia and various hemoglobinopathies [147–149]. Elevated MBHb levels were noted not only in pathological but also in certain physiological processes, among which are pregnancy [150], heavy muscular strains [41, 151], and natural erythrocyte aging [97]. The greatest degree of Hb binding to membranes occurred in aging cells with low levels of antioxidant enzymes. There is a direct correlation between the severity of the pathology and the MBHb level in all situations.

The MBHb level can be another reasonably simple criterion for erythrocyte reactivity. Let us give some examples illustrating the potential use of MBHb in clinical diagnostics. In the works of Pivovarov et al. [47, 139], it was proposed to use data on the MBHb content to calculate the coefficient of erythrocyte resistance to functional load (tissue hypoxia). A direct correlation was observed between the MBHb level and the severity of coronary heart disease (CHD) [49, 139]. Also, significant differences were revealed in the change in the MBHb content after functional load: there was a decrease in MBHb in healthy donors (from 3.6 to 0.9%), and, conversely, an increase (from 8.2 to 10.9%) in patients [47]. Thus, the MBHb level is an additional characteristic of CHD severity that makes it possible to assess the stress degree of the body's adaptive systems to ischemia in a patient with angina. It was proposed that the data be assessed on the MBHb level and integral index of the structural and functional state of erythrocyte membranes in patients with chronic obstructive pulmonary disease be calculated [50].

A positive correlation between the MBHb level and the intensity of oxidative stress in erythrocytes was described. In patients, an elevated level of MBHb positively correlated with an increase in leukocytes, lipid peroxidation, and the osmotic fragility of erythrocytes. In patients with chronic renal failure, an increase in MBHb correlated with an increase in C-reactive protein [144].

With a simple method to detect MBHb in blood samples [51], the range of normal values for MBHb was set at 3.3–4.9% [152]. The MBHb level was measured in patients of the National Medical Research Centre of oncology. The patient sample included a group of patients with malignant tumors of various localization (n = 125). Among the surveyed cancer patients, a discrepancy in the MBHb level was observed in 61%, while it was 38% in the control group (n = 26). The differences were significant (t = 1.7; p < 0.05). Among patients with diagnosed anemia (anemia of chronic diseases, iron deficiency anemia, microangiopathic hemolytic anemia) (n = 23), 74% had a discrepancy with the norm; in the control group (n = 23), it was 35% (t = 2.05; p < 0.05). Data on the MBHb content in the group of cancer patients were included in the developed pilot version of a digital expert system for anemia diagnosis in cancer diseases [153]. Such a system, along with the use of artificial neural networks [154], is recognized as one of the most promising areas of hematological diagnosis [155].

Despite the numerous studies of membrane-bound Hb, this indicator has not yet become commonly used in diagnostic practice. The main reasons for this lie in the absence of a standardized method to determine MBHb and an approved understanding of the normal range. It is also necessary to study the dynamics of MBHb changes under normal conditions and in pathology. The use of MBHb definition is most effective to assess the functional state of athletes [41, 111] and to improve the diagnosis and treatment of CHD [47, 49, 55, 139, 142].

CONCLUSIONS

Despite the long history of Hb studies, its signaling function has not been obvious for a long time, although the gas-sensory properties of Hb-like proteins have long been discussed in the scientific literature. If the signaling function is supplementary for erythrocyte Hb, participation in the redox signaling pathways is the main purpose for some members of the superfamily [156]; the properties of these Hbs are modulated via the modification (usually by oxidation) of surface cysteines.

The problem of oxygen-dependent regulation of erythrocyte metabolism has been discussed for over two decades. In this time, a vast array of experimental data has been accumulated; they confirm the key role of Hb in this process. In a recent paper [157], experiments on mice provided direct evidence that the deoxyHb-Band 3 complex is a trigger for various intracellular processes that adapt the erythrocyte properties to the oxygen conditions inside the body.

In summary, one can safely conclude that Hb is a real gas and redox sensor protein that perceives changes in the external and internal environment, generates information signals, transmits them to metabolic structures, and thereby performs self-regulation of intracellular metabolic processes. With its help, the interactions between such parameters as oxygen binding, ATP synthesis, pH regulation, deformability, and cell redox state are carried out. The Hb signaling func-

tion is realized through its reversible binding to the membrane. Mutual equilibrium transitions are possible between soluble and bound Hb due to the small difference in the free energies of these two forms. This ancient mechanism of self-regulation of living systems is the main one in erythrocytes, since they lack a nuclear apparatus, and a change in metabolism through the synthesis of new proteins is impossible. With the help of protein–membrane interactions, even processes such as programmed cell death (in the case of an erythrocyte, eryptosis), the cellular clock defining a cell's lifespan [96], and its circadian rhythms [158] are regulated. The study of these processes confirms the involvement of the cytoplasm in the metabolism regulation.

The erythrocyte is an example of a well-developed mechanism for the formation of an adaptive response using proteins and membrane structures. A question arises here regarding the end of the adaptive response and the beginning of pathology. The reversible incorporation of Hb into the membrane is aimed at optimization of the irreversible delivery of oxygen to tissues with cellular repair and the elimination of old and damaged cells. The near-membrane protein layer formed by Hb oxidation products may be a factor supporting the integrity of the native membrane. It is possible that such erythrocytes acquire greater stability, sacrificing functionality. Conversely, a high MBHb level leads to the violation of several erythrocyte characteristics: the charge balance, the membrane barrier properties, and the rheological properties. However, even membrane destabilization and the subsequent lysis of single cells can be physiologically justified, e.g., in conditions of hypoxia. Therefore, it is necessary to consider that the shifts in the MBHb level observed in various disease states do not always have a pathological orientation and may reflect compensatory–adaptive processes that are implemented in order to protect the body and/or cell in the changed conditions. The data on the range of MBHb changes corresponding to the adaptation zones can increase the informational significance of this indicator for diagnostics.

ACKNOWLEDGMENTS

This study was supported in part by the Russian Foundation for Basic Research, project no. 8-34-00561_mol_a.

COMPLIANCE WITH ETHICAL STANDARDS

The authors declare that they have no conflict of interest. This article does not contain any studies involving animals or human participants performed by any of the authors.

REFERENCES

1. Kurganov, B.I., *Organized Multienzyme Systems: Catalytic Properties*, New York: Academic Press, 1985.
2. Kurganov, B.I. and Lyubarev, A.E., *Biokhimiya*, 1991, vol. 56, no. 1, pp. 19–32.
3. Kaprel'yants, A.S., *Biol. Nauki*, 1988, vol. 6, pp. 5–13.
4. Oparin, A.I., Manskaja, S.M., and Magaram, M., *Biochem. Z.*, 1933, vol. 265, pp. 21–28.
5. Chung, H.S., Wang, S.B., Venkatraman, V., Murray, C.I., and Van Eyk, J.E., *Circ. Res.*, 2013, vol. 112, pp. 382–392.
6. Kosmachevskaya, O.V., Shumaev, K.B., and Topunov, A.F., *Biochemistry* (*Moscow*), 2019, vol. 84, Suppl. 1, pp. S206–S224.
7. Yang, J., Carroll, K.S., and Liebler, D.C., *Mol. Cell. Proteomics*, 2016, vol. 15. Article ID 1. doi 10.1074/mcp.O115.056051
8. Benesch, R.E. and Benesch, R., *Biochemistry*, 1962, vol. 1, pp. 735–738.
9. Kannan, R., Labotka, R., and Low, P.S., *J. Biol. Chem.*, 1988, vol. 263, pp. 13766–13773.
10. Zaitseva, O.I., Tereshchenko, V.P., Manchuk, V.T., Prakhin, E.I., Evert, L.S., and Nyagashkina, E.I., *Fundam. Issled., Biol. Nauki*, 2004, no. 6, pp. 8–21.
11. Petibois, C. and Déléris, G., *Arch. Med. Res.*, 2005, vol. 36, pp. 524–531.
12. Martusevich, A.A., Deryugina, A.V., and Martusevich, A.K., *J. Stress Physiol. Biochem.*, 2016, vol. 12, pp. 5–11.
13. Hoffman, J.F., *J. Gen. Physiol.*, 1958, vol. 42, pp. 9–28.
14. Anderson, H.M. and Turner, J.C., *Nature*, 1959, vol. 183, pp. 112–113.
15. Anderson, H.M. and Turner, J.C., *J. Clin. Invest.*, 1960, vol. 39, pp. 1–7.
16. Fung, L.W., *Biochemistry*, 1981, vol. 20, pp. 7162–7166.
17. Eisinger, J., Flores, J., and Salhany, J.M., *Proc. Natl. Acad. Sci. U. S. A.*, 1982, vol. 79, pp. 408–412.
18. Walder, J.A., Chatterjee, R., Steck, T.L., Low, P.S., Musso, G.F., Kaiser, E.T., Rogers, P.H., and Arnone, A., *J. Biol. Chem.*, 1984, vol. 259, pp. 10238–10246.
19. Chu, H., Breite, A., Ciraolo, P., Franco, R.S., and Low, P.S., *Blood*, 2008, vol. 111, pp. 932–938.
20. Cao, Z., Bell, J.B., Mohanty, J.G., Nagababu, E., and Rifkind, J.M., *Am. J. Physiol. Heart. Circ. Physiol.*, 2009, vol. 297, pp. 1494–1503.
21. Sega, M.F., Chu, H., Christian, J.A., and Low, P.S., *Blood Cells Mol. Dis.*, 2015, vol. 55, pp. 266–271.
22. Chan, E. and Desforges, J.F., *Blood*, 1974, vol. 44, p. 926.
23. Sharma, R. and Premachandra, B.R., *Biochem. Med. Metab. Biol.*, 1991, vol. 46, pp. 33–44.
24. Chaimanee, P. and Yuthavong, Y., *FEBS Lett.*, 1977, vol. 78, pp. 119–123.
25. Mishra, K., Chakrabarti, A., and Das, P.K., *J. Phys. Chem. B*, 2017, vol. 121, pp. 7797–7802.
26. Shaklai, N., Yguerabide, J., and Ranney, H.M., *Biochemistry*, 1977, vol. 16, pp. 5593–5597.

27. Rauenbuehler, P.B., Cordes, K.A., and Salhany, J.M., *Biochim. Biophys. Acta—Biomembranes*, 1982, vol. 692, pp. 361–370.
28. Datta, P., Chakrabarty, S., Chakrabarty, A., and Chakrabarty, A., *Biochim. Biophys. Acta*, 2008, vol. 1778, pp. 1–9.
29. Lebbar, I., Stetzkowski-Marden, F., Mauffret, O., and Cassoly, R., *Eur. J. Biochem.*, 1987, vol. 170, pp. 273–277.
30. Tuvia, S., Levin, S., and Korenstein, R., *Biophys. J.*, 1992, vol. 63, pp. 599–602.
31. Reithmeier, R.A., Casey, J.R., Kalli, A.C., Sansom, M.S.P., Alguel, Y., and Iwata, S., *Biochim. Biophys. Acta*, 2016, vol. 1858, pp. 1507–1532.
32. Spring, F.A., Bruce, L.J., Anstee, D.J., and Tanner, M.J.A., *Biochem. J.*, 1992, vol. 288, pp. 713–716.
33. Ferru, E., Giger, K., Pantaleo, A., Campanella, E., Grey, J., Ritchie, K., Vono, R., Turrini, F., and Low, P.S., *Blood*, 2011, vol. 117, pp. 5998–6006.
34. Pantaleo, A., Ferru, E., Pau, M.C., Khadjavi, A., Mandili, G., Matte, A., Spano, A., De Franceschi, L., Pippia, P., and Turrini, F., *Oxid. Med. Cell. Longev.*, 2016. Article ID 6051093. doi 10.1155/2016/6051093
35. Walder, J.A., Chatterjee, R., Steck, T.L., Low, P.S., Musso, G.F., Kaiser, E.T., Rogers, P.H., and Arnone, A., *J. Biol. Chem.*, 1984, vol. 259, pp. 10238–10246.
36. Tsuneshige, A., Imai, K., and Tyuma, I., *J. Biochem.*, 1987, vol. 101, pp. 695–704.
37. Demehin, A.A., Abugo, O.O., Jayakumar, J.R., and Rifkind, J.M., *Biochemistry*, 2002, vol. 41 P, pp. 8630–8637.
38. Shaklai, N. and Ranney, H.R., *Isr. J. Med. Sci.*, 1978, vol. 14, pp. 1152–1156.
39. Kumar, S. and Bandyopadhyay, U., *Toxicol. Lett.*, 2005, vol. 157, pp. 175–188.
40. Kriebardis, A.G., Antonelou, M.H., Stamoulis, K.E., Economou-Petersen, E., Margaritis, L.H., and Papassideri, I.S., *J. Cell. Mol. Med.*, 2007, vol. 11, pp. 148–155.
41. Welbourn, E.M., Wilson, M.T., Yusof, A., Metodiev, M.V., and Cooper, C.E., *Free Radic. Biol. Med.*, 2017, vol. 103, pp. 95–106.
42. Giardina, B., Scatena, R., Clementi, M.E., Ramacci, M.T., Maccari, F., Cerroni, L., and Condo, S.G., *Adv. Exp. Med. Biol.*, 1991, vol. 307, pp. 75–84.
43. Topunov, A.F. and Golubeva, L.I., *Usp. Biol. Khim.*, 1989, vol. 30, pp. 239–252.
44. Nikolić, M., Stanić, D., Antonijević, N., and Niketić, V., *Clin. Biochem.*, 2004, vol. 37, pp. 22–26.
45. Drabkin, D.R., *Am. J. Med. Sci.*, 1945, vol. 209, pp. 268–270.
46. Toktamysova, Z.S. and Birzhanova, N.Kh., *Biofizika*, 1990, vol. 35, no. 5, pp. 1019–1020.
47. Pivovarov, Yu.I., Kuznetsova, E.E., Gorokhova, V.G., and Koryakina, L.B., RF Patent no. 2008108370, 2008.
48. Sergeeva, A.S., Pivovarov, Yu.I., Kuril'skaya, T.E., and Kuznetsova, E.E., *Ross. Kardiol. Zh.*, 2014, vol. 115, no. 11, pp. 13–18.
49. Chuiko, E.S., Orlova, G.M., Kuznetsova, E.E., Gorokhova, V.G., and Koryakina, L.B., *Sib. Med. Zh.* (Irkutsk), 2015, no. 7, pp. 101–104.
50. Davydkin, I.L., Seleznev, A.V., Romasheva, E.P., and Mishina, N.A., in *Osnovy klinicheskoi gemostaziologii* (Basics of Clinical Hemostasiology), Samara: Ofort, 2009, pp. 77–96.
51. Nasybullina, E.I., Kosmachevskaya, O.V., and Topunov, A.F., *Trudy Karel. Nauch. Tsentra Ross. Akad. Nauk*, 2018, no. 4, pp. 93–104.
52. Sozarukova, M.M., Vladimirov, G.K., and Izmailov, D.Yu., in *Proc. Int. Sci. Conf. "Science and Practice: New Discoveries," Czechia, Karlovy Vary–Russia, Moscow*, Shvec, I.M., Ismagilova, L.A., Gur, V.A., Eds., Kirov: Mezhdunar. Tsentr Nauchno-Issled. Proektov, 2015, pp. 771–781.
53. Sears, D.A. and Lewis, P.C., *J. Lab. Clin. Med.*, 1980, vol. 96, pp. 318–327.
54. Gromov, P.S., Zakharov, S.F., Shishkin, S.S., and Il'inskii, R.V., *Biokhimiya*, 1988, vol. 53, no. 8, pp. 1316–1326.
55. Brazhe, N.A., Abdali, S., Brazhe, A.R., Luneva, O.G., Bryzgalova, N.Y., Parshina, E.Y., Sosnovtseva, O.V., and Maksimov, G.V., *Biophys. J.*, 2009, vol. 97, pp. 3206–3214.
56. Giardina, B., Messana, I., Scatena, R., and Castagnola, M., *Crit. Rev. Biochem. Mol. Biol.*, 1995, vol. 30, pp. 165–196.
57. De Rosa, M.C., Carelli, AlinoviC., Galtieri, A., Scatena, R., and Giardina, B., *Gene*, 2007, vol. 398, pp. 162–171.
58. Huang, Z., Louderback, J.G., Goyal, M., Azizi, F., King, S.B., and Kim-Shapiro, D.B., *Biochim. Biophys. Acta*, 2001, vol. 1568, pp. 252–560.
59. Vaughn, M.W., Huang, K.-T., Kuo, L., and Liao, J.C., *J. Biol. Chem.*, 2000, vol. 275, pp. 2342–2234.
60. Han, T.H., Hyduke, D.R., Vaughn, M.W., Fukuto, J.M., and Liao, J.C., *Proc. Natl. Acad. Sci. U. S. A.*, 2002, vol. 99, pp. 7763–7768.
61. Campanella, M.E., Chu, H., and Low, P.S., *Proc. Natl. Acad. Sci. U. S. A.*, 2005, vol. 102, pp. 2402–2407.
62. Kurganov, B.I. and Lyubarev, A.E., *Mol. Biol.*, 1988, vol. 22, no. 6, pp. 1605–1613.
63. Puchulu-Campanella, E., Chu, H., Anstee, D.J., Galan, J.A., Tao, W.A., and Low, P.S., *J. Biol. Chem.*, 2013, vol. 288, pp. 848–858.
64. Low, P.S., Rathinavelu, P., and Harrison, M.L., *J. Biol. Chem.*, 1993, vol. 68, pp. 14627–14631.
65. Chu, H. and Low, P.S., *Biochem. J.*, 2006, vol. 400, pp. 143–151.
66. Messana, I., Orlando, M., Cassiano, L., Pennacchietti, L., Zuppi, C., Castagnola, M., and Giardina, B., *FEBS Lett.*, 1996, vol. 390, pp. 25–28.
67. Weber, R.E., Voelter, W., Fago, A., Echner, H., Campanella, E., and Low, P.S., *Am. J. Physiol. Regul. Integr. Comp. Physiol.*, 2004, vol. 287, pp. 454–464.
68. Irzhak, L.I., *Gemoglobiny i ikh svoistva* (Hemoglobins and Their Properties), Moscow: Nauka, 1975.

69. Konev, S.V., *Strukturnaya labil'nost' biologicheskikh membran i regulyatornye protsessy* (Structural Lability of Biological Membranes and Regulatory Processes), Minsk: Nauka i Tekhnika, 1987.
70. Sikora, J., Orlov, S.N., Furuya, K., and Grygorczyk, R., *Blood*, 2014, vol. 124, pp. 2150–2157.
71. Luneva, O.G., Sidorenko, S.V., Ponomarchuk, O.O., Tverskoy, A.M., Cherkashin, A.A., Rodnenkov, O.V., Alekseeva, N.V., Deev, L.I., Maksimov, G.V., Grygorczyk, R., and Orlov, S.N., *Cell Physiol. Biochem.*, 2016, vol. 39, pp. 81–88.
72. Grygorczyk, R. and Orlov, S.N., *Front. Physiol.*, 2017, vol. 8. Article ID 1110. doi 10.3389/fphys.2017.01110
73. Jia, L., Bonaventura, C., Bonaventura, J., and Stamler, J.S., *Nature*, 1996, vol. 380, pp. 221–226.
74. Stamler, J.S., Singel, D.J., and Piantadosi, C.A., *Nature Medicine*, 2008, vol. 14, no. 10, pp. 1008–1009.
75. Huang, K.T., Keszler, A., Patel, N., Patel, R.P., Gladwin, M.T., Kim-Shapiro, D.B., and Hogg, N., *J. Biol. Chem.*, 2005, vol. 280, pp. 31126–31131.
76. Gladwin, M.T., Raat, N.J., Shiva, S., Dezfulian, C., Hogg, N., Kim-Shapiro, D.B., and Patel, R.P., *Am. J. Physiol. Heart Circ. Physiol.*, 2006, vol. 291, pp. 2026–2035.
77. Shiva, S., *Redox Biol.*, 2013, vol. 1, pp. 40–44.
78. Bergfeld, G.R. and Forrester, T., *Cardiovasc. Res.*, 1992, vol. 26, pp. 40–47.
79. Ramdani, G. and Langsley, G., *Biomed. J.*, 2014, vol. 37, pp. 284–292.
80. Pawloski, J.R., Hess, D.T., and Stamler, J.S., *Proc. Natl. Acad. Sci. U. S. A.*, 2005, vol. 102, pp. 2531–2536.
81. Thevenin, B.J.-M., Willardson, B.M., and Low, P.S., *J. Biol. Chem.*, 1989, vol. 264, pp. 15886–15892.
82. Salhany, J.M., *Biochemistry*, 2008, vol. 47, pp. 6059–6072.
83. Sridharan, M., Adderley, S.P., Bowles, E.A., Egan, T.M., Stephenson, A.H., Ellsworth, M.L., and Sprague, R.S., *Am. J. Physiol. Heart Circ. Physiol.*, 2010, vol. 299, pp. 1146–1152.
84. Luneva, O.G., Sidorenko, S.V., Maksimov, G.V., Grigorchik, R., and Orlov, S.N., *Biol. Membr.*, 2015, vol. 32, no. 4, pp. 223–234.
85. Wei, H.S., Kang, H., Rasheed, I.Y., Zhou, S., Lou, N., Gershteyn, A., McConnell, E.D., Wang, Y., Richardson, K.E., Palmer, A.F., Xu, C., Wan, J., and Nedergaard, M., *Neuron*, 2016, vol. 91, pp. 851–862.
86. Stefanovic, M., Puchulu-Campanella, E., Kodippili, G., and Low, P.S., *Biochem. J.*, 2013, vol. 449, pp. 143–150.
87. Ito, H., Murakami, R., Sakuma, S., Tsai, C.D., Gutsmann, T., Brandenburg, K., Poschl, J.M., Arai, F., Kaneko, M., and Tanaka, M., *Sci. Rep.*, 2017, vol. 7. Article ID 43134. doi 10.1038/srep43134
88. Nagababu, E., Mohanty, J.G., Bhamidipaty, S., Ostera, G.R., and Rifkind, J.M., *Life Sci.*, 2010, vol. 86, pp. 133–138.
89. Miyazawa, T., Suzuki, T., Fujimoto, K., and Kinoshita, M., *Mech. Ageing Dev.*, 1996, vol. 86, pp. 145–150.
90. Luneva, O.G., Brazhe, N.A., Fadyukova, O.E., Alakhaya, M.Ya., Baizhumanov, A.A., Parshina, E.Yu., Demidova, A.E., Koshelev, V.B., and Maksimov, G.V., *Dokl. Biochem. Biophys.*, 2005, vol. 405, no. 6, pp. 465–467.
91. Rifkind, J.M. and Nagababu, E., *Antioxid. Redox Signal.*, 2013, vol. 18, pp. 2274–2283.
92. Dutra, F.F. and Bozza, M.T., *Front. Pharmacol.*, 2014, vol. 5. Article ID 115. doi 10.3389/fphar.2014.00115
93. Shperling, I.A., Ryazantseva, N.V., Novitskii, V.V., Mikhalenko, A.N., Shevtsova, N.M., Naslednikova, I.O., Miller, A.A., and Filippova, O.N., *Vestn. Ross. Voenno-Med. Akad.*, 2011, vol. 3, no. 35, pp. 156–162.
94. Kiefmann, R., Rifkind, J.M., Nagababu, E., and Bhattacharya, J., *Blood*, 2008, vol. 111, pp. 5205–5214.
95. Klei, T.R., Meinderts, S.M., Berg, T.K., and van Bruggen, R., *Front. Immunol.*, 2017, vol. 8. Article ID 73. doi doi 10.3389/fimmu.2017.00073
96. Badior, K.E. and Casey, J.R., *IUBMB Life*, 2018, vol. 70, pp. 32–40.
97. Waugh, S.M. and Low, P.S., *Biochemistry*, 1985, vol. 24, pp. 34–39.
98. McPherson, R.A., Sawyer, W.H., and Tilley, L., *Biochemistry*, 1992, vol. 31, pp. 512–518.
99. Bosman, G.J., Lasonder, E., Groenen-Dopp, Y.A., Willekens, F.L., Werre, J.M., and Novotný, V.M., *J. Proteomics*, 2010, vol. 73, pp. 396–402.
100. Arashiki, N., Kimata, N., Manno, S., Mohandas, N., and Takakuwa, Y., *Biochemistry*, 2013, vol. 52, pp. 5760–5769.
101. Ferru, E., Pantaleo, A., Carta, F., Mannu, F., Khadjavi, A., Gallo, V., Ronzoni, L., Graziadei, G., Cappellini, M.D., and Turrini, F., *Haematologica*, 2014, vol. 99, pp. 570–578.
102. Willekens, F.L., Werre, J.M., Groenen- Döpp, Y.A., Roerdinkholder-Stoelwinder, B., de Pauw, B., and Bosman, G.J., *Br. J. Haematol.*, 2008, vol. 141, pp. 549–556.
103. Belevich, E.I., Kostin, D.G., and Slobozhanina, E.I., *Usp. Sovrem. Biol.*, 2014, vol. 134, no. 2, pp. 149–157.
104. Briglia, M., Rossi, M.A., and Faggio, C., *Curr. Med. Chem.*, 2017, vol. 24, pp. 937–942.
105. Lang, E., Bissinger, R., Qadri, S.M., and Lang, F., *Int. J. Cancer*, 2017, vol. 141, pp. 1522–1528.
106. Nicolay, J.P., Liebig, G., Niemoeller, O.M., Koka, S., Ghashghaeinia, M., Wieder, T., Haendeler, J., Busse, R., and Lang, F., *Pflugers Arch.*, 2008, vol. 456, pp. 293–305.
107. Colclasure, G.C. and Parker, J.C., *J. Gen. Physiol.*, 1992, vol. 100, pp. 1–10.
108. Vitoux, D., Beuzard, Y., and Brugnara, C., *J. Membr. Biol.*, 1999, vol. 167, pp. 233–240.
109. Gibson, J.S., Cossins, A.R., and Ellory, J.C., *J. Exp. Biol.*, 2000, vol. 203, pp. 1395–1407.
110. Barvitenko, N.N., Adragna, N.C., and Weber, R.E., *Cell Physiol. Biochem.*, 2005, vol. 15, pp. 1–18.
111. Borovskaya, M.K., Kuznetsova, E.E., Gorokhova, V.G., Koryakina, L.B., Kuril'skaya, T.E., and Pivovarov, Yu.I., *Byull. VSNTs SO RAMN*, 2010, vol. 73, pp. 334–354.
112. Knutton, S., Finean, J.B., Coleman, R., and Limbrick, A.R., *J. Cell Sci.*, 1970, vol. 7, pp. 357–371.

113. Komissarchik, Ya.Yu., Levin, S.V., Sviridov, B.E., Sabalyauskas, I.Yu., and Aiditite, G.S., in *Obshchie mekhanizmy kletochnykh reaktsii na povrezhdayushchie vozdeistviya* (General Mechanisms of Cellular Responses to Damaging Effects), Leningrad: Inst. Tsitol., 1977, pp. 29–31.

114. Jarolim, P., Lahav, M., Liu, S.C., and Palek, J., *Blood*, 1990, vol. 76, pp. 2125–2131.

115. Liu, S.C. and Palek, J., *J. Biol. Chem.*, 1984, vol. 259, pp. 11556–11562.

116. Kirschner-Zilber, I., Rabizadeh, E., and Shaklai, N., *Biochim. Biophys. Acta*, 1982, vol. 690, pp. 20–30.

117. Dadosh, N. and Shaklai, N., *J. Muscle Res. Cell. Motil.*, 1987, vol. 9, pp. 86–92.

118. Calabrese, E.J., *Microb. Cell*, 2014, vol. 1, pp. 145–149.

119. Nasonov, D.N., *Mestnaya reaktsiya protoplazmy i rasprostranyayushcheesya vozbuzhdenie* (Local Protoplasm Reaction and Distributing Excitation), Moscow: AkademIzdat, 1962.

120. Kosmachevskaya, O.V., Nasybullina, E.I., Blindar', V.N., and Topunov, A.F., *Aktual'nye voprosy eksperimental'noi biologii i meditsiny* (Current Issues of Experimental Biology and Medicine), Sukhum: Dom Pechati, 2017, pp. 421–429.

121. Belous, A.M., Bondarenko, V.A., Bondarenko, T.P., and Babiichuk, L.A., *Kriobiol. Kriomed.*, 1983, no. 12, pp. 13–24.

122. Vanin, A.F., *Dinitrozil'nye kompleksy zheleza s tiolsoderzhashchimi ligandami: fizikokhimiya, biologiya, meditsina* (Dinitrosyl Iron Complexes with Thiol-Containing Ligands: Physical Chemistry, Biology, and Medicine), Moscow: Inst. Komp. Issled., 2015.

123. Shumaev, K.B., Gubkin, A.A., Serezhenkov, V.A., Lobysheva, I.I., Kosmachevskaya, O.V., Ruuge, E.K., Lankin, V.Z., Topunov, A.F., and Vanin, A.F., *Nitric Oxide*, 2008, vol. 18, pp. 37–46.

124. Shumaev, K.B., Kosmachevskaya, O.V., Timoshin, A.A., Vanin, A.F., and Topunov, A.F., *Methods Enzymol.*, 2008, vol. 436, pp. 445–461.

125. Shumaev, K.B., Kosmachevskaya, O.V., Nasybullina, E.I., Gromov, S.V., Novikov, A.A., and Topunov, A.F., *J. Biol. Inorg. Chem.*, 2017, vol. 22, pp. 153–160.

126. Martusevich, A.K., Solov'eva, A.G., Peretyagin, S.P., and Davydyuk, A.V., *Biofizika*, 2014, vol. 59, no. 6, pp. 1173–1179.

127. Shamova, E.V., Bichan, O.D., Drozd, E.S., Gorudko, I.V., Chizhik, S.A., Shumaev, K.B., Cherenkevich, S.N., and Vanin, A.F., *Biofizika*, 2011, vol. 56, no. 2, pp. 265–271.

128. Shumaev, K.B., Petrova, N.E., Zabbarova, I.V., Vanin, A.F., Topunov, A.F., Lankin, V.Z., and Ruuge, E.K., *Biochemistry* (Moscow), 2004, vol. 69, no. 5, pp. 569–574.

129. Shumaev, K.B., Gorudko, I.V., Kosmachevskaya, O.V., Panasenko, O.M., Pugachenko, I.S., Topunov, A.F., and Ruuge, E.K., *Aktual'nye voprosy eksperimental'noi biologii i meditsiny* (Current Issues of Experimental Biology and Medicine), Sukhum: Dom Pechati, 2017, pp. 445–452.

130. Orbach, A., Zelig, O., Yedgar, S., and Barshtein, G., *Transfus. Med. Hemother.*, 2017, vol. 44, pp. 183–187.

131. Stec, D.E., Drummond, H.A., and Vera, T., *Hypertension*, 2008, vol. 51, pp. 597–604.

132. Otterbein, L.E., Soares, M.P., Yamashita, K., and Bach, F.H., *Trends Immunol.*, 2003, vol. 24, pp. 449–455.

133. Konrad, F.M., Zwergel, C., Ngamsri, K.C., and Reutershan, J., *Front. Immunol.*, 2017, vol. 8. Article ID 1874. doi doi 10.3389/fimmu.2017.01874

134. Shurkhina, E.S., Nesterenko, V.M., Tsvetaeva, N.V., and Nikulina, O.F., *Klin. Lab. Diagn.*, 2014, no. 7, pp. 41–46.

135. Zaitseva, O.I., Tereshchenko, V.P., Manchuk, V.T., Prakhin, E.I., Evert, L.S., and Nyagashkina, E.I., *Fundam. Issled., Biol. Nauki*, 2004, no. 6, pp. 18–21.

136. Sullivan, S.G. and Stern, A., *Biochim. Biophys. Acta*, 1984, vol. 774, pp. 215–220.

137. Rocha, S., Costa, E., Coimbra, S., Nascimento, H., Catarino, C., Rocha-Pereira, P., Quintanilha, A., Belo, L., and Santos-Silva, A., *Blood Cells, Mol. Dis.*, 2009, vol. 43, pp. 68–73.

138. Sergeeva, A.S., Pivovarov, Yu.I., and Babushkina, I.V., *Acta Biomed. Sci.*, 2015, no. 4, pp. 12–17.

139. Pivovarov, Yu.I., Kuznetsova, E.E., Gorokhova, V.G., Sergeeva, A.S., Babushkina, I.V., Koryakina, L.B., and Andreeva, E.O., *Byull. VSNTs SO RAMN*, 2016, vol. 1, no. 4, pp. 61–67.

140. Santos-Silva, A., Castro, E.M.B., Teixeira, N.A., Guerra, F.C., and Quintanilha, A., *Atherosclerosis*, vol. 116, no. is. 1995, pp. 199–209.

141. Santos-Silva, A., Rebelo, I., Castro, E., Belo, L., Catarino, C., Monteiro, I., Almeida, M.D., and Quintanilha, A., *Clin. Chim. Acta*, 2002, vol. 320, pp. 29–35.

142. Pivovarov, Yu.I., Kuznetsova, E.E., Koryakina, L.B., Gorokhova, V.G., and Kuril'skaya, T.E., *Tromboz Gemostaz Reol.*, 2013, no. 2, pp. 39–45.

143. Rocha-Pereira, P., Santos-Silva, A., Rebelo, I., Figneiredo, A., Quintanilha, A., and Teixeira, F., *Br. J. Dermatol.*, 2004, vol. 150, pp. 232–244.

144. Costa, E., Rocha, S., Rocha-Pereira, P., Castro, E., Miranda, V., Sameiro, FariaM., Loureiro, A., Quintanilha, A., Belo, L., and Santos-Silva, A., *Open Clin. Chem. J.*, 2008, vol. 1, pp. 57–63.

145. Vinogradov, D.B., Panachev, I.V., Izarovskii, B.V., Kozochkin, D.A., and Tseilikman, O.B., *Vopr. Narkol.*, 2010, no. 5, pp. 44–50.

146. Bryszewska, M., *J. Clin. Chem. Clin. Biochem.*, 1988, vol. 26, pp. 809–813.

147. Sears, D.A. and Luthra, M.G., *J. Lab. Clin. Med.*, 1983, vol. 102, pp. 694–698.

148. Scott, M.D., Berg, J.J., Repka, T., Rouyer-Fessard, P., Hebbel, R.P., Beuzard, Y., and Lubin, B.H., *J. Clin. Invest.*, 1993, vol. 91, pp. 1706–1712.

149. Hebbel, R.P., *Blood*, 1991, vol. 77, pp. 214–237.

150. Catarino, C., Rebelo, I., Belo, L., Rocha-Pereira, P., Rocha, S., Bayer, CastroE., Patricio, B., Quintanilha, A., and Santos-Silva, A., *J. Perinat. Med.*, 2009, vol. 37, pp. 19–27.

151. Santos-Silva, A., Rebelo, I., Castro, E.M.B., Belo, L., Guerra, A., Rego, C., and Quintanilha, A., *Clin. Chim. Acta*, 2001, vol. 306, pp. 119–126.

152. Kosmachevskaya, O.V., Nasybullina, E.I., Blindar', V.N., Shumaev, K.B., and Topunov, A.F., in *Novye informatsionnye tekhnologii v meditsine, biologii, farmakologii i ekologii* (New Information Technologies in Medicine, Biology, Pharmacology, and Ecology), Moscow: Inst. Novykh Inform. Tekhnol., 2016, pp. 172–180.

153. Nasybullina, E.I., Nikitaev, V.G., Pronichev, A.N., Blindar, V.N., Kosmachevskaya, O.V., and Topunov, A.F., *Bull. Lebedev Phys. Inst.*, 2015, vol. 42, no. 7, pp. 206–208.

154. Amendolia, S.R., Brunetti, A., Carta, P., Cossu, G., Ganadu, M.L., Golosio, B., Mura, G.M., and Pirastru, M.G., *Med. Decis. Making*, 2002, vol. 22, pp. 18–26.

155. Lippi, G. and Plebani, M., *J. Lab. Precis. Med.*, 2018, vol. 3. Article ID 68. doi doi 10.21037/jlpm.2018.07.09

156. Reeder, B.J., *Antioxid. Redox. Signal.*, 2017, vol. 26, pp. 763–776.

157. Chu, H., McKenna, M.M., Krump, N.A., Zheng, S., Mendelsohn, L., Thein, S.L., Garrett, L.J., Bodine, D.M., and Low, P.S., *Blood*, 2016, vol. 128, pp. 2708–2716.

158. Bayer, S.B., Low, F.M., Hampton, M.B., and Winterbourn, C.C., *Free Radic. Res.*, 2016, vol. 50, pp. 1329–1339.

Translated by I. Shipounova