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Imbalance in the Enzymatic System of Production and Consumption of Active Oxygen Species in Liver of AKR Mice with Spontaneous Leucosis

L. S. Vartanyan*, S. M. Gurevich, A. I. Kozachenko, L. G. Nagler, and E. B. Burlakova

Emanuel Institute of Biochemical Physics, Russian Academy of Sciences, ul. Kosygina 4, Moscow, 117977 Russia; fax: (095) 137-4101; E-mail: chembio@glasnet.ru

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Abstract—Activities of protective antioxidant enzymes, the rate of superoxide formation (v) in microsomal membranes and submitochondrial particles (SMP), and the concentrations of reduced and oxidized glutathione in cytosol were studied in the liver of AKR mice during the development of spontaneous leucosis. It was found that in the latent period of leucosis (mice of 3-6 months of age) the glutathione reductase (GR) activity in cytosol and mitochondria decreased and v in SMP increased. The increase in v in SMP did not result in the induction of Mn-SOD. In this stage of leucosis, the activities of Cu,Zn-SOD, GSH-Px, and G-6-PDH in cytosol were unchanged; at the same time, the GR activity and the concentration of reduced glutathione smoothly decreased. In the stage of developed leucosis (mice of 7-9 months of age), non-synchronous changes in the antioxidant system resulting in the shift of metabolism towards the prooxidant state were found. Comparison of our findings and the literature data demonstrates that the observed decrease in the SOD/GSH-Px ratio, the decrease in GR activity, and the increase in the v/Mn-SOD activity ratio are typical for pre-neoplastic changes in cell metabolism.

Key words: antioxidant enzymes, glucose-6-phosphate dehydrogenase, glutathione, superoxide radicals, mouse liver, AKR mice, leucosis

Radical products of metabolism, especially oxygen radicals, can cause changes in the genome that are neoplastic [1, 2]. The protective antiradical enzymatic system includes a wide group of enzymes localized in various subcellular organelles. The protective system includes a family of superoxide dismutases, namely, Cu,Zn-SOD localized in cytosol, extracellular SOD in blood plasma, and Mn-SOD in mitochondrial matrix. Superoxide dismutase catalyzes reduction of superoxide radicals ($O_2^{\cdot-}$), hydrogen peroxide being produced in the reaction. Superoxide dismutase is considered an integral protective enzyme because $O_2^{\cdot-}$, which is a SOD substrate, is produced not only in the electron transport chain in microsomes, mitochondria, and nuclei, during phagocytosis, and in endothelium cells, but also under oxidative stress and on the autooxidation of low molecular weight metabolites such as reduced glutathione [3].

Another group of protective enzymes includes glutathione-dependent enzymes that take part in detoxication of hydrogen peroxide as well as organic peroxides. Enzymes of the glutathione peroxidase (GSH-Px) family are present in cytosol and mitochondria. Reduced glutathione, which is a donor of hydrogen for glutathione peroxidase, is an important endogenous antioxidant itself. Oxidized glutathione, produced in reactions catalyzed by GSH-Px, is reduced by NADPH in the reaction catalyzed by glutathione reductase (GR) in cytosol and mitochondria. One of the main sources of NADPH is a reaction catalyzed by glucose-6-phosphate dehydrogenase (G-6-PDH), a cytosolic enzyme; that is why the activity of this enzyme also should be taken into account when the functioning of protective enzymes is considered.

Along with tumor development, phase changes in the activity of antioxidant enzymes have been found in other organs of the tumor-bearers [4-6]. In the case of

Abbreviations: SMP) submitochondrial particles; SOD) superoxide dismutase; GSH-Px) glutathione peroxidase; GR) glutathione reductase; G-6-PDH) glucose-6-phosphate dehydrogenase.

* To whom correspondence should be addressed.

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Ehrlich ascites carcinoma, the activity of GSH-Px and GR in liver increased in the stage preceding the maximum rate of tumor growth and then decreased to control values and lower. The similar changes were found in rats when chemical carcinogenesis was induced with 3,4-benzpyrene [4], while the activity of Cu,Zn-SOD remained constant. Phase changes in the activity of Cu,Zn-SOD and GR were found in erythrocytes of rats with developing Morris hepatoma [5]. In the case of Pliss sarcoma, decrease in Cu,Zn-SOD activity by 30-40% was found in rat liver [7]. Earlier it was shown that the development of Ehrlich ascites carcinoma in liver is accompanied by significant changes in the ratio between the rate of superoxide production and the activity of SOD of corresponding compartment [8]. The ratio decreased in microsomes and nuclei and increased 2-3-fold in mitochondria; this fact suggested an imbalance in systems of production and detoxication of superoxide radicals.

There are many papers reporting changes in the activity of antioxidant enzymes in the blood of oncological patients. The data are contradictory, but this might be explained by rough estimation of disease stage and sometimes by the influence of chemotherapy. The trend in SOD activity apparently can depend not only on the stage of the disease, but also on the type of tumor [9, 10].

It was reported that in patients with malignant diseases of blood and with some kinds of solid tumors an increase in the activity of erythrocyte SOD was found. However, after chemotherapy this parameter returned to norm [11, 12]. In cases of lymphoma and chronic leukemia, no changes in the erythrocyte SOD activity were found [13]. A number of researchers observed a decrease in activity of antioxidant enzymes in the blood of patients with leucosis of various kinds [14-17].

In the present work, the enzymatic antioxidant system of AKR mice during the development of spontaneous leucosis has been studied. The origin and clinical manifestations of leucosis in mice are similar to those in humans. Thus, the study of biochemical parameters in the course of leucosis in mice is very important. Because active forms of oxygen are products of normal metabolism, antioxidant enzymes perform a regulatory function. The maintenance of a stationary concentration of superoxide radicals and peroxides depend on the balanced functioning of all the chain of antioxidant enzymes. The aim of this work was to determine how the entire antioxidant system of liver responded to tumor development and which part of the protective system was most sensitive to leucosis.

MATERIALS AND METHODS

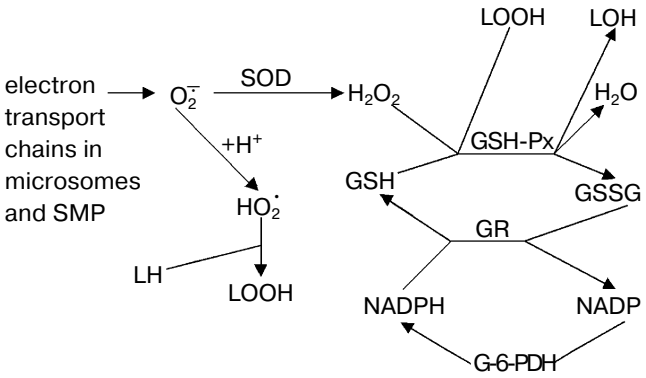
Mice were supplied by the Research Laboratory of Experimental and Biological Models of the Russian Academy of Medical Sciences. The average lifespans of females and males were reported to be 271 and 292.8 days, respectively [18]. The maximum rate of disease occurrence was registered at 7-11 months of age.

Five series of experiments were performed. Biochemical parameters at each point were determined in independent experiments. For mice of 4, 7, and 8 months of age, two observations were made and the average values were plotted. Each point on the plot is a result of the experiment on 7-9 mice; 150 mice were examined altogether. The error of the measurements of the enzyme activity evaluated by using Student's criterion was in the range from 10% for GR up to 30% for SOD. The error of measurements of rates of free radical production was less than 10%.

The animals were sacrificed by decapitation and the liver was extracted. Microsomes and mitochondria were isolated from the perfused liver according to the routine described earlier [19-20]. Submitochondrial particles (SMP) were precipitated from the mitochondrial fraction after sonication with a UZDN-2T ultrasound disintegrator (Russia) [20]; activities of Mn-SOD and mitochondrial GSH-Px and GR were measured in the supernatant. Activities of Cu,Zn-SOD, G-6-PDH, and cytosolic GSH-Px and GR were assayed in the cytosol. The activity of SOD was determined by inhibition of the reduction of nitro blue tetrazolium by superoxide radicals produced during the oxidation of xanthine catalyzed by xanthine oxidase. The amount of sampled material (in milliliters) required for 50% inhibition of the xanthine oxidase reaction per mg protein content in the sample was used as the unit of activity [21]. The activity of G-6-PDH was determined by accumulation of NADPH [22], the activity of GSH-Px by oxidation of NADPH in a conjugated glutathione reductase system with *tret*-butyl hydroperoxide as substrate [23], and the activity of GR by NADPH oxidation [24]. For G-6-PDH, GSH-Px, and GR, one unit of activity corresponds to the enzyme amount that catalyzes transformation of 1 μmol of substrate in 1 min. The concentration of reduced glutathione was measured by spectrophotometry [25]. In microsomes and SMP, the rate of $O_2^{\cdot-}$ radical production was assayed by NADPH- and NADH-dependent oxidation of 2,2,6,6-tetramethyl-4-oxopiperidine hydrochloride into corresponding nitroxyl radical, which was registered with the ESR technique [20, 26].

RESULTS AND DISCUSSION

Antioxidant enzymes form a metabolic chain in which a product of one unit is a substrate for the next unit of the chain presented in the scheme (LH, polyunsaturated lipids; LOOH, hydroperoxides):



The state of the antioxidant system of liver was examined over the lifespan of the animals. The earliest characteristics were determined in mice 3 months of age. Changes in the activity (*A*) of antioxidant enzymes and the rates (*v*) of superoxide radical production in microsomes and SMP depending on animals' age are presented in Figs. 1-6. It is worth noting that males and females showed quite similar dependences. As seen in the figures, two periods in the state of the antioxidant system of liver can be noted: 3-6 months and 7-9 months.

The first period corresponds to the stage that is defined by thymus changes as pre-leucosis, and the second period corresponds to the active development of leucosis [27].

The results presented in the figures show that the stage of pre-leucosis is especially characterized by the constant activity of the key cytosolic antioxidant enzymes: Cu,Zn-SOD and GSH-Px (Figs. 1a and 2a). However, the constancy of GSH-Px activity was accompanied by decreased GR activity (Fig. 3a) and lowered level of reduced glutathione (Fig. 4a). At the stage of pre-leucosis, the concentration of oxidized glutathione remained unchanged (Fig. 4b). The activity of G-6-PDH in this period also remained unchanged (Fig. 5).

In mitochondria at the stage of pre-leucosis, a similar picture was observed: the activity of Mn-SOD remained unchanged (Fig. 1b), the activity of GSH-Px slightly increased (Fig. 2b), and the activity of GR fell slightly (Fig. 3b). Unfortunately, we do not have any data on changes in the glutathione concentration in mitochondria.

Along with changes in the activity of the antioxidant enzymes, we also examined the state of enzymatic systems of superoxide production in microsomes and mitochondria. The activity of enzymatic systems of O_2^- production was estimated by the rates of radical production in the electron transport chain in microsomes and mitochondria. In microsomes, the value of *v* remained almost unchanged over the whole period of observations (Fig. 6a). The changes in *v* in SMP are presented in Fig. 6b. As seen in the figure, in SMP the value of *v* changed with an extremum, reaching a maximum in the 4th or 5th month in the life of the mice. The maximum coincided in time with the maximum in the weight of the thymus in the AKR mice; the increase in thymus weight is supposed to be linked with formation of immune response to the development of the malignant process [18]. It is worth noting that even in the 3-month-old AKR mice the level of radical production in SMP is significantly higher than in mice of some other lines, while *v* values in microsomes and Cu,Zn-SOD are similar. The activity of Mn-SOD in AKR mice is a little higher (table). Apparently, the

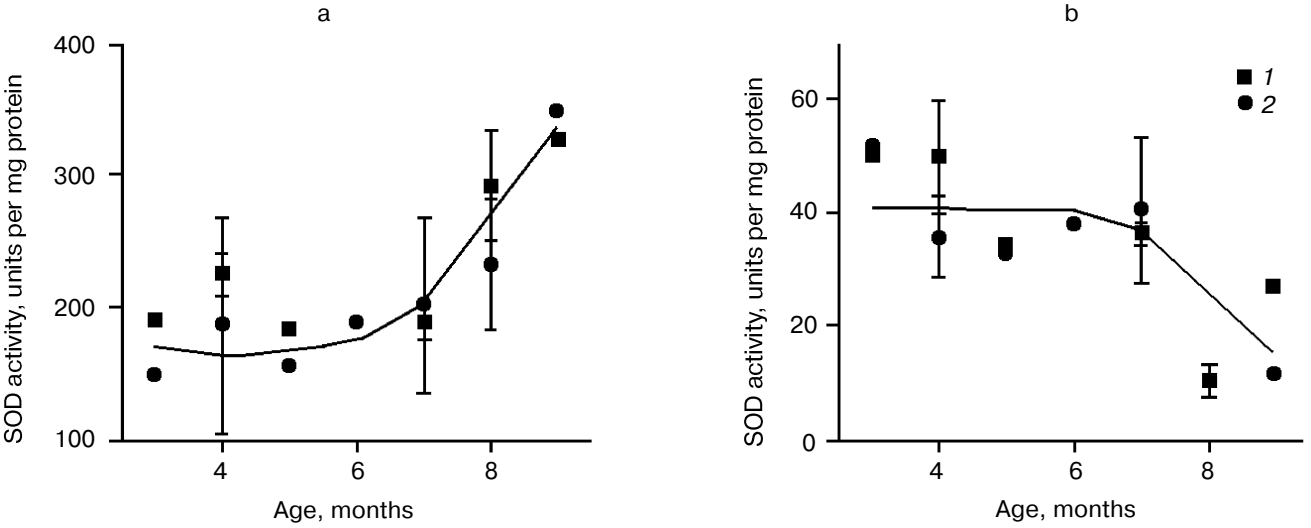


Fig. 1. Changes in the activities of Cu,Zn-SOD (a) and Mn-SOD (b) in the liver of AKR mice (females (*1*) and males (*2*)) during the development of spontaneous leucosis.

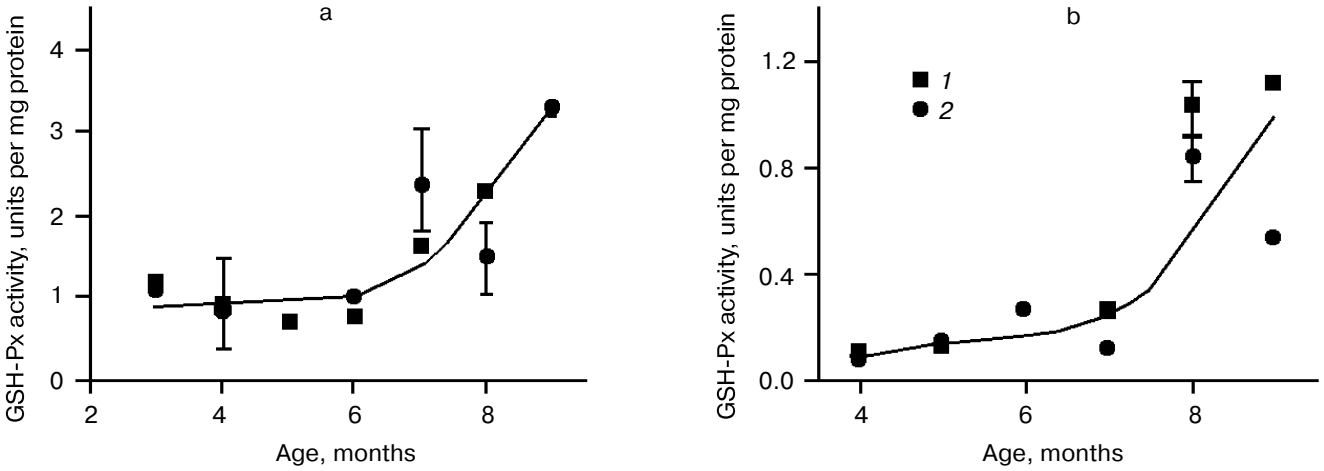


Fig. 2. Changes in the activities of cytosolic GSH-Px (a) and mitochondrial GSH-Px (b) in the liver of AKR mice (females (*1*) and males (*2*)) during the development of spontaneous leucosis.

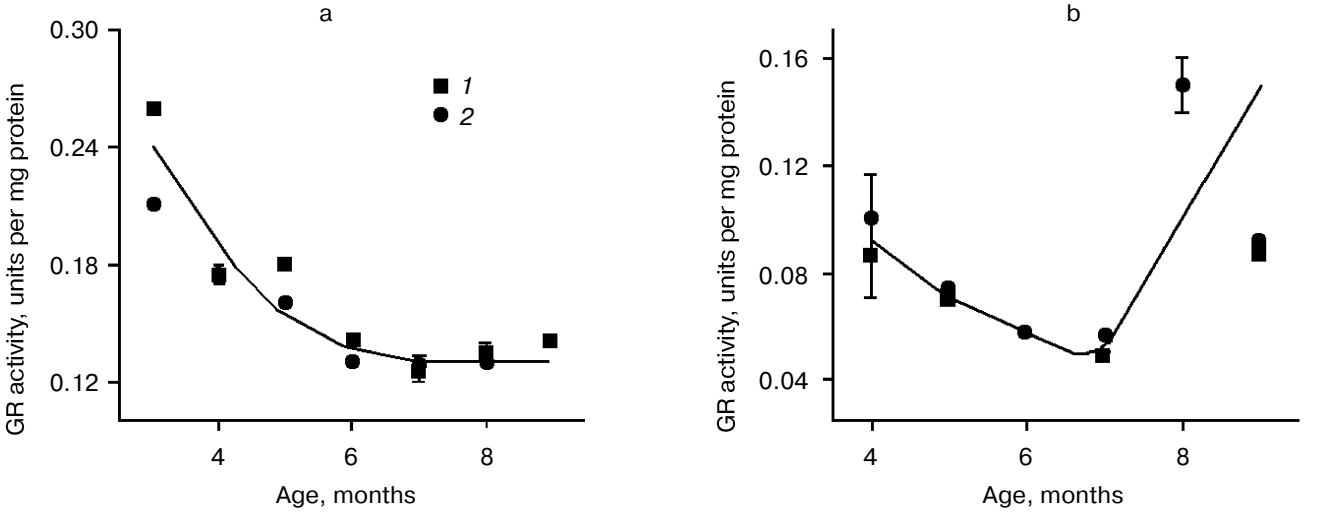


Fig. 3. Changes in the activities of cytosolic GR (a) and mitochondrial GR (b) in the liver of AKR mice (females (*1*) and males (*2*)) during the development of spontaneous leucosis.

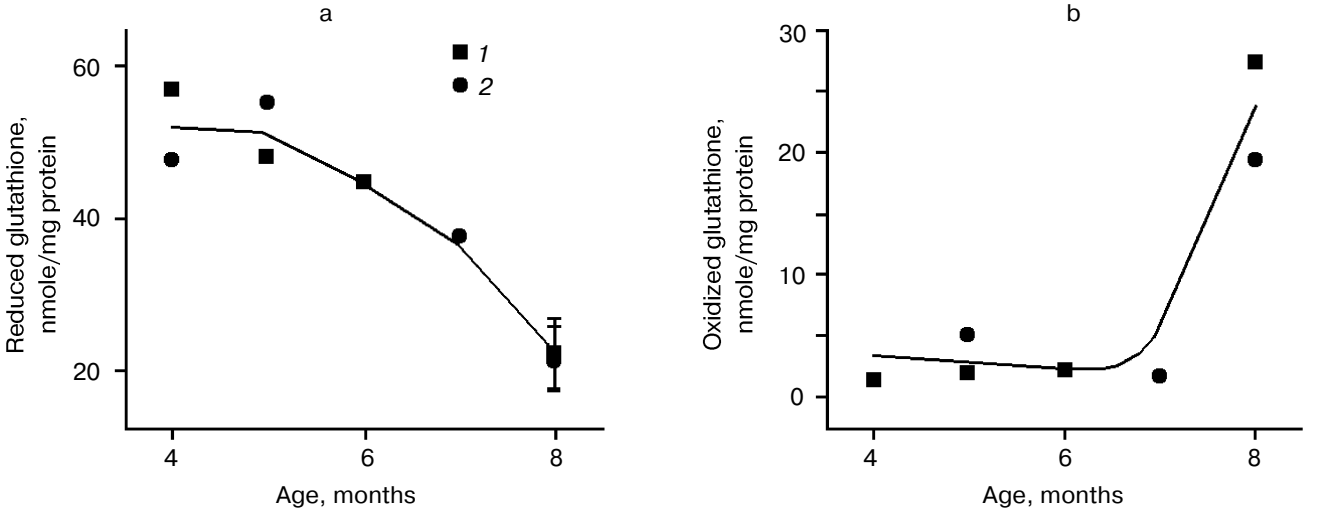


Fig. 4. Changes in the concentrations of reduced (a) and oxidized (b) glutathione in cytosol in the liver of AKR mice (females (*1*) and males (*2*)) during the development of spontaneous leucosis.

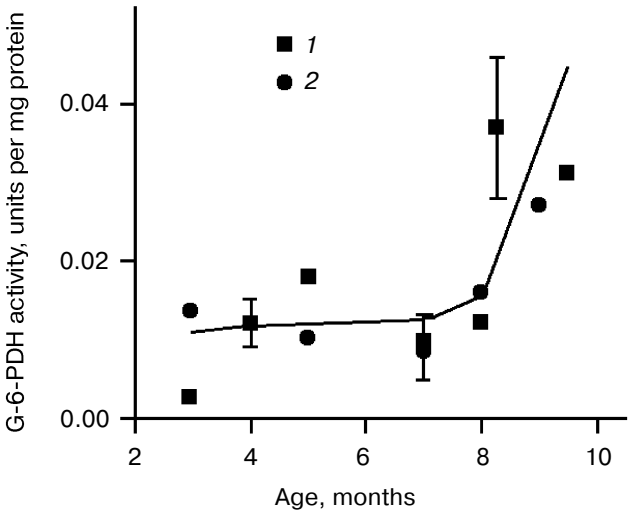


Fig. 5. Changes in the activities of cytosol G-6-PDH in the liver of AKR mice (females (*1*) and males (*2*)) during the development of spontaneous leucosis.

increase in *v* in SMP can be considered as an early sign of the pre-leucosis stage.

In the mice after 7 months of age, drastic changes in all parts of the antioxidant system were found. By 9 months, in cytosol the activity of SOD, GSH-Px, and G-6-PDH sharply increased (Figs. 1a, 2a, 5), the activity of GR was stable at a low level (Fig. 3a), while the fraction of oxidized glutathione became much higher (Fig. 4).

In mitochondria, changes in the activity of glutathione-dependent enzymes were more pronounced than in cytosol: the activity of GSH-Px increased by an order of magnitude (Fig. 2b). The activity of mitochondrial GR, which decreased at the first stage, increased to levels higher than the initial level (Fig. 3b). It is noteworthy that during the stage of leucosis progression, the difference in the activity of glutathione-dependent enzymes in cytosol and mitochondria were only quantitative, whereas the dynamics of SOD activity in these two structures differed qualitatively. Unlike all other enzymes, the activity of Mn-SOD decreased 4-5-fold in the late times of observation (Fig. 1b). Earlier a similar picture was found for Mn-SOD in liver of tumor-bearing animals during the development of Ehrlich ascites carcinoma [8].

We chose the ratio between the rate of superoxide production and the activity of SOD of corresponding compartmentalization (v/A) as an index of the efficiency of antioxidant protection, as we had done earlier [8]. In the period between 3 and 6 months, the value of v/A in mitochondria changed in accordance with values of v and exceeded 5-6-fold the v/A value for inline mice and for F(C57/B1/CBA) mice. Within the interval between 7 and 9 months, both the activity of Mn-SOD and the v value decreased, but as v decreased more slowly, the ratio v/A increased and by the ninth 9th month twice exceeded the ratio for 4-month-old animals.

In a stationary mode, the rate of superoxide production is equal to the rate of its consumption:

$$v = d[O_2^-]/dt = k\,[O_2^-]\,[SOD],$$

where k is the rate constant of dismutation of superoxide radicals. From this it follows that a stationary superoxide concentration is defined as: $[O_2^-] = v/k\,[SOD]$.

The activity of SOD measured in the experiments is proportional to SOD concentration ([SOD]) expressed in relative units. Thus, the increase in the v/A ratio in mito-

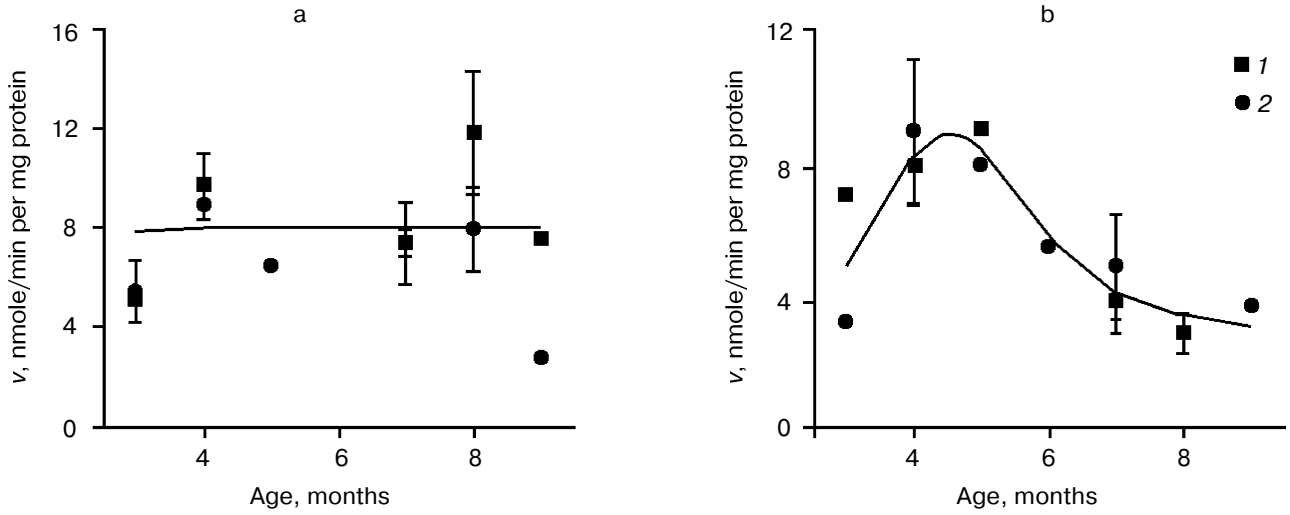


Fig. 6. Changes in the rates of superoxide production in microsomes (a) and mitochondria (b) in the liver of AKR mice (females (*1*) and males (*2*)) during the development of spontaneous leucosis.

Characteristics of the system of production and consumption of superoxide radicals in mice of different lines

Mouse line	Activity of Cu,Zn-SOD, units per mg protein	Activity of Mn-SOD, units per mg protein	*v* in microsomes, nmol/min per mg protein	*v* in SMP, nmol/min per mg protein
Mouse inline	164 ± 23	37 ± 3.3	5.4 ± 0.4	1.54 ± 0.09
F_1 (C57/Bl/CBA)	256 ± 21	25.3 ± 5.0	4.8 ± 1.1	0.8 ± 0.18
AKR	190 ± 17	50 ± 9	5.3 ± 1.2	5.3 ± 1.4

chondria means an increase in the stationary concentration of superoxide radicals in these subcellular organelles and may indicate a decrease in efficiency of the antioxidant protection both in the latent period of leucosis development and at the stage when a significant number of animals die.

In accordance with the above scheme of metabolism of active oxygen species, a certain correlation between the activities of SOD and GSH-Px, GSH-Px and GR, and GR and G-6-PDH should exist in normalcy. However, during the development of leucosis the observed changes in activities of individual antioxidant enzymes were not synchronized; as a result, in cytosol the ratio of SOD activity to GSH-Px activity decrease 2-fold by the 9th month compared to its value in the 4th month. For the same period, in mitochondria this ratio decreased 10-fold. Change in the ratio between GSH-Px and GR is also a manifestation of imbalance in the antioxidant system. In cytosol and mitochondria, the ratio of GSH-Px activity to GR activity increased 5-fold. Such a shift in the activity of GSH-Px and GR is probably one of the causes of the observed decrease in the concentration of reduced glutathione. At the same time, by the 9th month of the animals' lives the ratio of activities G-6-PDH/GR increased 4-fold, so there was no lack in NADPH for the glutathione reductase reaction.

The development of leucosis was accompanied by changes in the ratio between the activity of the same enzyme in cytosol and mitochondria. At 8 and 9 months the activity of Cu,Zn-SOD sharply increased compared to the activity of Mn-SOD; the ratio of the GR activity in cytosol to the GR activity in mitochondria decreased.

The question arises to what extent the observed imbalance in the antioxidant system of liver of a tumor-bearer is typical for development of tumors of various genesis. The stages of the changes in the systems of production and detoxication of active oxygen species observed in the present work were compared with the curve of survival of AKR mice [18]. The comparison showed that the transition from the first stage (with no significant changes) to the second stage (with evident changes) occurred at 7 month, i.e., in a period when death incidence became significant (15-20%).

We compared the relationships in the leucosis development described above with the changes in the antioxidant enzymatic system of mice liver observed during the development of Ehrlich ascites carcinoma and during benzpyrene-induced chemical carcinogenesis (the data of references [4, 8]). All three cases showed a decrease in the SOD/GSH-Px ratio in cytosol and mitochondria, increase in the cytosolic share of SOD compared to the mitochondrial share, and increase in the GSH-Px/GR ratio. During the development of Ehrlich ascites carcinoma as well as during the development of spontaneous leucosis the *v/A* ratio increased in SMP.

Substrate induction of GSH-Px can be considered as one of the causes of the decrease in the SOD/GSH-Px ratio. Changes in the concentration of lipid hydroperoxides (data of reference [28]) were compared with changes in the GSH-Px activity (data of reference [4]); in the liver of a tumor-bearer with Ehrlich ascites carcinoma at the stage of maximum tumor growth the ratio of lipid hydroperoxide concentration to GSH-Px activity increased 5-fold compared to control. The increased growth of the hydroperoxide concentration indicates the possibility of substrate induction of GSH-Px. The induction of the GSH-Px activity in mice injected with hydroperoxide of arachidonic acid was shown in direct experiments [4]. In accordance with recent data, not only substrate can be involved in GSH-Px induction. It was shown that a promoter of the cytosolic GSH-Px gene had a center for binding of p53 protein, a tumor suppressor. Thus, a link was found between a GSH-Px regulator of lipid peroxidation (LPO) and p53, which is involved in genome stability and in regulation of proliferation, differentiation, and apoptosis [29].

The described changes in ratios between the activities of the antioxidant enzymes during the development of spontaneous leucosis are similar to pre-neoplastic changes. This suggestion is supported by the following findings. The values of SOD and GSH-Px activities presented in [30] were compared; in normal fibroblasts transformed spontaneously, the SOD/GSH-Px ratio decreased by 7-8-fold, while in fibroblasts transformed *in vitro* by Rous sarcoma virus the ratio decreased by more than 30-fold. Similarly, using the data of reference [31],

one can see a 2-fold decrease of the SOD/GSH-Px ratio during the process of induced myeloid differentiation of human HL-60 cells. Apparently, a relatively small growth of the Cu,Zn-SOD activity compared to the GSH-Px activity leads to the formation of a phenotype with a low LPO level. The decrease in the SOD/GSH-Px ratio in the liver cells of a tumor-bearer may be a compensatory reaction of the organism in response to enhancement of LPO. According to [31], the activity of GR decreased during myeloid differentiation of human HL-60 cells similarly to the picture we observed in the latent period of leucosis. Earlier a shift in metabolism in the metastases-free tissues of a tumor bearer towards the metabolism typical for tumors was found by studying other biochemical characteristics [32].

In a recent review by Deichman [30], evidence was presented that during malignance a new phenotype with higher level of antioxidant protection is formed. We suppose that this high level could be reached in different ways. In this connection, it is important to reveal parameters that characterize the functioning of antioxidant enzymes as an integral system. The ratio of the activities SOD/GSH-Px probably can be considered as one such parameter.

Numerous data have shown stable negative feedback between Cu,Zn-SOD and Mn-SOD activities. We revealed this feedback in the liver of a tumor bearer with Ehrlich ascites carcinoma [8], during liver regeneration after partial hepatectomy in rats [33], and under treatment of mice with the antioxidant ionol [34]. Negative feedback was also found in macrophages kept in NO-producing media [35] and during overexpression of Cu,Zn-SOD in the culture of mice fibroblasts [36]. In normalcy, oppositely directed changes in the activities of Cu,Zn-SOD and Mn-SOD accompany a cell passing through different stages of the cellular cycle [33, 37]. It was shown that in the process of rat brain development the activity of Cu,Zn-SOD decreased while the activity of Mn-SOD increased [38]. As seen in Fig. 1b, during the development of spontaneous leucosis in the period from 7 to 9 months, when the activity of Cu,Zn-SOD increased, the activity of Mn-SOD fell to 20% of the initial level. It is worth noting that mutant mice lacking Mn-SOD are nonviable and die within 10 days after birth [39]. The results presented in this paper show that changes in the enzymatic antioxidant system of liver are regularly linked with the development of leucosis. The changes involve both enzymes of superoxide metabolism and glutathione-dependent enzymes regulating the metabolism of hydroperoxides in cytosol and mitochondria. The main conclusions of the work are as follows.

During the development of leucosis, most changes in regulation of production and detoxication of active oxygen species take place in mitochondria. The increase in stationary superoxide concentration provides conditions for the development of oxidative stress in these subcellular organelles. The growth of the GSH-Px/GR ratio in cytosol and mitochondria and the decrease in the concentration of reduced glutathione in cytosol also provide evidence in favor of the formation of a prooxidant state in the liver.

As analysis of the literature shows, the findings revealed in the present work such as the decrease in the ratio of SOD/GSH-Px activities in cytosol and mitochondria, the low level of GR activity, and the increase in the stationary concentration of superoxide radicals in mitochondria are typical for pre-neoplastic changes in cell metabolism.

Much of the data accumulated in the literature suggests that antioxidant enzymes act as a system participating in the maintenance of the balance between cell proliferation, LPO, and apoptosis [40, 41].

REFERENCES

1. Emanuel, N. M., and Lipchina, L. P. (1958) *Dokl. Akad. Nauk SSSR*, **121**, 141-144.
2. Sun, J. (1990) *Free Radical Biol. Med.*, **8**, 583-599.
3. Winterbourn, C. C. (1995) in *The Oxygen Paradox* (Davies, K. J. F., and Ursini, F., eds.) Cleup University Press, Padova, Italy, pp. 23-31.
4. Lankin, V. Z., Polyakov, V. M., and Gurevich, S. M. (1977) in *Lipids: Structure*, *Biosynthesis*, *Transformations and Functions* (Severin, S. E., ed.) [in Russian], Nauka, Moscow, pp. 93-103.
5. Batko, J., Warchl, T., and Karon, K. (1996) *Acta Biochim. Pol.*, **43**, 403-405.
6. Rana, M. P., Ghosh, R., and Catterjee, M. (1994) *Oncology*, **51**, 25-29.
7. Siminyan, M. A., Galstyan, D. A., and Demirchoglyan, I. G. (1985) *Biokhimiya*, **50**, 768-773.
8. Gurevich, S. M., Vartanyan, L. S., and Nagler, L. G. (1993) *Voprosy Med. Khim.*, **39**, 16-20.
9. Arruda, V. R., Saltes, T. S., Costa, F. F., and Saad, S. T. (1996) *Neoplasma*, **43**, 99-102.
10. Zima, T., Spicka, I., Stipek, S., Crkovska, J., Platenic, J., Merta, M., and Tesar, V. (1996) *Neoplasma*, **43**, 69-73.
11. Conzales, R., Anclair, C., Volsin, E., Dhermy, D., and Boivin, P. (1984) *Cancer Res.*, **44**, 4137-4139.
12. Franco, M. E., Carrillo, M. C., Hernandez, C., Medina, C., Ibarra, B., Vaga, G., and Cantu, J. M. (1990) *Arch. Invest. Med.*, **21**, 285-288.
13. Omata, F., Nakazawa, H., Nakano, M., and Arimori, S. (1990) *Tokai J. Exp. Clin. Med.*, **15**, 99-106.
14. Saito, T., Kurasaki, M., Kaji, H., and Saito, K. (1984) *Cancer Lett.*, **24**, 141-146.
15. Bewick, M., Cautie, W., and Tudhope, G. R. (1987) *Br. J. Haematol.*, **65**, 347-350.
16. Saito, T. (1987) *Hokkaido Igaku Zassi*, **62**, 257-268.
17. Rzymowska, J. (1995) *Neoplasma*, **42**, 53-56.
18. Burlakova, E. B., and Erokhin, V. N. (2001) *Radiats. Biol. Radioekol.*, **41**, No. 4.
19. Archakov, A. I., Devchinsky, V. M., Karuzina, I. I., Ivkov, N. N., Aleksandrova, E. F., Doronin, P. P., and Sorokina, M. L. (1968) *Biokhimiya*, **33**, 479-488.

20. Rashba, Yu. E., Vartanyan, L. S., Baider L. M., and Krinitskaya, L. A. (1989) *Biofizika*, **34**, 57-62.
21. Vartanyan, L. S., and Gurevich, S. M. (1991) *Voprosy Med. Khim.*, **5**, 23-26.
22. Kornberg, A. (1950) *J. Biol. Chem.*, **182**, 805-813.
23. Lankin, V. Z., and Gurevich, S. M. (1976) *Dokl. Akad. Nauk SSSR*, **216**, 705-708.
24. Carlberg, J., and Mannervik, B. (1985) *Meth. Enzymol.*, **113**, 484-490.
25. Tietze, F. (1969) *Anal. Biochem.*, **27**, 502-522.
26. Rashba, Yu. E., Chernikov, V. A., Baider, L. M., and Vartanyan, L. S. (1986) *Biol. Membr. (Moscow)*, **3**, 838-845.
27. Blandova, Z. K., Dushkin, V. A., Malashenko, A. M., and Shmidt, E. F. (1983) *Lines of Laboratory Animals for Medical and Biological Studies* [in Russian], Nauka, Moscow.
28. Kozlov, Yu. P., Danilov, V. S., Kagan, V. E., and Sitkovskii, M. V. (1972) in *Free-Radical Lipid Oxidation in Biological Membranes* [in Russian], Moscow State University, Moscow.
29. Tan, M., Swaroop, M., Guan, K., Oberley, L. W., and Sun, J. (1999) *J. Biol. Chem.*, **274**, 12061-12066.
30. Deichman, G. I. (2000) *Biochemistry (Moscow)*, **65**, 78-94.
31. Splier, Ch., and Newburger, P. E. (1986) *Arch. Biochem. Biophys.*, **251**, 551-557.
32. Shapot, V. S. (1975) in *Biochemical Aspects of Tumor Growth* [in Russian], Meditsina, Moscow, pp. 138-145.
33. Vartanyan, L. S., Sadovnikova, I. P., Gurevich, S. M., and Sokolova, I. S. (1992) *Biokhimiya*, **57**, 671-678.
34. Vartanyan, L. S., and Gurevich, S. M. (1999) *Voprosy Med. Khim.*, **45**, 314-320.
35. Brockhaus, F., and Brune, B. (1999) *Biochem. J.*, **338**, 295-303.
36. Kelner, M. J., and Bagnell, R. (1990) *J. Biol. Chem.*, **265**, 10872-10875.
37. Oberley, T. D., Schultz, J. L., Li, N., and Oberley, L. W. (1995) *Free Radical Biol. Med.*, **19**, 53-65.
38. Vanella, A., Geremia, E., D'Urso, G., and Pinturo, R. (1992) *Gerontology*, **28**, 108-113.
39. Huang, T. T., Yasunami, M., Carlsson, E. I., Gillespic, A. M., Reaume, A. G., Hoffman, E. K., Chau, P. H., Scott, R. W., and Epstein, C. I. (1997) *Arch. Biochem. Biophys.*, **344**, 424-432.
40. Burdon, R. H. (1995) *Free Radical Biol. Med.*, **18**, 775-794.
41. Allen, R. G., and Tresini, M. (2000) *Free Radical Biol. Med.*, **28**, 463-499.