2. S. P. Kovalenko, V. V. Gorn, V. A. Karginov, *et al.*, *Bioorgan. Khim.*, **14**, № 7, 910-915 (1988).
3. S. P. Kovalenko, I. A. Lisnyak, and N. P. Mertvetsov, *Ibid*, **15**, № 4, 492-498 (1989).
4. Ya. S. Shwartsman, *Byull. Eksp. Biol.*, № 12, 75-77 (1966).
5. J. W. Felt, D. J. Strydom, R. R. Lobb, *et al.*, *Biochemistry*, **24**, № 20, 5480-5486 (1985).
6. J. Folcman and M. Klagsbrun, *Science*, **235**, № 4787, 442-447 (1987).
7. S. M. Rubak, J. W. Felt, Q. -Z. Yao, and B. L. Vallee, *Biochem. Biophys. Res. Commun.*, **146**, № 3, 1240-1248 (1987).
8. R. Shapiro, D. J. Strydom, K. A. Olsen, and B. L. Vallee, *Biochemistry*, **26**, № 16, 5141-5146 (1987).
9. R. Shapiro, J. W. Harper, E. A. Fox, *et al.*, *Analyt. Biochem.*, **175**, № 2, 450-461 (1988).
10. H. L. Weiner, L. H. Weiner, and J. Swain, *Science*, **237**, № 4812, 280-282 (1987).

Peculiarities of Sodium Nitroprusside Biotransformation in Tumor-Bearing Animals

M. Ya. Demidov, A. L. Kleshchev, and K. R. Sedov

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Earlier we showed, using the EPR method, a phenomenon of specific biotransformation of sodium nitroprusside by tumor cells. The essence of the phenomenon lies in the formation in the intercellular space of ferric dinitrosyl complexes with paired RS groups of proteins [3], so-called 2.03 complexes [1]. The specific biotransformation of nitroprusside was revealed and confirmed in several models of mouse ascites tumors and in experiments with human leukemia cells [3].

The aim of this work was to study the expression of the phenomenon in models of experimental solid tumors *in vivo*.

MATERIALS AND METHODS

The following tumor models were used: sarcoma-180 (recipients - F_1(C57Bl/6×CBA/cal) mice, 18th day post-transplantation) and Walker sarcoma (recipients - Wistar rats, 30th day post-transplantation). The tumors were purchased from the Cancer Research Center of the Russian Academy of Medical Sciences. They were maintained by subcutaneous injection of 0.2 ml of 20% tumor cell suspension in saline. We also used outbred white mice with spontaneous adenomatosis obtained from the Central Department of Laboratory Animals of the Russian Academy of Medical Sciences. Healthy animals of the corresponding breed served as a control. Sodium nitroprusside (Sigma, USA) was admin-

TABLE 1. Prevalence of NP−1 EPR Signals and/or 2.03 Complexes in the Plasma of Healthy and Tumor−Bearing Animals (%) after Intraperitoneal Administration of Sodium Nitroprusside (25−50 mg/kg)

Experimental group	NP-1	2.03 Complex	Change
Healthy animals (n=15, rats, mice)	0	0	0
Sarcoma−180 (n=9, mice)	56	44	100
Walker sarcoma (n=5, rats)	50	50	100
Spontaneous adenomatosis (n=8, mice)	100	0	100

Institute of Medical Problems of the North, Siberian Department of the Russian Academy of Medical Sciences, Krasnoyarsk

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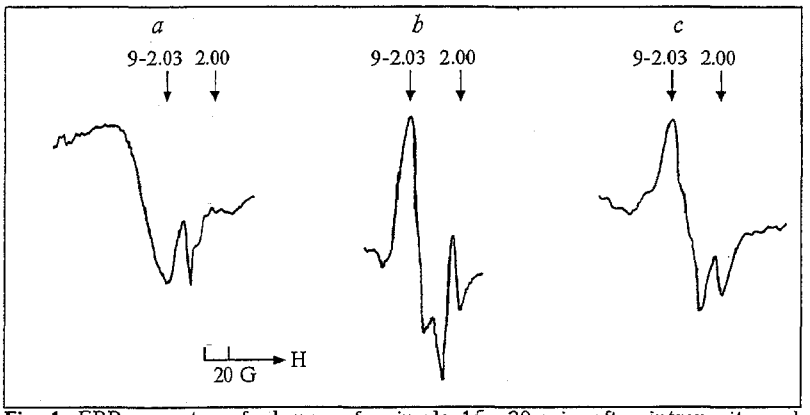

Fig. 1. EPR-spectra of plasma of animals 15-30 min after intraperitoneal administration of sodium nitroprusside (25-50 mg/kg). *a*) healthy mouse; *b*) mouse bearing sarcoma-180; *c*) mouse with spontaneous adenomatosis. Spectrum recorded on a Rubin radiospectrometer, x-range, ultrahigh frequency power 10 mW, modulation amplitude 5 G, display speed 100 G per min, T = 77 K.

istered intraperitoneally in sublethal doses. Mice received a single-shot injection of 0.5 mg per 20 g body weight in a volume of 0.5 m; rats were repeatedly injected with a dose of 1-2 mg per 200 g body weight during 3 hours, the total dose reaching 10 mg per 200 g. The animals were decapitated 15 to 30 min after the last injection. The samples to be EPR-analyzed (plasma, liver, kidneys, heart) were obtained immediately after the sacrifice after Foster *et al.* [4] and stored in liquid nitrogen. EPR spectra were registered in a Rubin radiospectrometer, in the X-ray region at T-77 K.

RESULTS

As in the earlier study [2], after the injection of sodium nitroprusside to healthy animals, a singlet EPR signal was registered in the organ specimens (liver, kidneys, heart); it was characterized by poorly resolved components in the central region and maximum values of g-parameter equal to 2.037 and 2.012, the signal assigned by the authors to a variant of reduced nitroprusside (NP-1). In addition, an EPR signal of the nitrosyl complexes of the heme-containing proteins was detected. The plasma exhibited the usual broad EPR signal with a g value of approximately 2.05, mediated by ceruloplasmin-harbored Cu^{2+} (Fig. 1, *a*).

These data show that the diamagnetic molecules of nitroprusside administered to the animals interact immediately with the cells and undergo a single-electron reduction followed by a transition to the paramagnetic state and subsequent degradation. It is likely that nitroprusside undergoes chemical reactions with the plasma and that the products of its degradaton by the cells (iron and nitrous oxide) do not enter the blood.

Unlike the situation with healthy animals, injection of sodium nitroprusside to the animals bearing sarcoma-180 and/or Walker sarcoma was always accompanied by a change in the EPR spectrum of the plasma and either the appearance of an EPR signal from NP-1 or an axial-symmetrical EPR signal with g_1 equal to 2.037, and g_2 2.012 of 2.03 complexes (Fig. 1, *b* and Table 1). The integral intensity of these EPR signals is consistent with the concentration of complexes, being of the order of 10^{-7}-10^{-6} M. In mice with spontaneous adenomatosis injected with sodium nitroprusside, only an NP-1-mediated EPR signal was registered in the plasma (Fig. 1, *c*).

The study of EPR spectra of the organs of nitroprusside-treated animals with tumors failed to reveal any difference from healthy animals. In the tissue of all the tumors a complex EPR signal from NP-1 and 2.03 complexes was observed.

Thus, the main distinctive features of nitroprusside biotransformation in the organism of animals with solid tumors were manifested in a change of the EPR spectra of the plasma (appearance of NP-1 or 2.03 complexes). In other words, one can state that the phenomenon of the specific nitroprusside reaction with ascites tumor cells [3] extends to solid tumors grown *in vivo*. However, in the first case the interaction of nitroprusside with tumor cells always leads to the formation in the intercellular space of 2.03 complexes, whereas in the case of a solid tumor grown *in vivo* such complexes are formed in the plasma of only some animals (Table 1). The registration of NP-1 in the plasma of other animals with solid malignant tumors as well as in all cases of spontaneous adenomatosis seems to reflect a new, as yet undescribed phenomenon. It is plausible to assume that the latter is explained by the appearance in the plasma of tumor-bearing animals of free sulfhydryl groups capable of reacting with nitroprusside.

REFERENCES

1. A. F. Vanin, *Nitrosyl Complexes of Nonheme Iron in Animal Tissues and in Microorganisms*, Doctoral Thesis, Moscow (1980).
2. A. F. Vanin, P. I. Mordvintsev, M. M. Shabarchina, *et al.*, *Biofizika*, **28**, № 6, 990-993 (1983).
3. A. L. Kleshchev, P. I. Mordvintsev, and A. F. Vanin *Vopr. Onkol.*, **34**, № 6, 736-737 (1988).
4. M. A. Foster, T. Pocklington, J. D. B. Miller, and J. R. Mallard, *Brit. J. Cancer*, **28**, 340-348 (1973).