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REVIEW

Positive (Regulatory) and Negative (Cytotoxic) Effects of Dinitrosyl Iron Complexes on Living Organisms

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Abstract—The proposed in our studies mechanism of dinitrosyl iron complex (DNIC) formation through the main step of disproportionation of two NO molecules in complex with Fe^{2+} ion leads to emergence of the resonance structure of dinitrosyl-iron fragment of DNIC, [$Fe^{2+}(NO)(NO^+)$]. The latter allowed suggesting capacity of these complexes to function as donor of both neutral NO molecules as well as nitrosonium cations (NO^+), which has been demonstrated in experiments. Analysis of biological activity of DNICs with thiol-containing ligands presented in this review demonstrates that NO molecules and nitrosonium cations released from the complexes exert respectively positive (regulatory) and negative (cytotoxic) effects on living organisms. It has been suggested to use dithiocarbamate derivatives to enhance selective release of nitrosonium cations from DNIC in living organisms followed by simultaneous incorporation of the released NO molecules into the biologically non-active mononitrosyl iron complexes with dithiocarbamate derivatives.

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INTRODUCTION. PHYSICOCHEMICAL FEATURES OF DINITROSYL IRON COMPLEXES DETERMINING THEIR ABILITY TO SERVE AS DONORS OF NO AND NO^+

Currently there is reason to believe that the dinitrosyl iron complexes (DNICs) with thiol-containing ligands functioning in living organisms as an "working" form of one of the universal regulators of metabolic processes – nitric oxide (NO) could exert both positive (regulatory) and negative (cytotoxic) effects on these organisms [1-5]. The aim of this review was to consider the basis of such multidirectional effects of DNICs on live organisms.

DNICs with thiol-containing ligands, mononuclear form of these complexes (M-DNICs) in particular, were discovered in the yeast cells in the early 1960s based on the anisotropic ESR signal with g-factor values characteristic for this form: $g_\perp = 2.04$; $g_\parallel = 2.014$; $g_{av} = 2.03$ ("2.03-signal") (Fig. 1) [6-10].

The nature of centers responsible for this signal was elucidated after it was shown in the study by Vanin [12] that the solutions of M-DNIC complexes with low molecular weight thiol containing ligands characterized with narrow symmetric ESR signal recorded at room temperature, demonstrate anisotropic EPR signal after freezing identical to the "2.03-signal" (Fig. 2). Analysis of the resolved hyperfine structure (HFS) of the ESR signal of these complexes at room temperature showed that the M-DNICs complexes with thiol-containing ligands include in addition to one iron atom two nitrosyl- and two thiol-containing ligands (chemical formula – $[(RS^-)_2Fe(NO)_2]$), which was in complete agreement with the data of X-ray diffraction analysis of the crystals of these complexes obtained later.

It was shown in the already mentioned publication of the results of ESR study of DNICs with low molecular

Abbreviations: DEDC, diethyldithiocarbamate; DNIC, dinitrosyl iron complex; GS-NO, S-nitrosoglutathione; GSH, glutathione; M-DNIC and B-DNIC, mono- and binuclear forms of dinitrosyl iron complex; MGD, N-methyl-D,L-glucamine dithiocarbamate; MNIC, mononitrosyl iron complexes; MS, mercaptosuccinate; NAC, N-acetyl-L-cysteine; NAC-NO, S-nitroso-N-acetyl-L-cysteine; NO^+, nitrosonium cation; TS, thiosulfate.

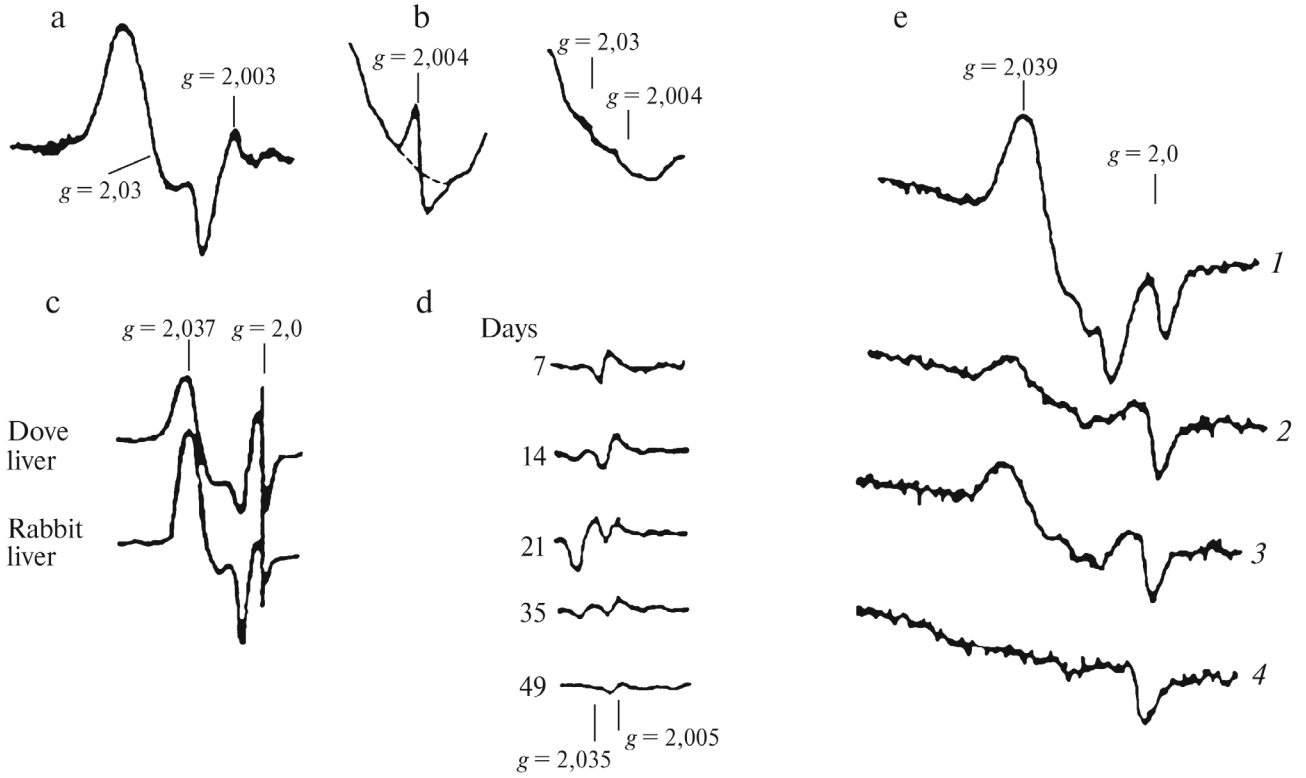


Fig. 1. "2.03-signal" first recorded in 1960s: a) in baker's yeast [6, 7]; b) in chemically induced hepatoma [8]; c) in dove and rabbit liver [9]; d) in rat liver 7-35 days after introduction of carcinogens in their diet [10]. e) "2.03-signal" recorded in 1990 in the activated mouse macrophages in the presence of the NO-synthase substrate, L-arginine (spectrum *1*), L-arginine and NO-synthase inhibitor N-methyl-L-arginine (NMLA) (spectrum *2*), in the absence of L-arginine and NMLA (spectrum *3*), and in the presence of only NMLA (spectrum *4*) [11].

weight thiols [13] that the M-DNICs with thiol-containing ligands are reversibly converted into the bi-nuclear forms of these complexes, B-DNICs (formula – $[(RS^-)_2Fe_2(NO)_4]$), with decrease of the thiol concentration in the solution. Unlike the M-DNICs, these forms are diamagnetic due to coupling of paramagnetic iron-dinitrosyl fragments in N-DNICs with participation of bridging sulfur atoms connecting these fragments.

Another feature is worth to mention. It was shown in the already cited publication [12] as well as in our further studies [14-16] that the DNICs ligands could include not only low molecular weight thiols, but also protein thiol groups with formation of corresponding protein-linked DNICs. Due to the low mobility of the protein component of these complexes at room temperature, which is insufficient for averaging anisotropy of g-factor, ESR signal of these complexes recorded at room temperature is anisotropic with shape identical to the "2.03-signal".

Obviously, biological activity of the DNICs with thiol-containing ligands exerting both positive and negative effects on live organisms is defined by the properties of electron shells of these complexes described by different medium-dependent resonance structures. Information about these structures could be obtained from the mechanisms of formation of paramagnetic mono-nuclear form of these complexes, M-DNICs.

As follows from the already mentioned publication [13], DNICs with thiol-containing ligands could emerge during treatment of aqueous solution of iron ions in bivalent state and thiols with gaseous NO. Considering that in the course of this synthesis NO molecules bind in pairs with Fe^{2+} ion, the emerging iron-dinitrosyl fragment contains even number (8) of electrons (6 d-electrons of Fe^{2+} + 2 electrons of two free radical NO molecules) at the upper molecular orbitals (MO), which, according to the Enemark–Feltham notion [17], corresponds to the formula of iron-dinitrosyl fragment $[Fe(NO)_2]^8$. In this case under conditions of sufficient separation of upper MOs the iron-dinitrosyl fragment in DNICs should be diamagnetic (ESR inactive), which does not correspond to the real low-spin paramagnetic state of M-DNIC with S = 1/2. To remove this contradiction one electron should be either added or deleted from the iron-dinitrosyl fragment, which would result in transformation of the diamagnetic electron configuration into paramagnetic one described in the Enemark–Feltham notion as $[Fe(NO)_2]^9$ or $[Fe(NO)_2]^7$, respectively.

The most natural way to replace $[Fe(NO)_2]^8$ configuration with $[Fe(NO)_2]^7$ in the course of DNIC synthesis is introduction of the reaction of disproportionation presented in Scheme 1 – single-electron mutual oxidation-reduction of the free-radical NO molecules with

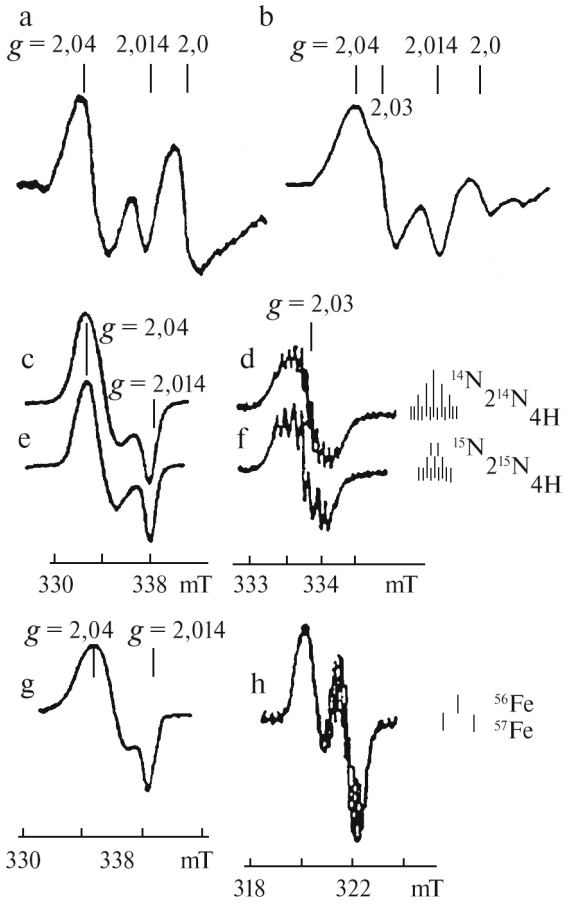


Fig. 2. "2.03-signal" (g = 2.04; 2.014) recorded in the wet rabbit liver (a) and wet yeast cells (b [14]; ESR spectra of DNIC complexes with cysteine containing ^{14}NO (c and d), ^{15}NO (e and f) [15], or ^{57}Fe (g and h) [16]. Spectra were recorded at room temperature (a, b, d, f, and h) and 77K (c, e, and g). Deciphering of HFS of the 2.03-signal from DNIC with cysteine is shown on the right. ESR signal with g = 2.0 (a and b) is due to endogenous free radicals.

participation of d-orbital electrons of the iron ion. This mechanism could be termed "oxidative" mechanism of DNICs formation [18-22].

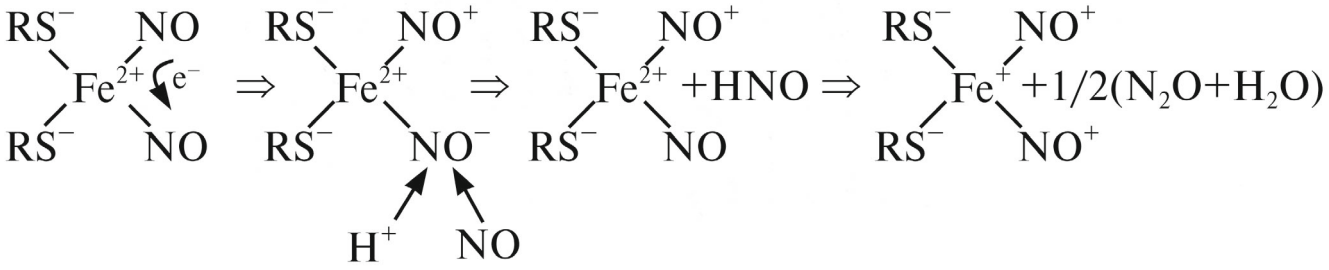
Hydrolysis of the nitroxyl anion produced as a result of disproportionation of NO molecules in these complexes results in formation of a nitroxyl molecule (HNO), which leaves its position as the iron ligand followed by occupation of the freed position with the third NO molecule completing synthesis of low-spin (with S = 1/2) mono-nuclear complexes (M-DNIC); according to the Scheme 1, one of the possible resonance structures is presented as $[(RS^-)_2Fe^{2+}(NO^+)(NO)]$.

According to Vanin et al. [16], the resonance structure $[Fe^{2+}(NO)(NO^+)]$ describing the state of dinitrosyl-iron fragment in the M-DNIC is also characteristic for the same fragments involved in the bi-nuclear form of DNICs (formula – $[(RS^-)_2Fe^{2+}{}_2(NO^+)_2(NO)_2]$).

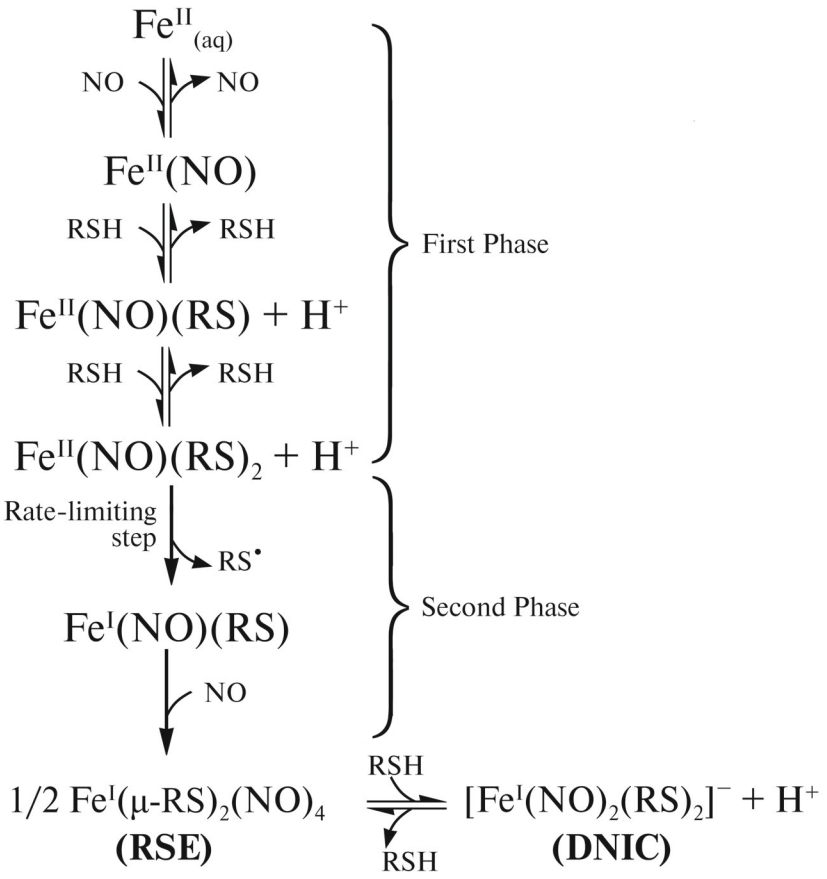
In principle, nitrosonium cation (NO^+) in the composition of the dinitrosyl-iron fragment could be also subjected to hydrolysis (binding with hydroxyl anion) resulting in its transformation in the course of this reaction into nitrite anion (under conditions of neutral pH values). However, such transformation does not occur in the DNICs with thiol-containing ligands due to the presence of thiol sulfur atoms in these complexes. High π-donor activity of these atoms transferring part of electron density to nitrosonium cations thus ensuring decrease of positive charge on these ligands prevents their binding with hydroxyl anions i.e., hydrolysis of these cations. As a result, stability of M-DNICs increases dramatically, which increases their life-time in cells and tissues [19]. Moreover, this provides the possibility of synthesis of stable exogenous DNICs that could be used in experiments with various live organisms.

Is it possible to synthesize low-spin DNICs with thiol-containing ligands (with S = 1/2) through one-electron reduction of the dinitrosyl iron fragment with initial $[Fe(NO)_2]^8$-configuration mentioned above? This possibility has been mentioned based on the results of experimental studies by Truzzi et al. [23]. In accordance with the scheme of DNIC formation suggested by them (Scheme 2), first a mono-nitrosyl iron complex is produced that includes two thiol-containing ligands with configuration of the mono-nitrosyl iron fragment $[Fe(NO)]^7$, and next a one-electron reduction of this fragment by the thiol-containing ligand in its composition occurs followed by substitution of this ligand with NO molecule and introduction of another thiol in the complex. This mechanism of DNIC formation could be termed a reductive mechanism.

As a result of such reductive mechanism M-DNICs emerge with $[Fe(NO)_2]^9$-configuration of dinitrosyl-iron fragment described by the resonance structure $[(RS^-)_2Fe^+(NO)_2]$.



Scheme 1. Oxidative mechanism of formation of M-DNICs with thiol-containing ligands in the reaction between Fe^{2+}, NO, and thiols.



Scheme 2. Reductive mechanism of DNIC formation in an aqueous solution as suggested by Truzzi et al. [23]. RSE, Roussine Red Salt thioester.

The main argument against this scheme is the fact that based on this mechanism it is impossible to explain formation of M-DNICs with the ligands of non-thiol nature such as phosphate, citrate, succinate, and finally, with water, which are characterized similar to the DNICs with thiol ligands by low-spin state with S = 1/2 and ESR signal although different form the 2.03-signal but with the same value of g-factor and approximately the same main values of this tensor [12, 13]. Obviously, it is very unlikely that the all mentioned non-thiol ligands are capable, similar to their thiol-containing analogues, to reduce mononitrosyl-iron fragment with $[Fe(NO)]^7$-configuration to $[Fe(NO)]^8$ followed by its transformation into $[Fe(NO)_2]^9$ configuration with S = 1/2. On the other hand, the mechanism of M-DNIC formation based on the reaction of disproportionation of NO molecules does not depend on the ability of anionic ligands to play a role of reducers.

Resonance structure of dinitrosyl iron fragments $[Fe^{2+}(NO^+)(NO)]$ in M- and B-DNICs emerging in accordance with the reaction mechanism of their formation involving disproportionation of NO molecules allows suggesting their ability to play a role of a donor of either neutral NO molecules or nitrosonium cation; moreover, based on the scheme of decomposition equilibrium (Scheme 3), they are formed in equal amounts [18-22].

$$[(RS^-)_2Fe^{2+}(NO^+)(NO)] \leftrightarrow Fe^{2+} + NO + \frac{NO^+ + RS^-}{RS^- - NO^+} + RS^-$$

Scheme 3. Chemical equilibrium between M-DNICs with thiol-containing ligands synthesized according to the "oxidative" mechanism and components of this complex.

Nitrosonium cations are incorporated into S-nitrosyls (RS-NO) in the presence of thiols, and are hydrolyzed into nitrite anions upon thiol removal.

Another situation, obviously, is realized in the course of equilibrium decomposition of M-B-DNICs with $[Fe(NO)_2]^9$-configuration of the dinitrosyl-iron fragments (Scheme 4).

$$[(RS^-)_2Fe^+(NO)_2] \leftrightarrow Fe^{2+} + NO + NO^- + 2RS^-$$

Scheme 4. Chemical equilibrium between M-DNICs with thiol-containing ligands synthesized according to the "reductive" mechanism and components of this complex.

In this case nitrosyl ligands are released in the form of neutral NO molecules and nitroxyl anions, but not in the form of nitrosonium cations. The latter is prevented, according to the Scheme 4, by localization of high electron density on the dinitrosyl-iron fragments according to the formula $[Fe(NO)_2]^9$ based on the Enemark–Feltham

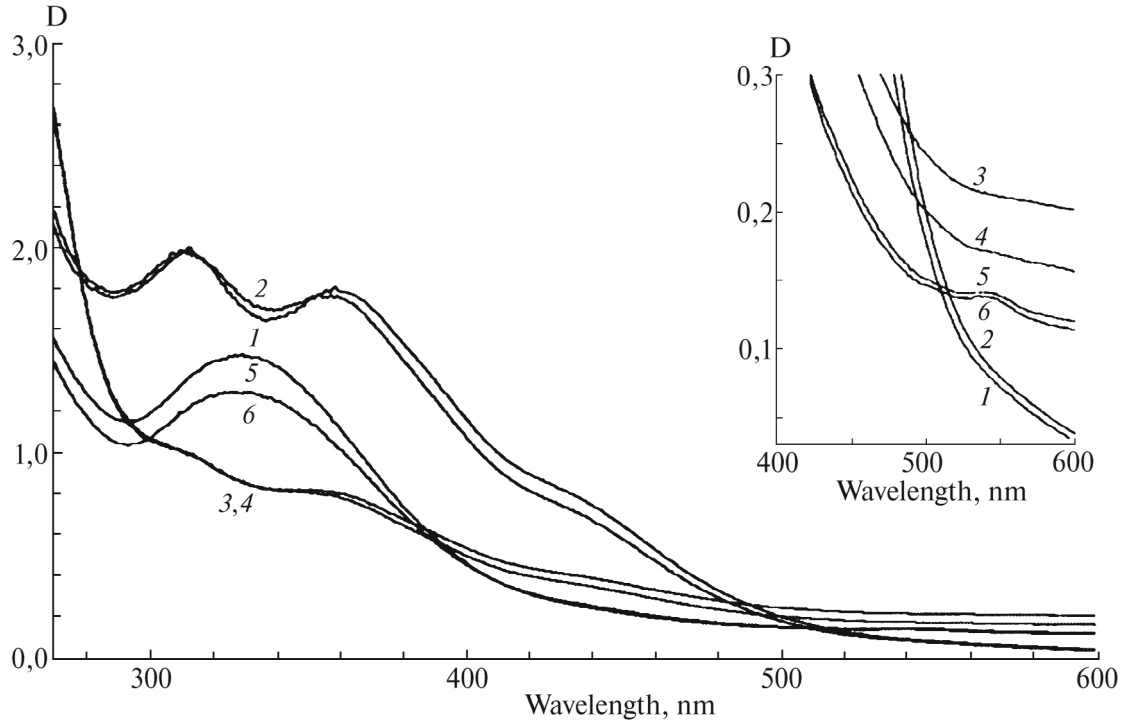


Fig. 3. Absorption spectra of 0.5 mM solution of B-DNIC-NAC containing 2.0- and 0.5-mM NAC not incorporated into the complexes (curves *1* and *2*, respectively). Curves *3-6* – absorption spectra of 2.0 mM solutions of the same complexes containing 8.0 mM (curves *3* and *4*) or 2.0 mM NAC (curves *5* and *6*) not incorporated in these complexes, which were subjected to heating at 80°C under anaerobic conditions for 7-9 min. Spectra *5* and *6* with bands at 334 and 543 nm (in inset) are due to the formed NAC-NO. Extinction coefficients for B-DNIC and NAC-NO at absorption bands 360 and 334 nm are, respectively, 3700 (in terms of one iron atom in B-DNIC) and 0.94 $M^{-1} \cdot cm^{-1}$ [22].

notions [17]. It could be suggested in this connection that if one would be able to demonstrate experimentally release of both NO molecules and nitrosonium cations from the M-DNICs with thiol ligands as well as from their bi-nuclear analogues, this could serve as an unambiguous proof of the existence of $[Fe(NO)_2]^7$-configuration in the dinitrosyl-iron fragments of these complexes. In other words, it could be stated that their synthesis is realized via mechanism of disproportionation of NO molecules that bind in pairs with the ions of two-valent iron followed by the processes indicated in Schemes 1 and 3.

Such type of research has been conducted [20-22] and fully verified this hypothesis. It was found that heating of the bi-nuclear form of DNICs with glutathione (GHS) or N-acetyl-L-cysteine (NAC) at 80°C and acidic solutions (pH 1-2) independent on the presence or absence of air in the system resulted, based on disappearance of their absorption spectra, in complete decomposition of these complexes with emergence of absorption bands typical for S-nitrosylated forms of GSH and NAC (GS-NO and NAC-NO) as demonstrated in Fig. 3 for B-DNIC–NAC [22].

As follows from Fig. 3, at minimal ratio between the B-DNIC-NAC concentration and free NAC (not incorporated into the complex) (1 : 1), heating of their solution under anaerobic conditions resulted in formation of NAC-NO at concentration corresponding to NAC-NO in the NO^+ form of half of nitrosyl ligands in B-DNICs (according to Scheme 3) (Fig. 3, curves *5* and *6* recorded in two independent experiments). Hence, this result demonstrated that decomposition of the DNICs with thiol-containing ligands indeed resulted in the release of nitrosonium cations, which is, according to the opinion of the author of this review, an unambiguous proof of realization of the suggested mechanism of formation of these complexes through the reaction of disproportionation of NO molecules (Scheme 1).

At higher concentration of free NAC (at the ratio NAC : B-DNIC-NAC of 4 : 1) no formation of NAC-NO was observed in the experiments (Fig. 3, curves *3* and *4* recorded in two independent experiments). It is likely that the excess of this thiol could initiate reduction of NO^+ into NO. Possible mechanism of this reduction will be discussed in the final part of this review.

As follows from Scheme 3, in addition to the release of nitrosonium cations from the decomposing DNICs, these complexes are able to serve as effective donors of NO molecules. Release of the latter from DNICs was demonstrated in the study by Borodulin et al. [24] with the help of developed method of detecting gaseous NO based on the characteristic UV absorption spectrum consisting of four narrow equidistant absorption bands in the range 220-190 nm (Fig. 4, right panel). These bands were recorded using a light beam directed along the axis of the spectrophotometer cuvette filled with gaseous NO or NO_2 (Fig. 4, left panel).

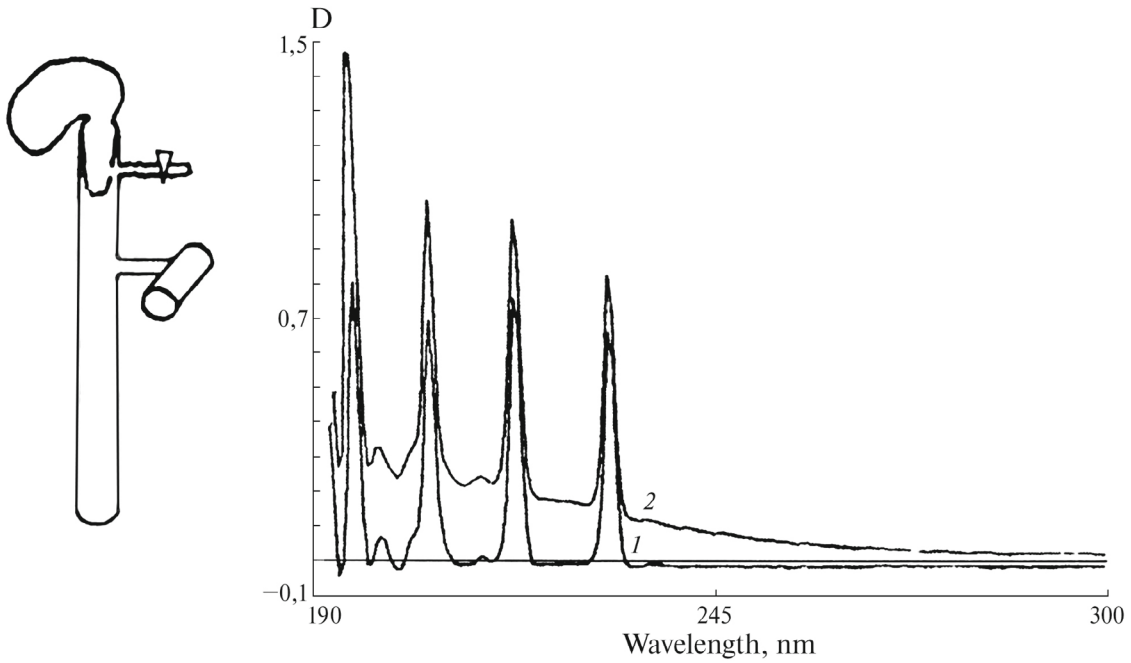
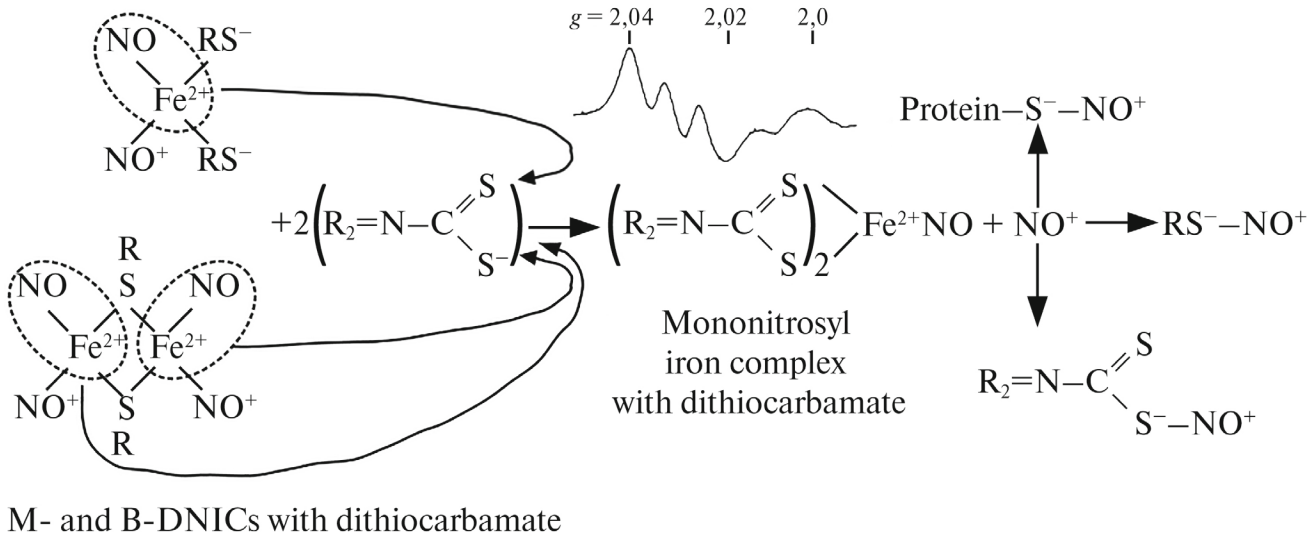


Fig. 4. Left panel: schematic presentation of the Thunberg apparatus used for measuring optical absorbance of NO and NO_2 in gaseous phase. Right panel: optical absorbance spectrum of NO (four narrow equidistant bands, curve *1*) and total optical absorbance of the NO and NO_2 gas mixture (curve *2*).

Blockade of the thiol groups in M- and B-DNICs with thiol-containing ligands with various thiol reagents or oxidation of these groups causing decomposition of DNICs results in hydrolysis of the nitrosonium cations released from the complexes at neutral pH levels into nitrites (due to the absence of thiols), while the other part of nitrosyl ligands is released in the form of gaseous NO, detected based on the presence of four equidistant optical absorption bands as shown in Fig. 4 [20, 22].

Ability of DNICs with thiol-containing ligands to serve in living organisms as donors of both nitrosonium cations as well as neutral NO molecules, obviously, makes it difficult to find out which of the DNIC components could be responsible for their biological actions. We have suggested an experimental approach to solve this problem, which allows elucidation biological effects of DNICs exerted only by the released nitrosonium cations. This approach involves the use of dithiocarbamate (DTC)



Scheme 5. Mechanism of transformation of B- and M-DNICs with thiol-containing ligands into MNICs with DEDC or MGD. NO^+ cations released in the process could participate in S-nitrosylation of low molecular weight thiols or protein thiols, as well as thiol groups in the composition of DEDC or MGD. ESR signal recorded at 77K with the values of g-factor 2.04 and 2.02 and with triplet hyperfine structure typical for MNICs with dithiocarbamate is presented in the upper panel [25].

derivatives (diethyldithiocarbamate, DEDC, or N-methyl-D,L-glucamine dithiocarbamate, MGD) in the experiments with DNICs [25]. As shown in Scheme 5, contact of these agents with DNICs results in attracting of the iron-mononitrosyl group, [Fe(NO)], from the nitrosyl-iron [$Fe(NO)_2$]-fragments of the complexes with formation of stable ESR-detectable mononitrosyl iron complexes (MNICs) with dithiocarbamate derivatives (R_2=NCS_2) with formula [(R_2=NCS_2)$_2$FeNO].

The formed highly stable MNIC with DEDC or MGD are biologically inactive, hence, neither NO molecules incorporated in them nor Fe^{2+} ions and dithiocarbamate ligands are released from them, and that is why they cannot exert any effects on live organisms. As to NO^+ cations released from the dinitrosyl iron fragments of DNICs in the process, they could participate in the reaction of S-nitrosylation presented in Scheme 5 with formation of corresponding S-nitrosothiols [25-27]. Moreover, nitrosonium cations could affect different cellular components as strong oxidizers [26, 27]. These processes are likely to induce various cytotoxic effects in the cells.

The experiments conducted by the researchers from the United Kingdom with the Swiss 3T3 fibroblast cell culture demonstrated that nitrosonium cations are 40-times more toxic dose-wise than NO molecules [28]. NO^+ donor, nitroprusside [$(CN^-)_5Fe^{2+}NO^+]^{2-}$, caused apoptosis in 50% of fibroblast cells already after 2-h incubation of these cells with 20 μM of nitroprusside, while the effect of the same magnitude was observed for the Swiss 3T3 fibroblast cells subjected to the action of 800 μM NO only after 24-h incubation with gaseous NO or NO donors (S-nitrosoglutathione, GS-NO, or S-nitroso-N-acetylpenicillamine, SNAP). Similar results were obtained for bacterial cells [29, 30]. Hence, there are grounds to suggest that nitrosonium ions produce more pronounced cytotoxic effect in comparison with NO. Considering that both these agents – NO^+ and NO – could be released from the DNICs with thiol-containing ligands, negative cytotoxic effects exerted by these complexes on live organisms most likely are associated with the nitrosonium cations released from them, while the regulatory effects are due to the neutral NO molecules released from DNICs.

POSITIVE, REGULATORY ACTION OF DNICs AS NO DONORS ON LIVING ORGANISMS

At present various positive regulatory effects of DNICs with thiol-containing ligands on different biological systems have been identified. In particular, their ability to increase viability of non-malignant cell cultures at concentration in the range 20-250 μM [31-34] was demonstrated, at the same time cytotoxic effect of these complexes on malignant cell cultures at the same range of concentration was shown [25, 35, 36]. Another example: it was found out that oral administration of DNICs with mercaptoethanol to mice followed by binding of these complexes with albumin or mucin resulted in their transport from stomach to brain thus inducing neurogenesis in hippocampus and enhancing cognitive abilities of these animals [37]. Unfortunately, the question on what components of DNICs (NO molecules or nitrosonium cations) mediated these effects was not addressed in this study. With regard to other examples of positive effects of DNICs with thiol-containing ligands on live organisms presented below, it can be stated with confidence that these effects indeed were due to the release of neutral NO molecules from these complexes.

Mainly this refers to vasodilatory [38-41] and hypotensive [42-47] effects of these complexes illustrated in Figs. 5 and 6.

As follows from Fig. 5, administration of DNIC with cysteine caused relaxation of the vessels starting with concentration of 10 nM. In the process, the rate of restoration of the vessel tone decreases with the increase of the content of free cysteine in the medium, which correlates with the increase of stability of DNICs in this medium.

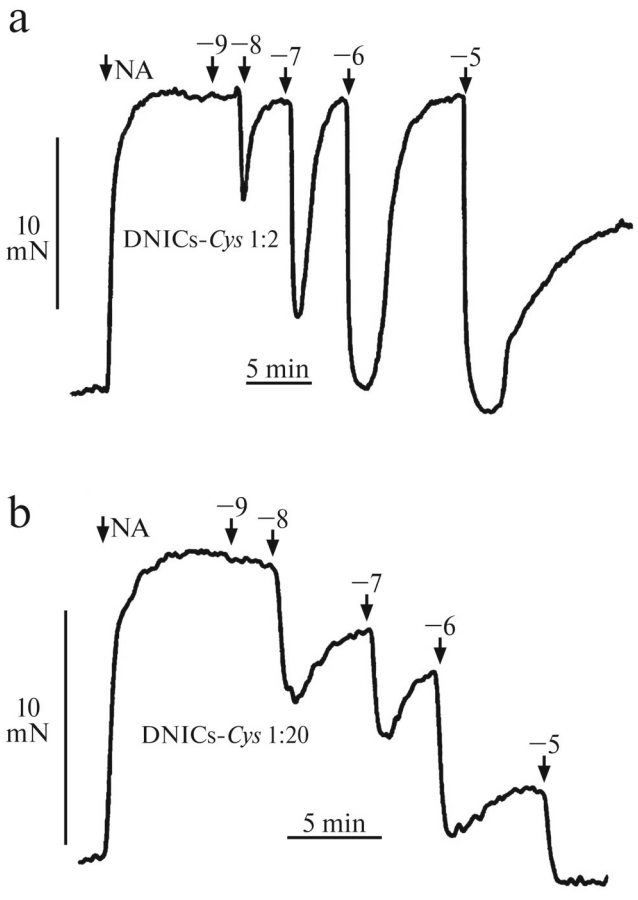


Fig. 5. Vasodilatory effects of DNICs with cysteine at a ratio of DNIC and non-bound (free) cysteine of 1 : 2 (a) or 1 : 20 (b) depending on the DNIC dose (from 1 nM to 10 μM) demonstrated using ring segment of the rat abdominal aorta with preserved endothelium that prior to measurements was constricted with noradrenaline (NA, 10^{-7} M).

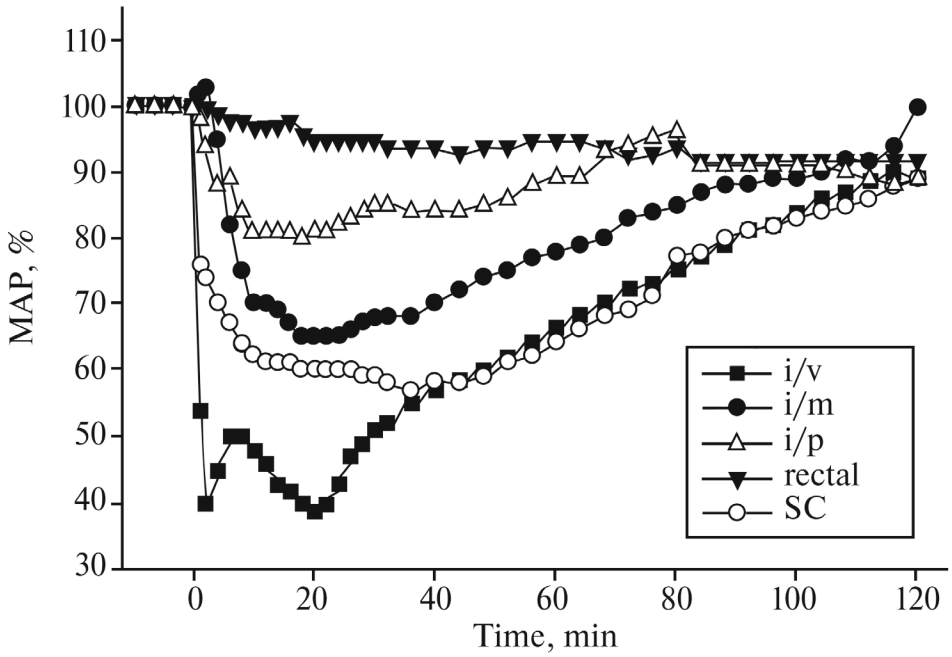


Fig. 6. Changes of mean arterial pressure (MAP, in % to initial) in rats during bolus intravenous (i/v), intramuscular (i/m), intraperitoneal (i/p), rectal (rectal) or subcutaneous (SC) injection of B-DNICs with glutathione to rats (dose 2 μmol per kg of body weight).

Vasodilatory activity of these complexes was totally suppressed in the presence of inhibitors of guanylate cyclase – heme-containing enzyme activated by molecular NO [39]. This fact indicated unambiguously that precisely the NO molecules released from DNICs are responsible for the vasodilatory effect of these complexes.

The vasodilatory action of DNICs with thiol-containing ligands was for the first time discovered by our research group in collaboration with Yu. P. Vedernikov from All-Union Scientific Center of Cardiology [38], later this result was reproduced in our collaborative study in Germany by Busse and Mülsch. At the same, the group of researchers from the United Kingdom independently reported the similar result [40]. It was logical to expect that high vasodilatory activity of DNICs should mediate hypotensive effect of these complexes, and this was confirmed in 1980s in our studies with the drugged (anesthetized) rats and, next, with the awake animals [42-44]. Detailed investigation of this effect was continued in collaboration with the researchers from All-Union Scientific Center of Cardiology in 2000s [45-47].

As an example, one of the results of these studies is presented in Fig. 6 demonstrating changes of mean arterial pressure (MAP) in rats depending on the method of administration of B-DNICs with glutathione (Fig. 6) [47].

Unlike in the case of intravenous administration of the complex during which a short-term phase (several min) decreases of MAP due to release of NO from the administered low molecular weight DNICs with GSH, in the cases of other methods of administration this phase was not observed. In this case hypotension was determined by the release of NO from protein DNICs formed as a result of transport of dinitrosyl iron [$Fe(NO)_2$]-fragments from low molecular weight DNICs to protein thiols producing protein-bound DNICs. High stability of the latter facilitated only slow release of NO molecules from them accompanied with the slow decrease of MAP and its slow restoration to the initial level. Similar kinetics of MAP changes was also observed in the case of i/v administration of DNICs with GSH following rapid transformation of the latter into the protein-bound DNICs, which were detected in animals based on the 2.03-signal that preserved its anisotropic shape during increase of the temperature of its recording from 77K to room temperature [45, 46].

The results of investigation of the effect of DEDC on kinetics of hypotensive effect of DNICs with thiosulfate on awake rats imply participation of NO molecules released from the DNICs in the process as determinants of the hypotensive effect of these complexes (Fig. 7a, curves *1* and *2*) [44]. As can be seen in Fig. 7a (curve *3*), i/v injection of this complex to the rats 1 min after administration of the DEDC solution results in practically complete abolishment of the hypotensive effect. Administration of DEDC after DNICs at the time points indicated in Fig. 7a (curves *4* and *5*) with arrows caused short-term decrease of arterial pressure followed by gradual restoration to initial level.

As shown in Scheme 5, contact of DNICs with dithiocarbamate derivatives (DTC) leads to formation of rather stable MNIC-DTC complexes with strongly attached mononitrosyl-iron group, thus preventing its components (NO molecule and Fe^{2+} ion) from exerting their effects on cells. Obviously, this particular incorporation of NO into

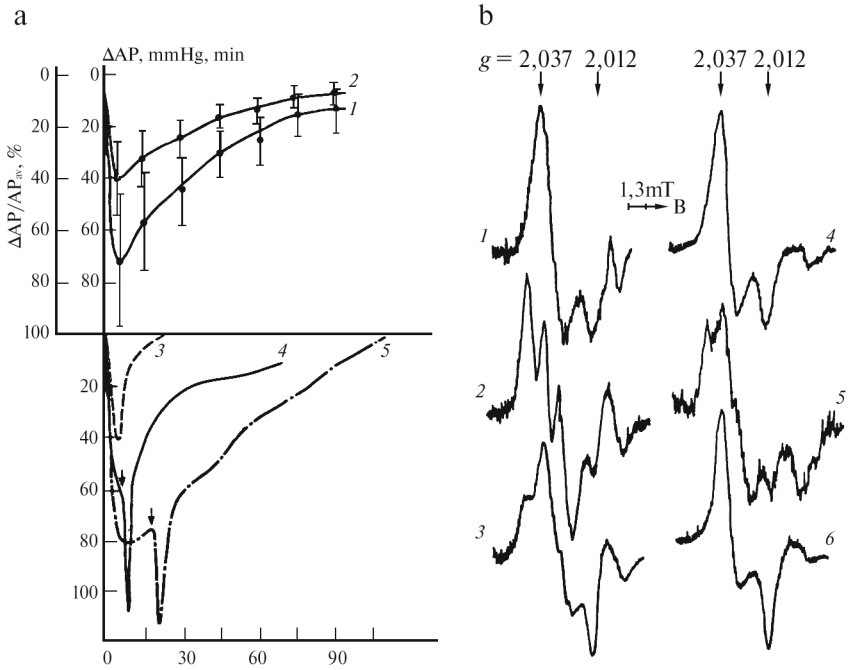


Fig. 7. Changes of arterial pressure (AP or ΔAP) in awake rats (a) after i/v administration of DNICs with thiosulfate at the dose of 10 μmol per kg of the animal body weight (curves *1* and *2*); DNIC was injected 1 min after i/v administration of DEDC (30 mg/kg) (curve *3*); DEDC was administered to animals after DNICs at the time points indicated with arrows (curves *4* and *5*). b) ESR spectra of blood (*1* and *4*), liver (*2* and *5*), and kidneys (*3* and *6*) obtained from rats after consecutive administration of DEDC and DNICs with 1-min interval (*1-3*) or consecutive administration of DNICs and DEDC with 1-min interval (*4-6*). Spectra are recorded at 77 K.

MNIC with DEDC led to sharp decrease of the hypotensive effect of DNICs administrated to pre-treated rats as shown in Fig. 7. This is also corroborated by the fact of transformation of the 2.03-signal in the tissues from these animals (Fig. 7b, spectra *1-3*): triplet ESR signal characteristic for MNIC-DEDC was recorded in the case of liver and kidney, while in the case of blood decrease of the amplitudes of the 2.03-signal at g = 2.012 was observed. We have observed similar decrease in the experiments investigating interaction of DEDC with DNICs bound in the rat blood with albumin [48]. With regard to additional short-term decrease of arterial pressure on administration of DEDC to rats after administration of DNICs (Fig. 7a, curves *4* and *5*), it was likely due to the rapid decomposition of S-nitrosothiols (RS-NO) formed in accordance with the Scheme 5 in the reaction DNICs with DEDC. NO molecules released from RS-NO were responsible for the observed short-term decrease of arterial pressure [44]. Part of the DNICs in tissues of animals could in these experiments remain inaccessible to DEDC, which is manifested by the lower contribution of the triplet ESR signal of MNICs-DEDC to the total ESR spectra presented in Fig. 7b (spectra *5* and *6*). These remaining DNICs facilitated delay in restoration of arterial pressure to the initial level (Fig. 7a, curves *4* and *5*).

Main results of these studies provided proof that hypotensive activity of DNICs in our experiments was due to NO molecules released from these complexes that further interacted with heme iron in guanylate cyclase thus activating this enzyme.

Similar conclusion could be drawn with regard the ability of DNICs with thiol-containing ligands to accelerate healing of at least skin wounds. The healing process mediated by DNICs was completely identical to the would healing stimulated by treatment with gaseous NO, or NO produced in the wounds from L-arginine under the action of inducible and constitutive NO-synthases [49, 50]. The same notion could be applied to the ability of DNICs with thiol-containing ligands to suppress thrombocyte aggregation and, moreover, initiate lysis of already formed thrombi [51, 52]. It must be mentioned here that DNICs are also capable to induce dramatical increase of erythrocyte elasticity thus facilitating their movement in capillaries as demonstrated by Shamova and co-authors [53] – the feature that could be used for improving microcirculation.

In all these biosystems positive, regulatory effects of DNICs are due to release of NO molecules from them, which, in turn, activate one of the most important regulatory enzyme in cells and tissues – guanylate cyclase, which is responsible for generation of one of the secondary messengers – cyclic guanosine monophosphate (cGMP) thus initiating a complex system of biochemical processes resulting in physiological effects caused by the interaction of guanylate cyclase with NO molecules, part of which could be originated from DNICs.

cGMP hydrolysis by the respective phosphodiesterase (PDE-5) blocked realization of these effects. On the other hand, suppression of activity of this enzyme, by, for example, papaverine, enhanced these effects,

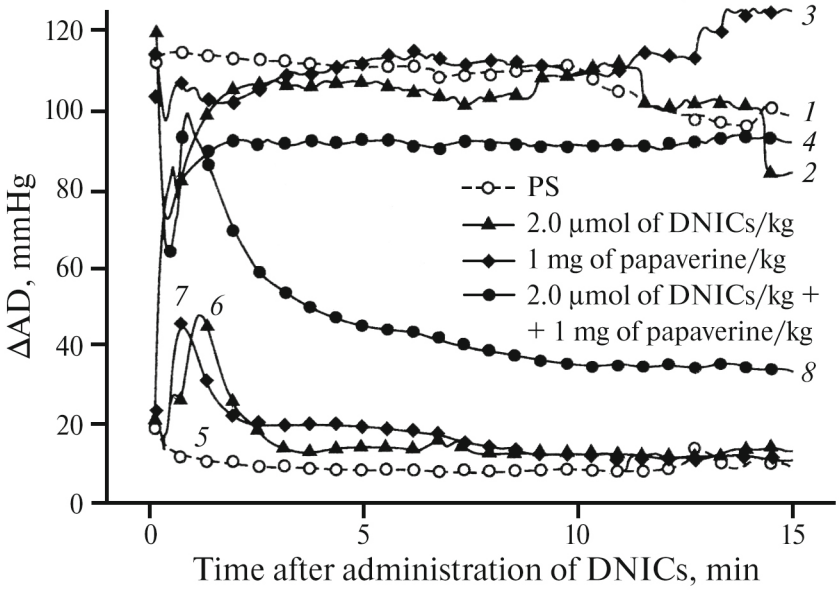


Fig. 8. Time-course of systemic mean arterial pressure (curves *1-4*) and intracavernosal blood pressure in cavernous penis bodies (curves *5-8*) during intracavernous injection (i/c) of: DNICs with cysteine to male rats at a dose 2 μmol per kg of animal body weight – curves *2* and *6*; papaverine (1 mg/kg of animal body weight) – curve *3* and *7*, combined administration of DNICs + papaverine at the same concentrations – curves *4* and *8*, and physiological solution (PS) – curves *1* and *5*.

such as significantly prolonged hypotensive effect caused by DNICs [54]. These peculiarities were clearly pronounced in our experiments with animals investigating erectile action of DNICs with thiol-containing ligands [54], results of which are presented in Fig. 8.

Injection of DNICs with cysteine in combination with papaverine (Viagra analogue) into the male rat penis resulted in sharp increase of the time of penile erection. Moreover, priapism (irreversible penal erection due to the absence of venous blood drain from penis) developed in 10 out of 17 animals participated in the experiment. The value of blood pressure in cavernous bodies reached the levels equal to the mean arterial pressure in the same animals. Interestingly enough, such penile erection response was also observed in the rats subjected to denervation of cavernous tissues [54].

NEGATIVE, CYTOTOXIC EFFECTS OF DNICs AS NO^+ DONORS ON CELLS AND TISSUES

Cytotoxic effects of DNICs as donors of nitrosonium ions were first demonstrated in the collaborative study of Russian and German scientists [35] for the culture of human Jurkat cells using M-DNICs with thiosulfate (M-DNIC–TS). As can be seen in Fig. 9, simultaneous administration of 0.1 mM M-DNIC–TS and 0.2 mM MGD resulted in the increase of the number of apoptotic cells to 60%. Considering that in accordance with the Scheme 5 at the indicated ratio between the amounts of M-DNIC and dithiocarbamate derivative (MGD) the mononitrosyl iron group from M-DNIC–TS should be completely incorporated into the biologically inactive MNIC-MGD with release of 0.1 mM of nitrosonium cations, it is possible to state that the observed 60%-level of apoptosis is due exclusively to the 0.1 mM NO^+ released from M-DNIC–TS.

Hence, MGD, which practically facilitates decomposition of M-DNIC–TS (as follows from the EPR spectra I-III presented in Fig. 9) increases dramatically cytotoxic effect of M-DNICs via release of nitrosonium ions. It is worth mentioning that the replacement of thiosulfate in the M-DNIC–TS with glutathione resulted in formation of more stable B-DNIC-GSH complex with significantly less pronounced cytotoxic activity (Fig. 9a, bar *3*).

Similar experiments conducted in our research group with the human tumor cells MCF-7 using B-DNICs with mercaptosuccinate (B-DNIC–MS) and B-DNIC–GSH also demonstrated that the addition of MGD to them decreased viability of the cells in the case of both complexes (viability was evaluated with MTT-test, i.e., based on activity of mitochondria [25]) (Fig. 10).

As follows from Fig. 10, 0.5 mM of nitrosonium ions released under the action of MGD from B-DNIC-MS decrease viability of the cells by 30% in accordance with the mechanism suggested above. In the experiments with B-DNIC-GSH this level was achieved using the complex/MGD ratio of 1 mM/3 mM. It cannot be ruled out that low efficiency of nitrosonium ions in this case was due to low penetration of MGD into the cells insufficient to exert toxic effect of nitrosonium ions emerging in the cells on mitochondria.

More effective suppression of cell viability (their death) in this culture was determined based on the

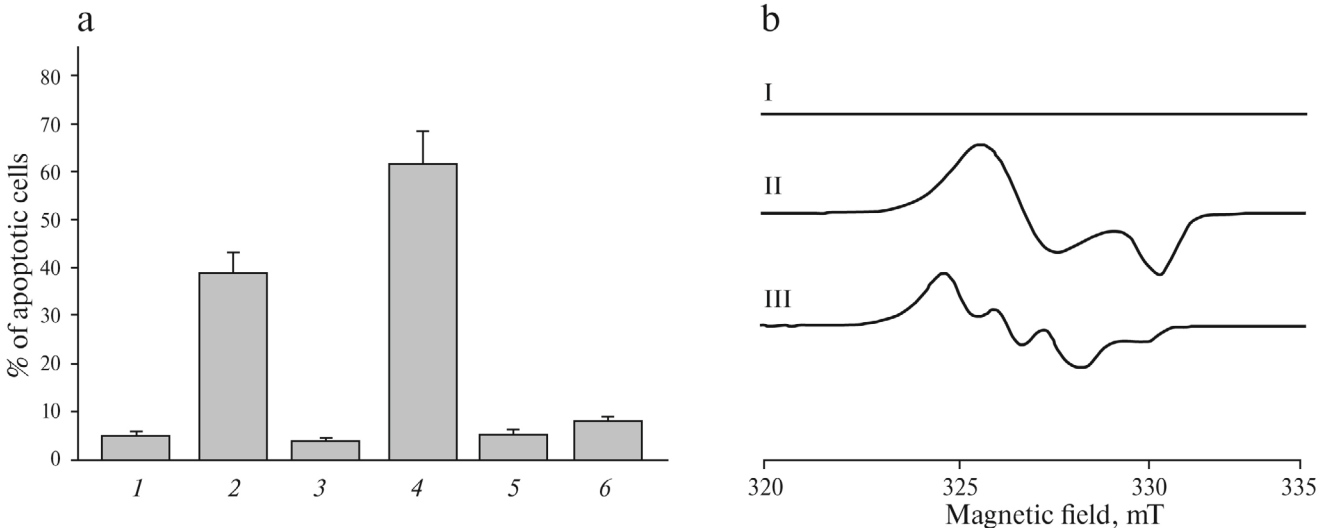


Fig. 9. Initiation of apoptosis in the Jurkat cell culture upon treatment with M-DNIC–TS and MGD. a) Fraction (in %) of apoptotic Jurkat cells in the control (*1*); after addition of 0.1 mM M-DNIC–TS (DNIC, *2*), 0.1 mM M-DNIC–TS C + 2 mM GSH (DNIC + GSH, *3*), M-DNIC–TS + 0.2 mM MGD (DNIC + MGD, *4*), 0.2 mM MGD (*5*), and 0.1 mM (Fe + thiol) (*6*). b) Transformation of the 2.03-signal (I), recorded in the cell culture after addition of M-DNIC–TS, into the ESR signal of MNIC-MGD (II) following addition of 0.2 mM MGD to the cell culture. III, control.

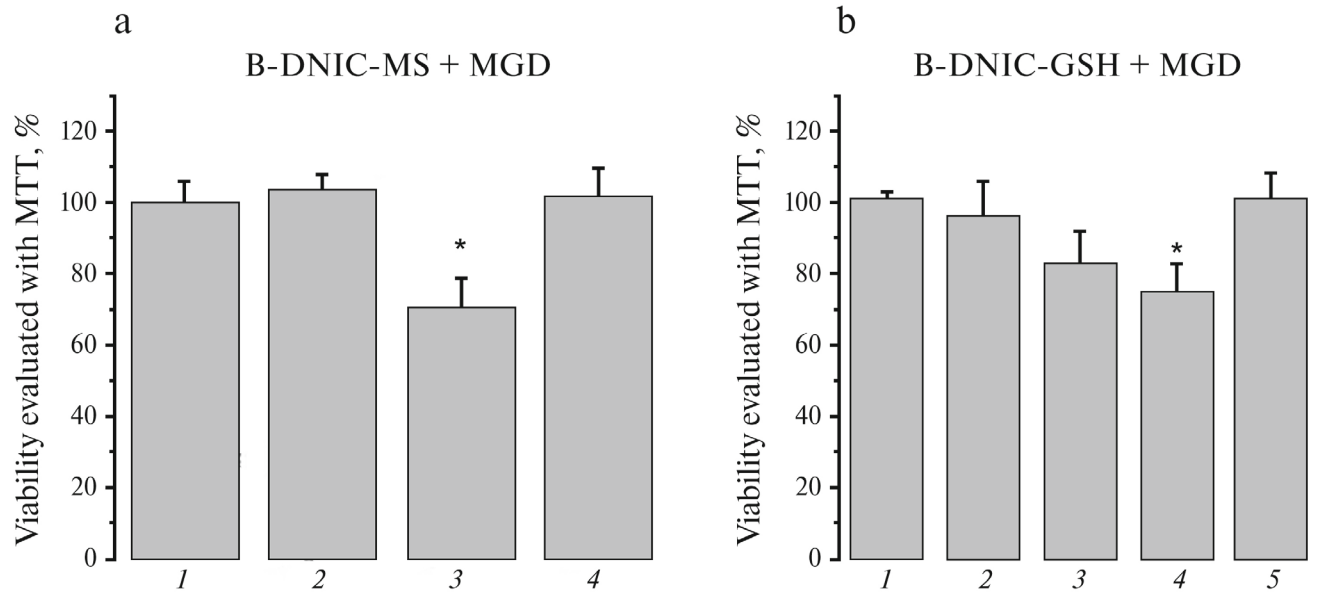


Fig. 10. Decrease of viability of MCF-7 cells under the action of B-DNIC-MS (a) and B- DNIC-GSH (b) after addition of MGD to them determined using MTT test. Ratio of concentrations of both B-DNIC and MGD (B-DNIC/MGD): a) 0 mM/0 mM (*1*); 0.5 mM/0 mM (*2*); 0.5 mM/1 mM (*3*); b) 0 mM/0 mM (*1*); 1 mM/0 mM (*2*); 1 mM/2mM (*3*); 1 mM/3 mM (*4*). Bars *4* in panel (a) and *5* in panel (b) characterize effect of MNIC/MGD at concentration of these complexes 0.5 (*4*) and 1.0 mM (*5*).

two-dimensional diagram obtained using flow-cytometry method [25] that evaluates degree of apoptosis (based on fluorescence of annexin V-FITC) and degree of necrosis (based on fluorescence propidium iodide) (Fig. 11).

As follows from Fig. 11b, percentage of apoptotic cells increases dramatically during incubation of cells with B-DNIC-MS + MGD (quadrants Q2-2 and Q2-4) reaching 80% as additionally demonstrated in Fig. 11c. Considering that the ratio of the complex concentration (determined with respect to one nitrosyl iron group in B-DNIC-MS) to MGD concentration was 1 : 2, all B-DNIC-MS were transformed into MNIC-MGD, and death of 80% of the cells was caused by 0.5 mM nitrosonium cations released from B-DNIC-MS in the course of this transformation.

Similar results were obtained in our laboratory during investigation of the effect of B-DNIC-GSH and DEDC on viability of the *Escherichia coli* TN530 cells [30] determined from the number of colony forming units (Fig. 12).

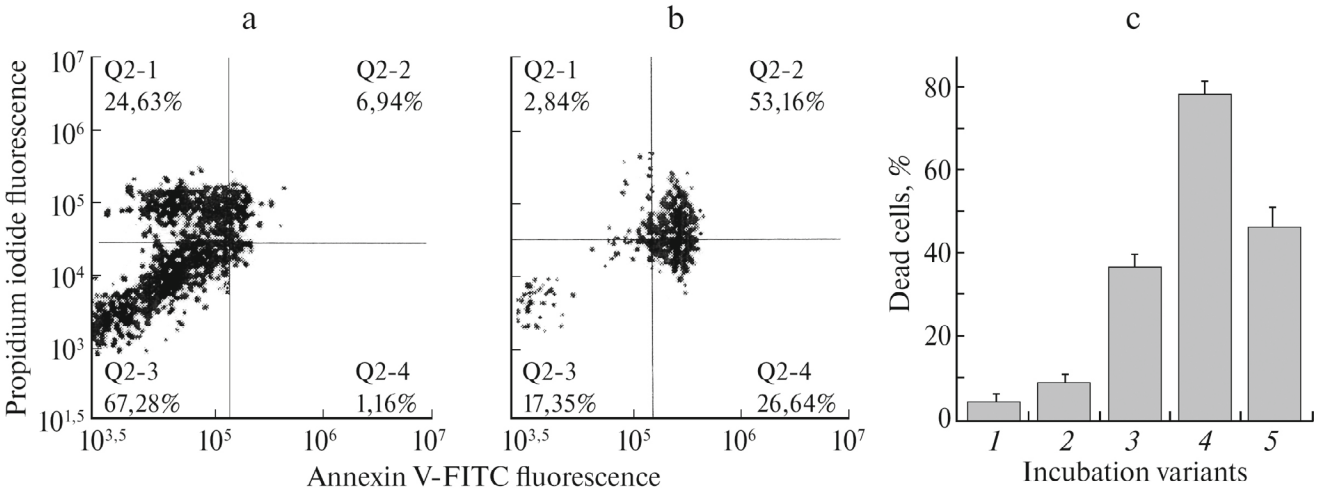


Fig. 11. Flow cytometry 2D scatter plot. Death of the MCF-7 cells after treatment with B-DNIC-MS (0.5 mM) and MGD (1.0 mM) (based on staining with annexin + propidium iodide mixture). Cells were incubated either with only B-DNIC-MS (a) or with B-DNIC-MS+MGD mixture (b). c) Percent of the dead cells: *1*) control; *2*) incubation with B-DNIC-MS; *3*) incubation with MGD; *4*) incubation with mixture B-DNIC-MS + MGD; *5*) combined effects of B-DNIC-MS and MGD (*2* + *3*) in the absence of interactions between them.

As follows from Fig. 12, complete loss of viability of bacterial cells was caused by 0.5 mM nitrosonium cations released from B-DNIC-GSH in the course of transformation of almost all of these complexes into MNIC-DEDC [30]. Such effective transformation of B-DNIC-GSH was likely due to effective penetration of DEDC into intracellular space where DNICs were localized. Unlike in the case of MGD, complexes of which with metals are water-soluble, similar complexes with DEDC are hydrophobic, which facilitated their penetration across cell membranes.

Ability of only DNICs with thiol-containing ligands to function in this system as donors of nitrosonium ions causing inactivation of bacterial cell has been illustrated in the following experiments. As follows from the results of experiments presented in Fig. 13 [30] simultaneous addition of 0.5 mM DEDC and 0.5 mM sodium nitrite or S-nitrosoglutathione (GS-NO) to *E. coli* cells does not cause changes in the number of colony forming units (Fig. 13, *6* and *7*, respectively) in comparison with the colony forming units calculated for the combined action of DEDC + $NaNO_2$ or DEDC + GS-NO in the absence of interaction between these agents (Fig. 13, *4* and *5*, respectively). At the same time, in the case when DEDC was added to the cell medium 40 min after $NaNO_2$ or GS-NO, inactivation of bacterial cells was observed (Fig. 13, *8* and *9*, respectively).

The latter occurred due to the fact that 40-min incubation of cells with $NaNO_2$ or GS-NO was sufficient for these agents, as NO donors, to mediate formation of DNICs with thiol-containing groups in the bacteria detected based on the presence of 2.03-signal. DEDC introduced at that moment mediated formation of MNIC-DETC in the process of interaction with the formed complexes accompanied with accumulation of NO^+ in the cells. The latter being generated directly inside the cells promoted their death.

In this connection the question arises, whether there are endogenous compounds capable, similar to the exogenous dithiocarbamate derivatives, of initiation of DNICs decomposition leading to the release from them of only nitrosonium cations rather than also of NO molecules? We have shown in our preliminary studies that superoxide anion could serve this function.

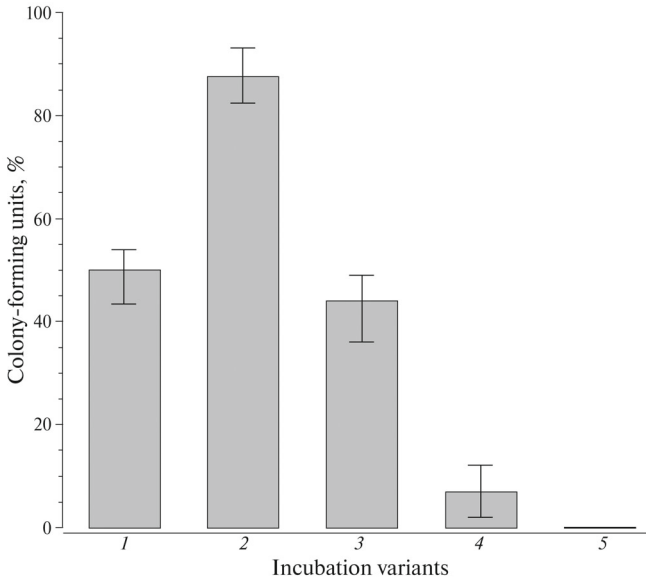


Fig. 12. Effect on the number of colony forming units (CFU) in the samples of *E. coli* of addition of: DEDC (2.5 mM) (*1*); B-DNIC-GSH (0.5 mM) (*2*); combined effect of DEDC + B-DNIC-GSH addition (*1* + *2*) in the absence of interaction between them (*3*); simultaneous addition of B-DNIC-GSH + DEDC (*4*); addition of B-DNIC-GSH followed by addition of DEDC after 40-min incubation (*5*).

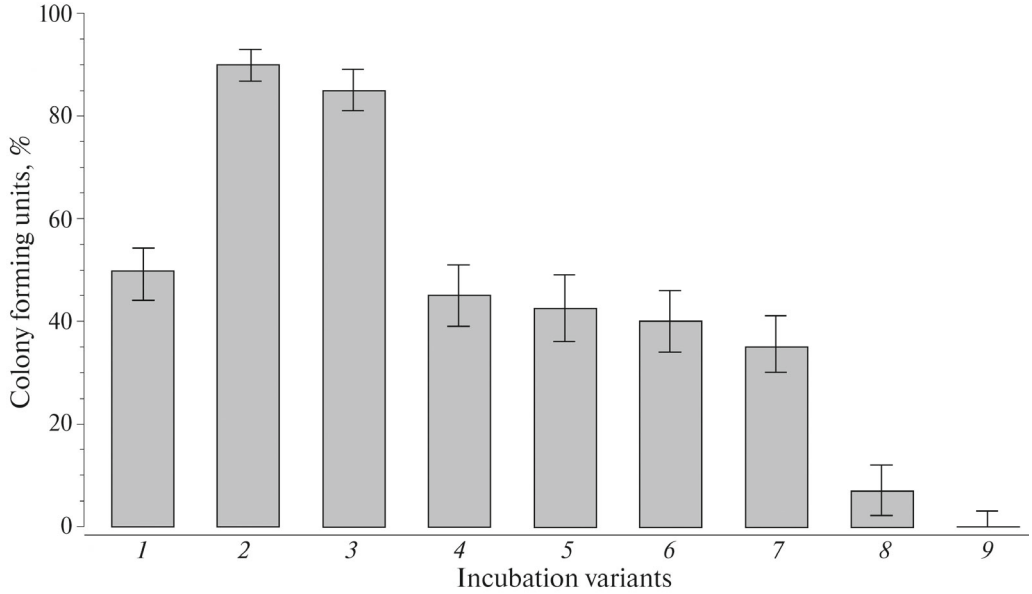


Fig. 13. Effect on the number of colony forming units (CFU) in the samples of *E. coli* of addition of: DEDC (2.5 mM, *1*); $NaNO_2$ (0.5 mM, *2*); GS-NO (0.5 mM, *3*); combined effect of DEDC + $NaNO_2$ (*1* + *2*) in the absence of interaction between them (*4*); combined effect of DEDC + GS-NO (*1* + *3*) in the absence of interaction between them (*5*); simultaneous addition of DEDC + $NaNO_2$ in the incubation medium (*6*); simultaneous addition of DEDC + GS-NO in the incubation medium (*7*); $NaNO_2$ + DEDC (DEDC added 40 min after $NaNO_2$, *8*); GS-NO + DEDC (DEDC added 40 min after GS-NO, *9*).

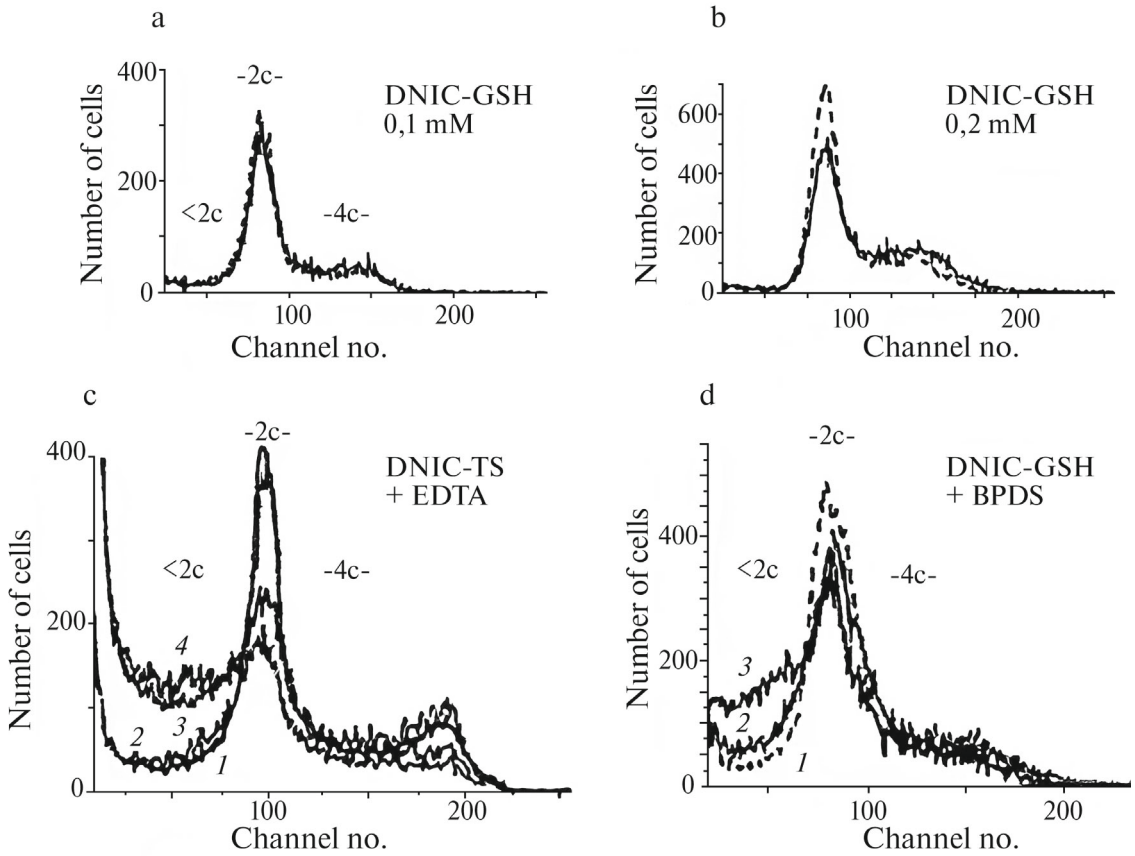


Fig. 14. Histograms illustrating lack of effects of 0.1 mM B-DNIC-GSH (a) and 0.2 mM B-DNIC-GSH (b) on the state of DNA in HeLa cells in the absence of iron chelators (preservation of cells with diploid DNA in the population). Solid line) control, dashed line) experiment. -2c and -4c) Subpopulation of cells with diploid and tetraploid DNA sets. c) Histogram illustrating proapoptotic effect of 0.1 mM DNIC-TS (curve *3*) and 0.2 mM DNIC-TS (curve *4*) in the presence of 0.5 mM EDTA. Effect is insignificant at DNIC-TS concentration 0.05 mM (curve *2*). Curve *1*) Control. d) Histogram illustrating proapoptotic effect of 0.2 mM B_DNIC-GSH in the presence and in the absence of 0.05 mM BPDS (curves *3* and *2*, respectively). Curve *1*) Control. Proapoptotic effect was manifested by the increase of population of cells with hyploid (<2c) DNA set [56].

Previously it was established in the study by Shumaev et al. [55] that these anions react with NO molecules in composition of dinitrosyl iron complexes bound to proteins with rather high speed ($\sim 10^7$ $M^{-1} \cdot cm^{-1}$). As a result, peroxinitrite appears in the iron coordination sphere, which could isomerize to nitrate being still in complex with iron leading to decomposition of DNIC accompanied by the release of nitrosonium cations.

We have shown in the recent study (paper in preparation) that indeed there is decomposition of DNIC with simultaneous accumulation of nitrite in solution at neutral pH occurring on contact of B-DNIC-GSH with excess of KO_2 as an O_2^- donor. Emergence of this product unambiguously implies release of nitrosonium cations from B-DNIC-GSH, which are rapidly hydrolyzed into nitrite. With regard to the possibility of binding these cations with glutathione molecules, which could result in formation of the respective S-nitrosothiol, this has not been observed. GS-NO did not emerge on acidification of the solution also. It has been expected that if part of glutathione in the composition of B-DNIC or existing in free form in the solution of these complexes remains present, acidification could result in formation of GS-NO from the nitrite and glutathione molecules, which could be detected based on absorption band at 334 nm [25]. Absence of the latter could be explained by oxidation of the thiol group in glutathione molecules by superoxide anions.

Another type of endogenous molecules that could cause decomposition of DNICs with release of NO and NO^+ includes iron chelators. As follows from the results of our experiments conducted in collaboration with the researchers in the Institute of Nuclear Physics in Gatchina [56], DNIC-TS and B-DNIC-GSH exerted cytotoxic action on the HeLa cells in the presence of such exogenous iron chelators as bathophenanthrolin disulfonate (BPDS) or EDTA, manifested by DNA degradation in these cells and development of apoptosis [56] (Fig. 14). The latter was evaluated based on quenching of fluorescence of ethidium bromide intercalated into the cellular DNA. Degree of this quenching was determined from the number of cells (ordinate axis in Fig. 14) that fluoresce with lower intensity (recorded in channel no. 75 and below, see Fig. 14).

In contrast to the effect of superoxide anions on DNICs, which should lead to release of predominantly nitrosonium cations form these complexes, destructive effect of iron chelators on these complexes should facilitate release of both nitrosonium cations and NO molecules in equal amounts as follows from the Scheme 3. Considering the fact that nitrosonium cations exhibit significantly higher cytotoxic effect than NO molecules, it could be suggested that the iron chelators should initiate sharp increase of cytotoxic effect of DNICs (similar to superoxide anions) by decomposing DNICs causing release of nitrosonium ions from them.

Unfortunately, for both these agents the abovementioned statement seems to be not totally correct. The fact is that in both those cases the thiol-containing components remain intact on decomposition of DNICs, and, as was mentioned previously considering results presented in Fig. 3, their high concentration could initiate reduction of NO^+ to NO. Thiols on their own cannot realize this process (due to violation of the law of total spin conservation of reagents and products of the chemical reaction). This process could be realized with participation of spin catalysts that are capable of transfer the spin of a thiol agent, for example, from its singlet state to triplet state. As a result (in accordance with the law of spin conservation in chemical reactions) transformation of NO^+ into NO becomes possible, and this is accompanied by transformation of thiol into thiyl radical (RS). With regard to the nature of spin catalysts, iron ions in DNIC composition could serve as such.

Hence, the issue on appearance of nitrosonium cations in the process of decomposition of DNICs with thiol-containing ligands in chemical, and even more so in biological processes remains at present quite complicated. It is currently impossible to discuss rationally the possibility of controlling various metabolic processes in live organisms by generating these agents. Based on these facts, the only approach to solving this problem seems to be the use of dithiocarbamate derivatives that are capable of initiating release of only nitrosonium cations (as biologically active agents) from DNICs upon their interaction (in accordance with the Scheme 5).

INTERACTION OF DNICs WITH ACTIVE CENTERS OF IRON-SULFUR PROTEINS

Unique role of nitric oxide in live organisms was elucidated in 1980s-1990s, which involved not only positive, regulatory effects, but these molecules also exhibit cytotoxic effect playing a role of main cytotoxic effector in the system of cell-mediated immunity. It was suggested that cytotoxic effects are determined by the ability of NO to destroy active centers of iron-sulfur proteins abundant in the respiration chain of mitochondria [57-60].

Attempts to verify this suggestion conducted in our laboratory in the late 1980s and early 1990s using animal tissues and macrophage samples were not successful. The results of ESR studies of the mouse macrophage respiratory chain exposed to gaseous NO presented in Fig. 15 contradicted this suggestion [61].

ESR spectra of the initial suspension of macrophages recorded at 16-30K presented in Fig. 15a are represented by the ESR signals of oxidized form of S-3 succinate dehydrogenase at $g = 2.022$ and oxidized from of aconitase at $g = 2.026$. Treatment of this suspension with dithionite in the presence of redox mediator, methyl viologen, resulted in the ESR spectra dominated by ESR signals of all

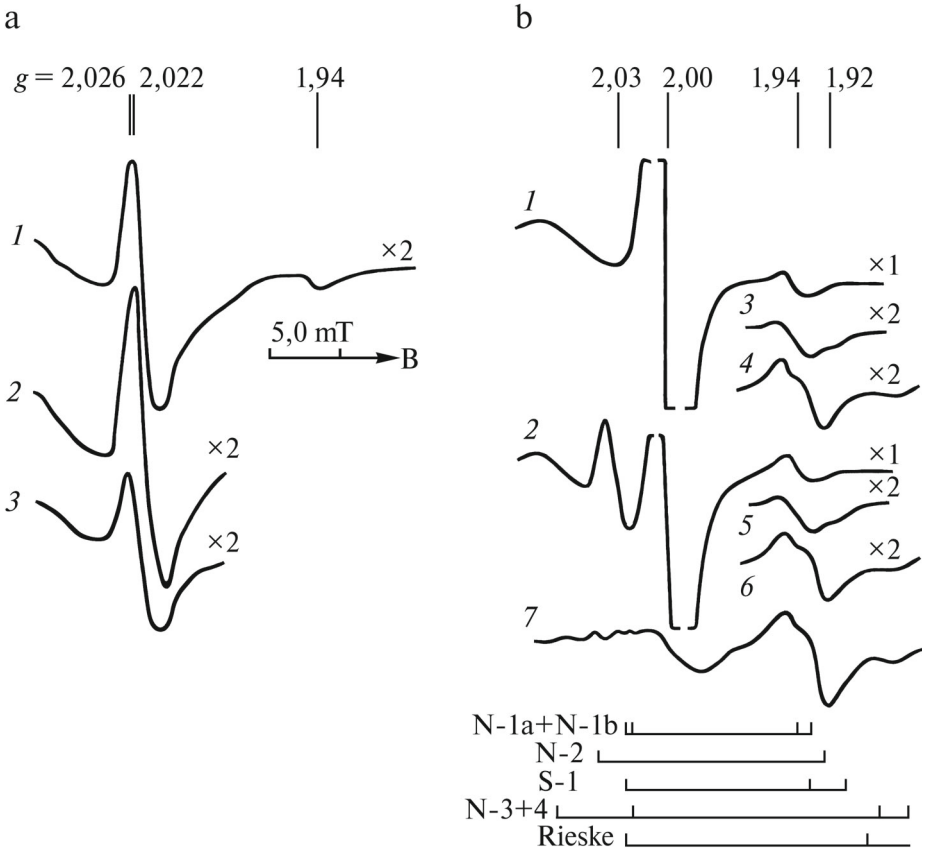


Fig. 15. a) ESR spectra of the initial (non-treated with dithionite as a reducer) recorded at 25K (*1*), 16K (*2*), and 30K (*3*). b) ESR spectra of the same suspension treated with dithionite + methyl viologen (*1*) followed by 5-min exposure to NO (*2*) recorded at 40K (*1*, *2*, *3*, and *5*) and 23K (*4*, *6*). ESR spectrum of isolated mouse liver recorded at 23K (*7*).

reduced sulfur-iron proteins of the macrophage respiratory chain, correlation of which with the signals of Green complexes (1-3) is presented in the right bottom corner of the figure. The DNIC 2.03 signal was observed in the ESR spectrum of macrophage suspension treated with dithionite and next with gaseous NO.

Analysis of these spectra demonstrated practically identical amounts of iron incorporated into the iron-sulfur proteins and iron in the composition of DNICs [61]. Based on this it was concluded that the emergence of DNICs in the macrophage suspension was not due to the destructive action of NO on active centers of iron-sulfur proteins followed by incorporation of the iron released from these centers into DNICs. The latter could be formed with participation of the weakly bound free form of iron – "non-heme, non-Fe-S iron" – discovered in 1980s by the Swedish scientists in animal mitochondria [62].

Similar experiments conducted in our laboratory with the isolated electron-transport protein adrenodoxin from bovine adrenal glands (Fig. 16) resulted in the same conclusions [63]. Treatment of this protein with gaseous NO after its reduction with dithionite did not results in the significant decrease of the ESR signal of this protein at g = 1.94 and 2.02 and emergence of the strong ESR signal of DNIC at g = 2.03, which would be due to decomposition of the [2Fe-2S] iron-sulfur cluster in adrenodoxin (Fig. 16, spectrum b). This signal overlapped with the ESR signal from nitrosyl complexes of admixtures of hemeproteins at g = 2,07.

ESR spectrum of the reduced adrenodoxin changed significantly on addition of the excess of Fe^{2+} salt followed by exposure to gaseous NO. Intensity of the ESR signal at g = 1.94 typical for the intact iron-sulfur cluster in adrenodoxin decreased to almost zero with simultaneous emergence of the intensive 2.03 signal (Fig. 16, spectrum c). The following repeated treatment of this solution with dithionite did not result in restoration of the signal at g = 1.94 to the initial level, but resulted in the appearance of the ESR signal g = 2.0 and 1.98, emerging, as was established later, due to the reduced form of B-DNIC bound to adrenodoxin [63].

In conclusion it can be stated that decomposition of the iron-sulfur cluster occurred in adrenodoxin in these experiments, and, in the process, iron ions released form the iron-sulfur centers were quantitatively incorporated into composition of the produced DNICs linked via thiol groups of apo-adrenodoxin. There were some grounds to suggest that the discovered decomposition could be due to the destruction of the iron-sulfur center by low molecular weight DNICs that had in their composition buffer ions, such as phosphate, as ligand of the additional iron.

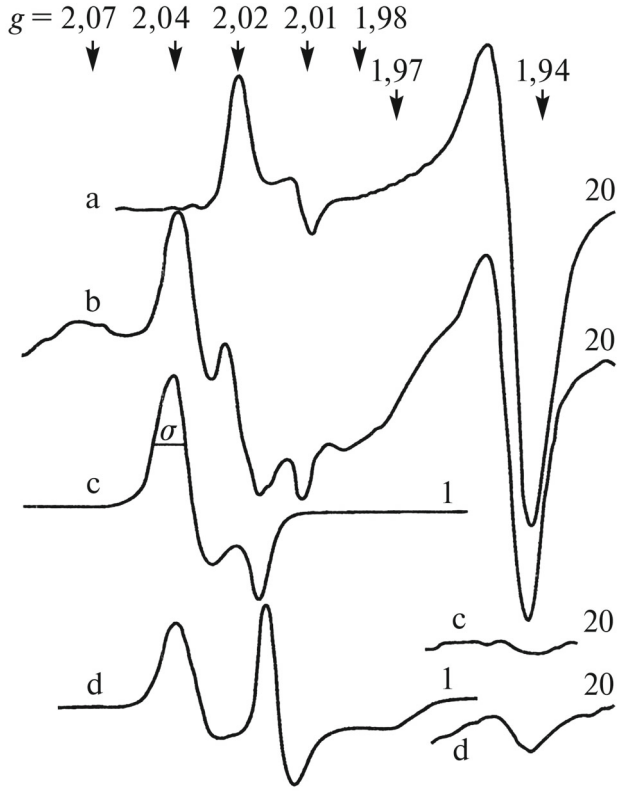


Fig. 16. ESR spectra of adrenodoxin solution reduced with dithionite (a) followed by treatment with NO (b) and Fe^{2+} + NO (c). d) Spectrum of the preparation (c) repeatedly treated with dithionite. c^{20} and d^{20}) Amplification of radiospectrometer in arbitrary units. Spectra recorded at 77K.

Indeed, addition of the previously synthesized DNIC with phosphate or cysteine to the adrenodoxin solution caused immediate decomposition of [2Fe-2S] in adrenodoxin in the absence of NO and appearance of the protein-bound DNIC.

These results allowed suggesting that breakdown of the adrenodoxin active center under the action of low molecular weight DNICs could occur via binding of the dinitrosyl iron fragments of these complexes with thiol groups in adrenodoxin, which initially linked [2Fe-2S] with the adrenodoxin protein globule. With regard to iron and inorganic sulfur (sulfur sulfide – S*), they, in accordance with the Scheme 6, should be released from the iron-sulfur cluster into environment (Scheme 6).

In this connection it is only logical to assume that in the presence of reducers capable of reducing the released iron to Fe^{2+}-state, this iron could continue the process of decomposition of [2Fe-2S]-centers by forming low molecular DNICs with NO, which began with participation of exogenous DNICs in other adrenodoxin molecules. As a result, the process of decomposition of iron-sulfur centers could become autocatalytic.

Relevant experiments with adrenodoxin solution fully confirmed this suggestion [63].

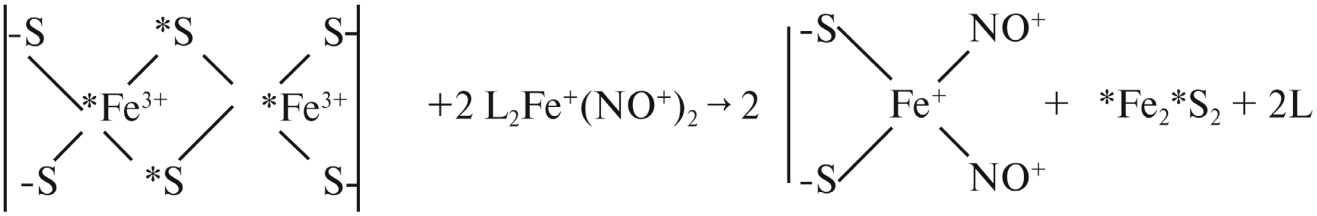
Finally, it can be stated that the results of these experiments demonstrated that NO itself is not capable of destroying iron-sulfur clusters in adrenodoxin: this process could be initiated only by the low molecular weight DNICs according to the mechanism presented in Scheme 6.

This statement is in full agreement with the results of our studies on the effects of gaseous NO on iron-sulfur proteins in composition of the macrophage respiratory chain described above. Nevertheless, a large amount of data is available in the literature on the ability of NO molecules themselves to destroy active centers of some iron-sulfur proteins with formation of DNICs in them [64-66]. These proteins belong to the family of protein regulators serving as transcription factors. It is presumed that composition of these proteins includes large number of acidic amino acid residues in the vicinity of iron-sulfur centers. Release of protons from the acidic residues weakens the bond between the iron atoms in these centers and inorganic sulfur atoms, which, in turn, enhances interaction between the iron atoms and NO molecules thus facilitating incorporation of both into DNICs.

CONCLUSION

One of the main statements of this review comes down to the fact that the DNICs produced in live organisms should be considered as main donors of nitrosonium cations. Moreover, considering that these complexes could have in their composition major part of molecular NO found in live organisms, DNICs could also play a role of main stabilizers and transporter of NO molecules to their biological targets.

Experimental data described above allows suggesting that NO molecules released from DNICs mainly function in live organisms as positive agents regulating vital metabolic processes. Regarding nitrosonium cations, after release from DNICs they function mainly as negative agents



Scheme 6. Mechanism of decomposition of bi-nuclear iron sulfur cluster under the action of DNICs.

responsible for cytotoxic effect of M- and B-DNICs with thiol-containing ligands produced in live organisms.

In this regard DNICs with thiol-containing ligands synthesized chemically could be considered as a basis for development of antibacterial and antiviral therapeutics, as well as antitumor drugs.

The abovementioned results on effective suppression of colony forming activity of *E. coli* cells (i.e., their infectivity) through sequential treatment of the suspension of bacterial cells first with DNICs with thiol-containing ligands, and after that with DEDC [30]. This sequence of treatments results in the release of mainly nitrosonium cations from DNICs rather than of NO molecules, which mediates antibacterial effect. Preliminary data have been obtained demonstrating that under these conditions significant suppression of the ability of some pathogenic bacteria to form biofilms protecting them from the action of different drugs such as antibiotics was observed.

In 2020 it was suggested by Vanin [67] that nitrosonium cations released from DNICs could exert antiviral action, including against the SARS-CoV-2 virus. It has been presumed that such function could be realized through the action of nitrosonium cations as S-nitrosylating agents on the host proteases vital for virus proliferation. Experiments conducted recently with animals infected by coronaviruses totally confirmed this suggestion [68].

The results of our studies investigating effects of DNICs with thiol-containing ligands on proliferation of benign tumor cells in rats with surgically induced endometriosis are of special interest. These studies conducted by Burgova et al. [68-71] resulted in the discovery of anti-endometriosis effect of DNICs with thiol-containing ligands. Obviously, the obtained results could be used as a basis for the development of drugs based in these complexes, which could be effective in treatment of endometriosis – disease affecting women of reproductive age and observed more and more frequently in recent years.

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