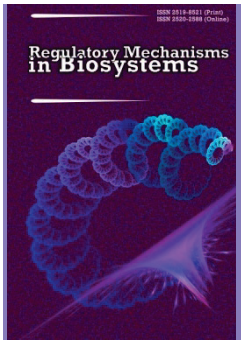

Regulatory Mechanisms in Biosystems

ISSN 2519-8521 (Print)
ISSN 2520-2588 (Online)
Regul. Mech. Biosyst.,
2024, 15(3), 496–503
doi: 10.15421/022470

Copper sulfate and carbon tetrachloride induces a uniform response at the level of the redox system and the nature of this response depends on age

E. G. Ivanov, K. M. Lebid-Biletska, A. I. Bozhkov, Y. V. Nikitchenko

V. N. Karazin Kharkiv National University, Kharkiv, Ukraine

Article info

Received 01.06.2024
Received in revised form 09.07.2024
Accepted 24.07.2024

V. N. Karazin Kharkiv National University, Pl. Svobody, 4, Kharkiv, 61002, Ukraine.
Tel.: +38050-402-36-21.
E-mail: niibio@karazin.ua

Ivanov, E. G., Lebid-Biletska, K. M., Bozhkov, A. I., & Nikitchenko, Y. V. (2024). Copper sulfate and carbon tetrachloride induces a uniform response at the level of the redox system and the nature of this response depends on age. Regulatory Mechanisms in Biosystems, 15(3), 496–503. doi:10.15421/022470

The concept of oxidative stress, which is a development of D. Harman's idea of random harmful tissue damage by free radicals, remains one of the most popular in the study of pathological processes, including age-dependent chronic changes. The work tested the hypothesis according to which, a shift in equilibrium towards pro-oxidants, i.e. oxidative stress, is the primary adaptive response of the body to exogenous toxic environmental factors. To test this, a number of indicators of the redox system were determined as a response to hepatotoxic compounds of different nature (copper sulfate and carbon tetrachloride) in young and old animals. The amount of lipid hydroperoxides and the activity of a number of antioxidant enzymes were determined in the blood serum of young (3 months) and old (20 months) rats before exposure (initial level) and after repeated sequential injections (3 injections) of copper sulfate at a dose of 1 mg/100 g body weight and carbon tetrachloride in a dose of 0.1 mL in 50% vegetable oil. It was found that in intact (initial level) old animals, the indicators of the redox system are shifted towards antioxidants. After three consecutive administrations of various hepatotoxic compounds, with an interval of 48 hours between administrations, the balance shifted towards pro-oxidants, regardless of the inducer, however, this effect was more pronounced in old animals compared to young ones, relative to their initial level. Such different reactivity of redox system indicators in animals of different ages led to the "evening out" of the initially different redox system indicators. We came to the conclusion that changing the balance in the pro-oxidant-antioxidant system is a universal, primary reaction of the body to endogenous or exogenous factors that perform regulatory functions and, depending on the temporal and functional characteristics of the body, are "transformed" into specific physiological manifestations.

Keywords: copper sulfate; carbon tetrachloride; hepatotoxicity; redox system; age; oxidative stress.

Introduction

There is a growing interest in the study of the mechanisms of ageing, not only to find the possibility of increasing life expectancy, but also because of a number of fundamental characteristics of this phenomenon: (1) ageing, or changes in the structural and functional characteristics of biological systems over time, is a characteristic of all biological systems, i.e. it is a universal biological phenomenon; (2) ageing is associated with the emergence of chronic diseases (Alzheimer's, cirrhosis of the liver, oncology, diabetes, etc.) due to irreversible metabolic states, i.e. it is linked to the global problem of metabolic irreversibility in biology; (3) knowledge of the peculiarities of the formation of age-dependent adaptive responses to exogenous environmental factors, including infectious ones, can significantly increase the effectiveness of treatment of not only chronic but also acute pathological states. It is well known that the success and intensity of scientific research is determined not only by its relevance, but also by the presence of productive ideas and hypotheses. One such productive and promising hypothesis in gerontology was the free radical hypothesis of ageing, proposed by D. Harman in 1956, according to which ageing is the result of random malignant damage to tissues by free radicals (Harman, 1956). During more than half a century of testing, this hypothesis, which is simple to understand, elegant in essence (based on natural processes of functioning of biological systems) and promising in practical terms (by regulating the production of free radicals in the body one can hope to control life expectancy), has been repeatedly modified and supplemented (Beckman & Ames, 1998; Harman, 2006; Ivanova & Yankova, 2013; Barja, 2014; Brygadyrenko & Ivanyshyn, 2015; Shostya & Siabro, 2022; Abdelazim & Abomughaid, 2024). Although this hypothesis did not turn out to be a fundamental gerontological theory, it proved to be extremely productive for a number of reasons. Firstly, it became the basis of a new scientific trend – free radical biology (Harman, 1956; Halliwell & Gutteridge, 2015; Averill-Bates, 2024), and secondly, it contributed to the formation of the concept of oxidative stress (Sies, 2020; Lushchak & Storey, 2021) and the study of its role in the development of pathologies (Alkadi, 2020; Hajam et al., 2022; Jomova et al., 2023) which has greatly expanded our understanding of the regulatory role of free radical reaction products (Dröge, 2002; Yoshikawa & You, 2024).

At the same time, the role of free radical processes in the mechanisms of ageing is somewhat exaggerated in the sense that the concept of peroxide processes, and especially of lipids (LPO), has been developed in model (*in vitro*) systems and no direct evidence of a direct pathogenetic role in the mechanisms of "natural" ageing has yet been demonstrated. Furthermore, the presence of powerful regulatory antioxidant systems in biological systems practically excludes the uncontrollable influence of LPO products in normal physiological processes. In this case, there is convincing evidence for a regulated rather than a chaotic LPO process. For example, in the natural process of eicosanoid synthesis, the resulting LPOs are fully metabolised, neutralised and do not accumulate, as would be expected according to the concept of uncontrolled LPO formation. The "oxidative burst" used by neutrophils to "neutralise" invading bacteria does not lead to an uncontrolled increase in oxidative processes in the body (Slauch, 2011). In this context, the emphasis in studies of free radical processes and their products has shifted from pathogenetic functions to studies of their involvement in the regulation of biological processes.

Relatively recently, it has been shown that the activity of aconitase (aconitate hydratase (EC 4.2.1.3), an enzyme of the hydratase family that catalyzes the reaction of isomerization of citrate to isocitrate through the formation of cis-aconitate in the Krebs cycle (Beinert et al., 1996) is inhibited against the background of oxidative stress and may serve as an indicator of the level of oxidative stress (Gardner et al., 1994).

In this case, it was of interest to study the possible "specificity" of changes in the indices of the redox system in animals of different ages in response to the action of various chemical compounds (in particular copper ions and tetrachloromethane) which have a hepatotoxic effect on the organism. The solution to this question is important from several points of view. Firstly, the action of hepatotoxic compounds is usually accompanied by the formation of reactions in the organism, which are manifested in the development of fibrosis and possibly cirrhosis, i.e. the formation of chronic pathologies. As is known, chronic pathologies can be attributed to age-dependent pathologies, and investigation of the mechanisms of irreversibility of chronic pathologies is directly related to the mechanisms of ageing (Bozhkov et al., 2017). Secondly, the study of the role of indicators of the redox system in the formation of the adaptive response of animals of different ages to the action of different in nature hepatotoxic compounds is a convenient model for the study of the physiological role of products of free radical reactions in the organism.

In this context, we tested the working hypothesis according to which the shift of the equilibrium in the redox system towards prooxidants is a primary, non-specific reaction of the organism to the action of exogenous environmental factors. The nature of the response, such a primary reaction of the organism, depends on the initial characteristics of the redox system of the organism at the moment of influence of these factors and, as is known, these characteristics of the redox system can be different in animals of different ages.

In order to test this hypothesis, the content of lipid hydroperoxides and the activity of a number of antioxidant enzymes in different compartments of liver cells and blood serum of intact animals of two age groups (3 and 20 months) were determined, as well as the changes in the indices of the redox system after chronic actions (three consecutive injections and an interval of 48 hours between injections) of copper sulfate and tetrachloromethane in animals of these two age groups.

Materials and methods

The experiments were carried out on male Wistar rats of two age groups: young – 3 months and old – 20 months. The animals were kept in standard vivarium conditions in compliance with the existing principles of animal experimentation in accordance with the "General Principles of Work on Animals" approved by the I National Congress on Bioethics (Kyiv, Ukraine, 2001) and agreed with the provisions of the "European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes" (Strasbourg, France, 1986) and after approval by the Bioethics Committee of the V.N. Karazin Kharkiv National University.

All animals were divided into 6 experimental groups (Fig. 1). The choice of doses and schedules of copper sulfate and tetrachloromethane injections was based on previous experiments designed to induce an adaptive response in animals. It was shown that at a dose of copper sulfate of 1 mg/100 g of body weight with a 48-hour interval between injections, after three consecutive injections in such animals, on the one hand, resistance was formed to the subsequent injection of a lethal dose of copper sulfate, indicating the formation of an adaptive response, and, on the other hand, it was accompanied by the formation of liver fibrosis in such animals (Bozhkov et al., 2014), which can be attributed to chronic age-dependent pathologies. It is known that copper ions are a metal with a transient valence and participate in redox processes of the organism, i.e. they can have a "specific" effect on the redox system of the organism.

It is known that tetrachloromethane (carbon tetrachloride) is an organochlorine compound with toxic effects, affecting the central nervous system, liver, kidneys, having a local irritant effect on the skin of the hands and mucous membranes, and being metabolised in the body to chloroform and carbon dioxide.

Consequently, these two different compounds differ in their properties and physiological manifestations. Theoretically, these compounds should also have different effects on the indices of the redox system. A comparative analysis of the impact of copper sulfate and tetrachloromethane on the indices of the redox system can inform our understanding of the 'specificity' or 'non-specificity' of the response of the redox system to different exogenous environmental factors. Moreover, the use of animals of different ages in the experimental design enables us to explore the influence of the initial metabolic characteristics of the organism on the formation of the adaptive response at the level of the redox system. The choice of the dose of tetrachloromethane was based on the dose and number of copper sulfate injections and was set similar to that of copper sulfate. Tetrachloromethane was given three times per os in a dose of 0.1 mL of 50% oil solution, with an interval of 48 h between injections, and 24 h after the last injection of the compounds studied, the animals were decapitated under ether anaesthesia, blood serum was collected, the liver was isolated and the parameters of research were determined, as shown in Figure 1.

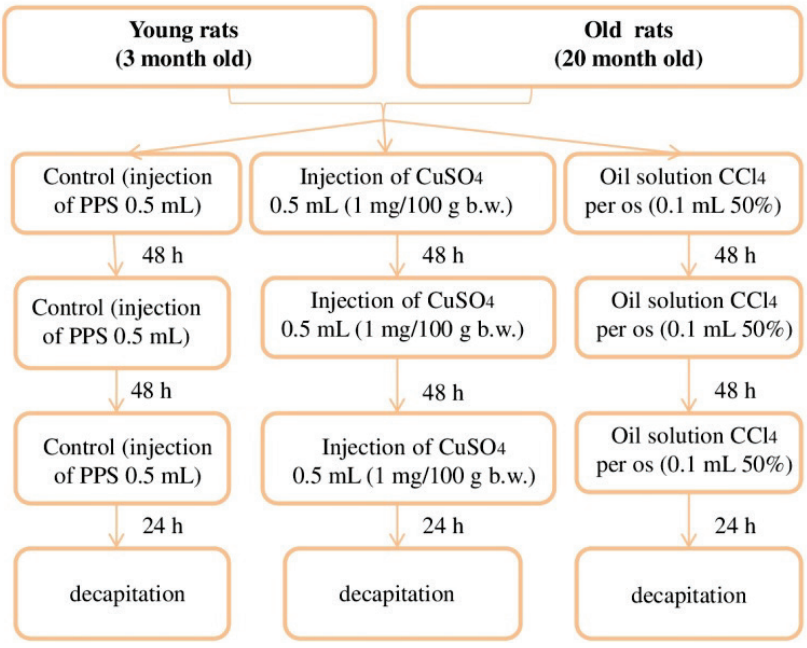

Fig. 1. Design of investigation: the sequence of administration of copper sulfate and tetrachloromethane to young and old animals and their doses

The manipulations with animals indicated in the experimental design were carried out in the morning from 9:00 to 11:00 before feeding under standard vivarium conditions. After completion of all manipulations, the animals were decapitated, blood was collected, and blood serum was obtained. Mitochondria and cytosol from liver cells were isolated according to the method of Kamath & Narayan (1972) and the prooxidant-antioxidant balance in blood serum, mitochondrial and cytosolic fractions of liver cells were measured.

The content of lipid hydroperoxides (LOOH) was determined by measuring the content of malondialdehyde (MDA) in liver mitochondria and cytosol was determined using the method of (Ohkawa et al., 1979), and in blood serum using the method of (Asakawa & Matsushita, 1980). The absorbance spectrum of the coloured product was recorded with a spectrophotometer (Specord UV VIS, Germany), measuring the difference in extinction at 535 and 520 nm. The malondialdehyde content was then calculated as an equivalent amount of MDA, based on a molar extinction coefficient of $1.56 \cdot 10^5$ $M^{-1} \cdot cm^{-1}$.

Aconitate (Aco) hydratase activity (EC 4.2.1.3) in mitochondria was measured spectrophotometrically using Specord UV VIS (Germany), using the method described by (Gardner et al., 1994; Varghese et al., 2003). Mitochondria were incubated for 6 minutes with stirring in a solution containing 50 mM Tris-HCl buffer (pH 8.0), 10 mM $MnCl_2$ and 10 mM isocitrate, then shaken in a thermostatic incubator at 37 °C. Activity was expressed in nanomoles of aconitate per milligram of protein.

Glutathione peroxidase (GPx) activity (EC 1.11.1.9) was measured in blood serum and mitochondrial, and cytosolic liver fractions spectrophotometrically at 340 nm (Specord UV VIS, Germany), using the method described by Paglia & Valentine (1967). The assay was conducted in a 50 mM potassium-sodium phosphate buffer (pH 7.4) containing 1 mM EDTA, 0.1 mM NADPH, 1 unit of yeast glutathione reductase, 1 mM GSH, 0.2% Triton X-100, 0.4 mM hydrogen peroxide, and 3 mM sodium azide to inhibit catalase. The temperature was maintained at 37 °C. The enzyme activity was expressed as nmol of NADPH consumed per minute per mg of protein or per ml of serum, using a molar extinction coefficient of 6.22×10^3 M^{-1} cm^{-1}.

Measurement of glutaredoxin (Grx) activity (EC 1.20.4.1): glutaredoxin activity in rat liver mitochondria was measured spectrophotometrically (Specord UV VIS, Germany) with slight modifications to the method described by Gallogly et al. (2010). The assay used a 50 mM potassium phosphate buffer (pH 8.0) containing 0.5 mM GSH, 0.2 mM NADPH, 0.4 units/mL yeast glutathione reductase, 1.25 mM cystine, and 0.2% Triton X-100, conducted at 37 °C. The activity was reported as nmol NADPH/min per mg of protein, with a molar extinction coefficient of 6.22×10^3 M^{-1} cm^{-1}.

Glutathione reductase (GR) activity (EC 1.6.4.2) in mitochondrial and postmitochondrial fractions of the liver was determined spectrophotometrically (Specord UV VIS, Germany) using the method by Carlberg & Mannervik (1975), which monitors the decrease in NADPH levels. The assay was performed in a medium containing 50 mM potassium phosphate buffer (pH 7.4), 1 mM EDTA, 0.16 mM NADPH, 1 mM GSSG, and 0.2% Triton X-100 at 37 °C. The enzyme activity was expressed as nmol NADPH consumed per minute per mg of protein, using a molar extinction coefficient of 6.22×10^3 M^{-1} cm^{-1}.

Determination of $NADP^+$-glucose-6-phosphate dehydrogenase (G6PDG) activity (EC 1.1.1.49): glucose-6-phosphate dehydrogenase (G6PDH) activity was measured in the postmitochondrial fraction of the liver by monitoring the rate of $NADP^+$ reduction using the method of Zaheer et al. (1967). The reaction was conducted in a 120 mM Tris-HCl buffer (pH 7.4) containing 10 mM $MgCl_2$, 2 mM glucose-6-phosphate, 0.9 mM NADP+, and 0.2% Triton X-100 at 37 °C. The activity was expressed as nmol NADPH produced per minute per mg of protein, considering a molar extinction coefficient of 6.22×10^3 M^{-1} cm^{-1}.

Determination of $NADP^+$-isocitrate dehydrogenase (ICDG) activity (EC 1.1.1.42): isocitrate dehydrogenase (ICDH) activity was measured in the mitochondrial and postmitochondrial fractions of the liver by the rate of $NADP^+$ reduction according to the method of Bauman et al. (1970). The assay was performed in a 34 mM Tris-HCl buffer (pH 7.4) containing 0.34 mM EDTA, 1.5 mM $MnCl_2$, 0.1 mM $NADP^+$, 1.5 mM isocitrate, and 0.2% Triton X-100 at 37 °C. The enzyme activity was expressed as nmol NADPH consumed per minute per mg of protein, using a molar extinction coefficient of 6.22×10^3 M^{-1} cm^{-1}.

The protein content of the samples was determined by the method of Lowry et al. (1951) by Folin Reaction modified by Miller (1959) for protein determination of large numbers of samples by addition of the color reagents.

Data are presented as group means and standard errors. Data analysis was carried out using Statistica 8 (Microsoft Corporation, Stanford). Significant differences between groups were determined using a nonparametric method using the Mann-Whitney U-test. Differences between comparison groups were considered significant at $P < 0.05$.

Results

Determination of some indices of the redox system in young and old intact (not exposed to any effects) animals.

It was found that the quantity of LOOH in the liver mitochondria of old intact rats was 37.1% lower than that of young animals (Fig. 2a). At the same time, the amount of LOOH in the liver cytosol fraction in young and old animals was the same (Fig. 2a).

It is known that mitochondria are the main contributors to the balance of lipid hydroperoxides in the organism. It was found that the content of LOOH in the blood serum, which reflects the general background at the level of the whole organism, was also 34.4% lower in old animals compared to young animals (Fig. 2a), i.e. the change in the amount of LOOH in serum and mitochondria with age coincided. At the same time, the absolute amount of LOOH in the blood serum of both young and old animals was 17-18 times higher compared to their amount in liver mitochondria, which indicates that along with liver mitochondria, other "sources" participate in the formation of the total pool of LOOH in the organism.

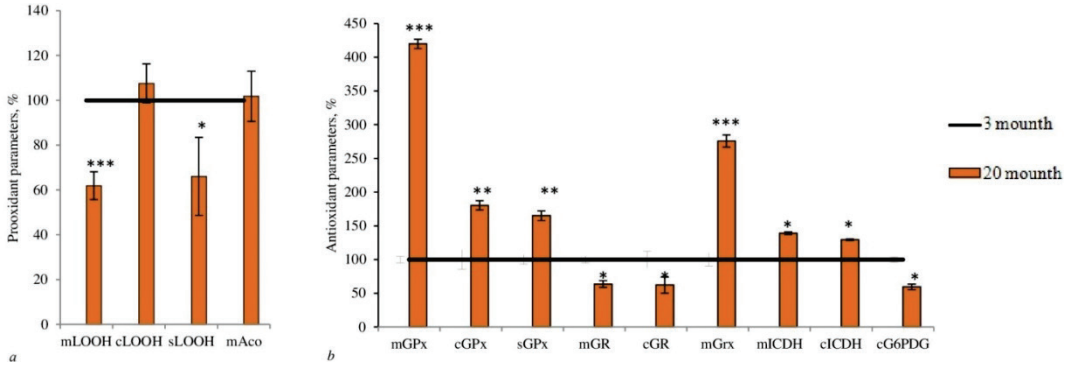

Fig. 2. Prooxidant content (LOOH-hydroperoxides in mitochondria (m LOOH), cytosol fraction (c LOOH) and blood serum (s LOOH), and aconitase activity in mitochondria mAco (*a*) as well as activity of antioxidant enzymes (glutathione peroxidase (GPx), glutaredoxin (Gpx), glutathione reductase (GR), glucose-6-phosphate dehydrogenase (G6PDG), isocitrate dehydrogenase (ICDG), in mitochondria, cytosol and blood serum (*b*); the values taken as 100% in 3-month-old rats and the change in these values in 20-month-old rats; mean values obtained from 8 determinations are presented; * – $P < 0.05$, ** – $P < 0.01$, *** – $P < 0.001$ (taking into account the Bonferroni amendment) changes are statistically significant compared to young rats

It was also established that aconitase activity in the mitochondria of intact old rats does not differ from its activity in intact young animals (Fig. 2a). This result may indicate that the differences in LOOH levels in both young and old animals are within homeostatic limits. The reduced LOOH levels observed in old animals may be due to increased activity of antioxidant enzymes rather than oxidative stress.

Indeed, the activity of glutathione peroxidase in mitochondria, cytosol and blood serum of old animals increased by 319.2%, 80.5% and 65.7%, respectively, compared with young animals (Fig. 2b). Glutaredoxin activity in liver mitochondria of old animals increased by 175.3% compared to young animals (Fig. 2b). At the same time, glutathione reductase activity in liver mitochondria and cytosol decreased by 37.1% in old compared to young intact animals (Fig. 2b). The activity of isocitrate dehydrogenase in mitochondria and cytosol, as well as that of glucose-6-phosphate dehydrogenase in cytosol and mitochondria of liver cells in aged animals, remained unchanged in comparison to that observed in younger animals (Fig. 2b). In old intact animals the content of LOOH did not increase, but on the contrary decreased in comparison with young animals and these quantitative variations do not reflect the presence of oxidative stress.

The lower amount of lipid hydroperoxides in old animals in mitochondria and blood serum is associated with a higher activity of a number of antioxidant enzymes and, first of all, with the activity of different isoforms of glutathione peroxidases.

Old intact animals form a different pattern of indicators of the redox system from young animals, i.e. the ratio between the elements of the pro-oxidant and antioxidant system is different in them. If we proceed from the "concept of the determining role of the initial metabolic level on the formation of the subsequent adaptive response of the organism", we can anticipate that in the case of extreme exogenous factors, in particular, copper sulfoxide and tetrachloromethane, the responses of young and old animals at the level of the redox system may differ.

The age-dependent nature of the response at the level of the redox system to repeated sequential actions of copper sulfoxide and tetrachloromethane.

The administration of copper sulfoxide to experimental animals in a threefold sequential manner at a dose of 1 mg/100 g body weight was accompanied by a 32.4% increase in the content of LOOH in liver mitochondria in young animals compared to their initial level (Fig. 3a). A threefold consecutive administration of tetrachloromethane to young animals at a dose of 0.1 mL of 50.2% oil solution per 100 g of body weight also resulted in an increase in the amount of LOOH in young animals by 35.6% (Fig. 3a).

If copper sulfoxide and tetrachloromethane were administered to old animals at the same dosage, the amount of LOOH in liver mitochondria increased in comparison to its initial level by 111.2% and 130.8%, respectively (Fig. 3a). Such different effects of increasing the amount of LOOH in mitochondria after the action of these hepatotoxic compounds led to an "equalization" of the initially different quantitative indices of hydroperoxides in young and old animals against the backdrop of the action of these hepatotoxic compounds (Fig. 3a).

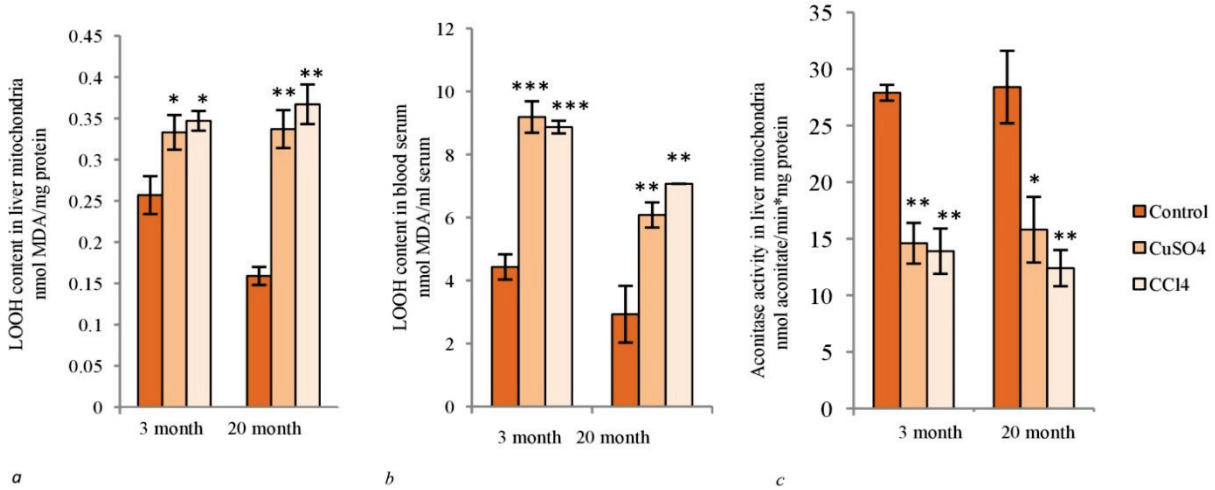

Fig. 3. The content of lipid hydroperoxides in mitochondria (*a*), the content of lipid hydroperoxides in blood serum (*b*), and the aconitase activity in mitochondria of liver cells (*c*) in young and old animals in the control group, 24 hours after threefold injection of copper sulfoxide and threefold injection of CCl_4: mean values from the experiments and their standard errors are presented; n = 8, x ± SE; * – P < 0.05, ** – P < 0.01, *** – P < 0.001 changes are statistically significant compared to control group of animals

Multiple consecutive injections of copper sulfoxide and tetrachloromethane into experimental animals were accompanied by an increase in the amount of LOOH in the blood serum, which was expressed to an even greater extent than in mitochondria (Fig. 3b). Thus, the concentration of LOOH in the blood serum of young animals 24 hours after the last injection of copper sulfoxide and tetrachloromethane was elevated by 108.2% and 101%, respectively, in comparison to their initial level (Fig. 3b). Under the same conditions, the amount of LOOH in the blood serum of old animals increased by 10.7% after the action of copper sulfoxide and by 14.1% after the action of tetrachlormethane (Fig. 3b). The increase in the number of LOOH in liver mitochondria occurred against the backdrop of a significant decrease in aconitase activity by 48% and 45% in the liver mitochondria of young animals after the action of copper sulfoxide and tetrachloromethane, respectively (Fig. 3c). In the liver mitochondria of old animals, copper sulfoxide and tetrachloromethane also exhibited a suppression of aconitase activity by 51.2% and 57.5%, respectively (Fig. 3c), which was comparable to that observed in young animals.

The increase in the rate of formation of oxidized products in the organism, in the context of copper sulfoxide and tetrachloromethane action, can be realized through either the inhibition of antioxidant defense systems and, most notably, the enzymatic link, or the induction of free-radical processes within the organism, which eventually leads to a shift in the pro-oxidant-antioxidant equilibrium towards pro-oxidants. The activity of glutathione peroxidase (one of the main antioxidant enzymes) in the liver mitochondria of young and old animals was insignificantly decreased compared to their initial levels, only by 22–25% regardless of age and nature of the toxicant (Fig. 4a). At the same time, the activity of this enzyme in the cytosol fraction in young animals did not significantly differ from the control level, while in old animals it was 20.2% lower than the control level (Fig. 4b).

The activity of glutathione peroxidase in the blood serum was decreased to the same extent in young and old animals regardless of the nature of the toxicant by 22–27% compared to their initial levels (Fig. 4c).

At the same time, the activity of glutathione reductase in the presence of hepatotoxic compounds was found to be significantly reduced only in the liver mitochondria of young animals (Fig. 4d), while in the cytosol fraction, it remained within the control values (Fig. 4e).

Against the backdrop of copper sulfoxide action, the activity of isocitrate dehydrogenase in the liver mitochondria of young rats decreased by 28.3% compared to their initial level, and in old animals, in which in the control it was 40.9% higher compared to young animals, it decreased against the background of copper sulfoxide action by 47.5% compared to their initial level and at this time did not differ from that of young animals, i.e. they were "equalized" (Fig. 5a).

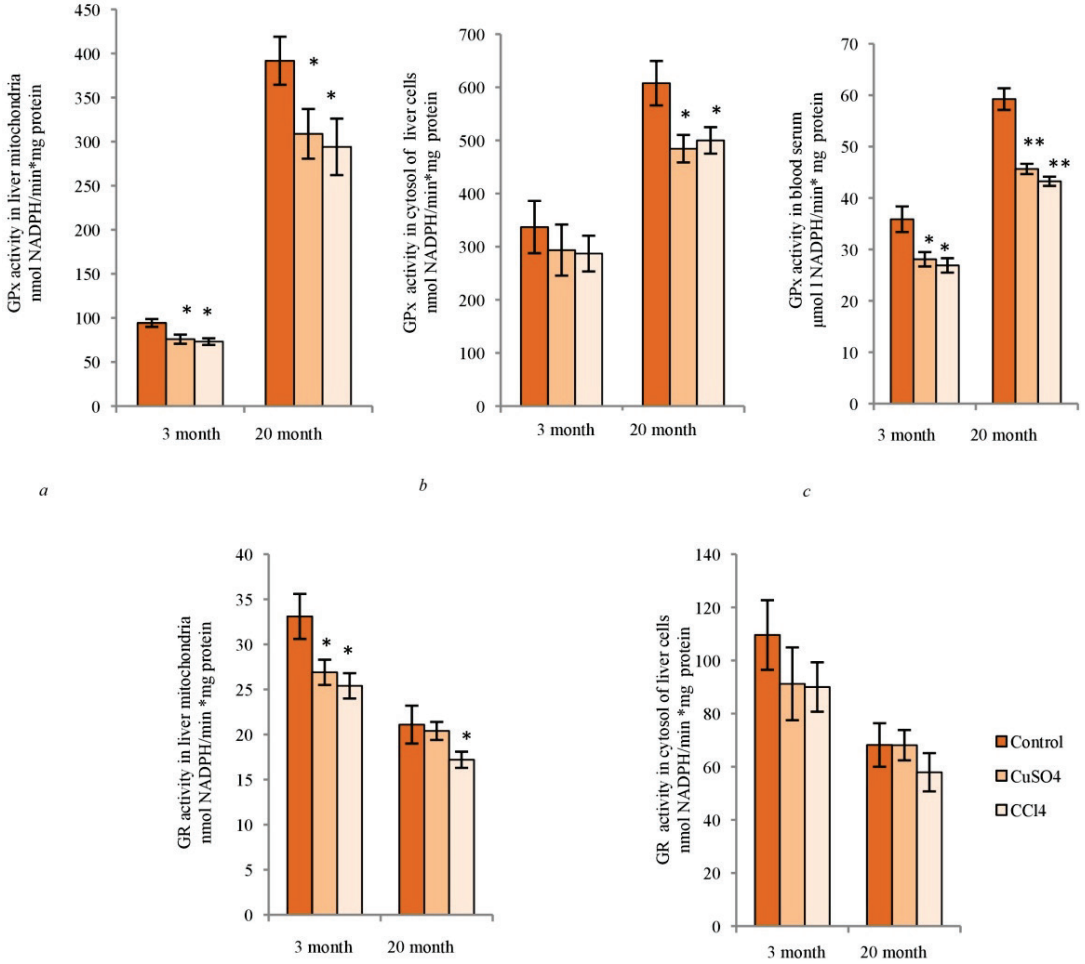

Fig. 4. The activity of glutathione peroxidase in liver mitochondria (*a*), cytosol (*b*) and blood serum (*c*), glutathione reductase activity in mitochondria (*d*) and cytosol (*e*) of liver cells in young and old animals in control, after threefold administration of copper sulfoxide and threefold administration of CCl_4: n = 8, x ± SE; * – P < 0.05, ** – P < 0.01 changes are statistically significant compared to control group of animals

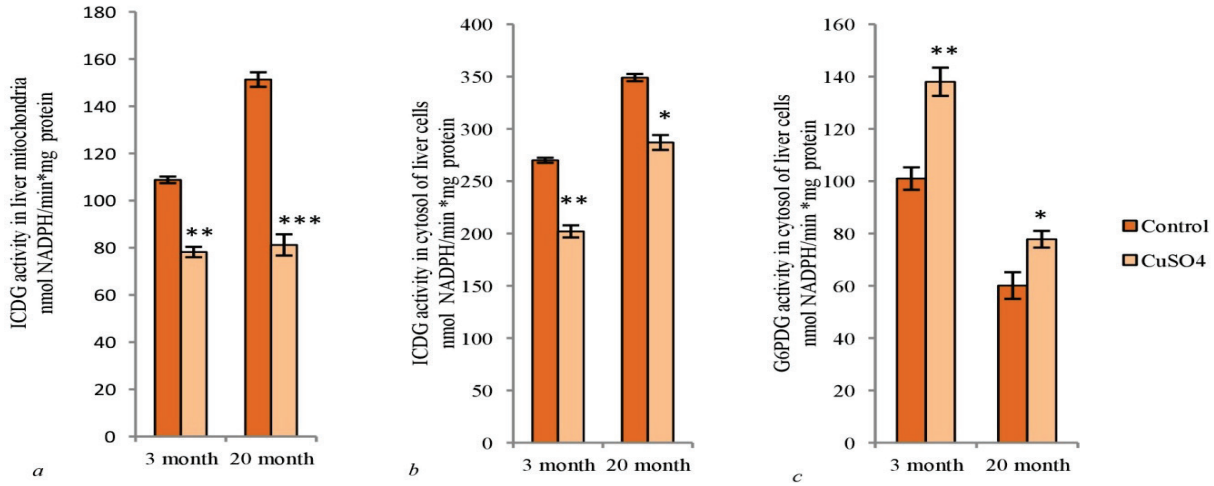

Fig. 5. Isocitrate dehydrogenase (*a*) in mitochondria of liver cells, as well as isocitrate dehydrogenase (*b*) and glucose-6-phosphate dehydrogenase (*c*) activity in cytosol of liver cells in young and old animals in control and after threefold administration of copper sulfoxide: n = 8, x ± SE; * – P < 0.05, ** – P < 0.01, *** – P < 0.001 changes are statistically significant compared to control group of animals

Isocitrate dehydrogenase, which is localized in the cytosol, also decreased compared to initial levels in young animals by 26.1%, and in old animals by 18% against the background of copper sulfate action (Fig. 5b). It should be noted that in old intact animals the activity of isocitrate dehydrogenase in cytosol as well as in mitochondria was higher in comparison with intact young animals, these differences in the activity of this enzyme in the cytosol fraction were also maintained under the action of copper (Fig. 5b).

At the same time, the activity of glucose-6-phosphate dehydrogenase, which provides the synthesis of reduced glutathione, in the cytosol fraction increased on the background of copper sulfate action by 36.6% in young animals in comparison with the initial level and by 29.4% in old animals in comparison with the initial level (Fig. 5c).

Consequently, the actions of copper sulfoxide and tetrachloromethane insignificantly inhibited the activity of a number of antioxidant enzymes, which was accompanied by a shift in the balance toward pro-oxidants, while they formed "new" different from the initial ratios.

It should be noted that the initial differences in the indicators of the redox system of young and old animals were almost completely eliminated after the action of these toxic compounds (Fig. 6).

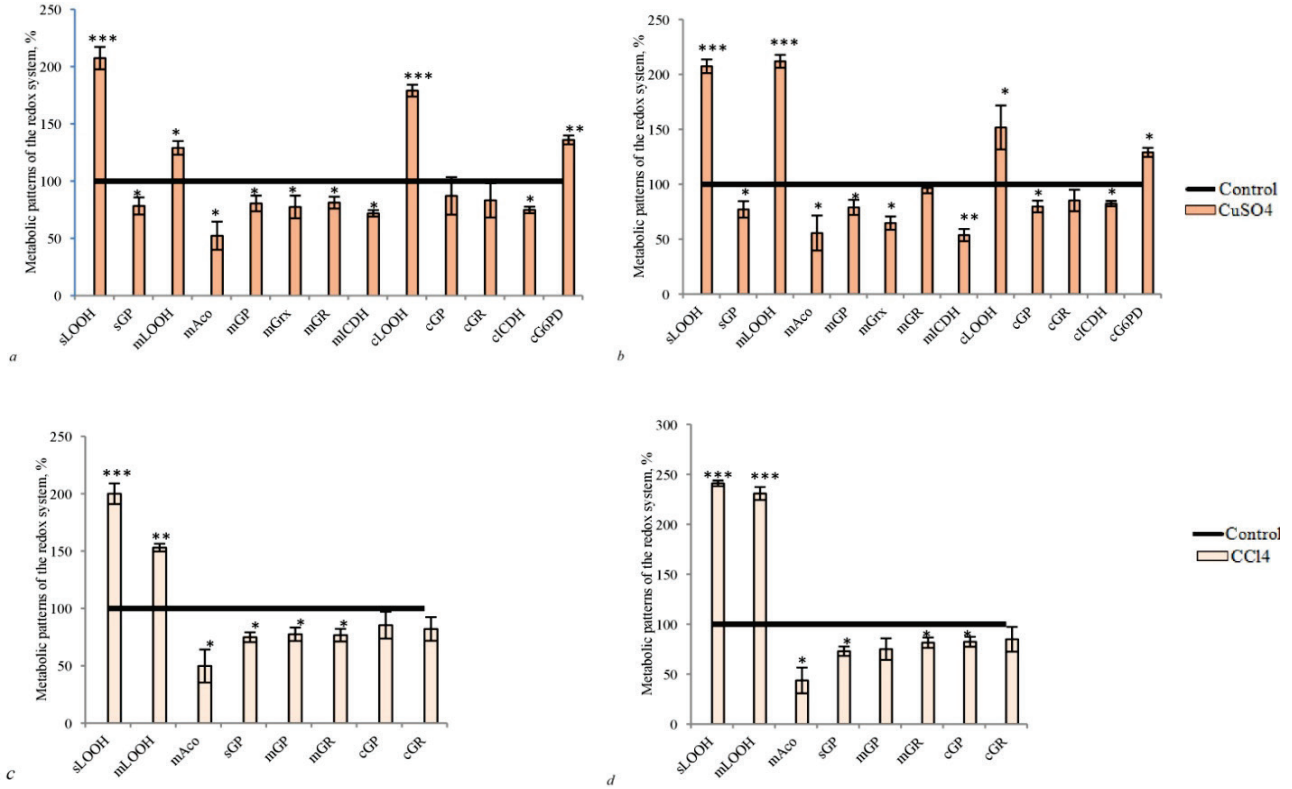

Fig. 6. The formation of new metabolic patterns of the redox system in young (*a*) and old (*b*) animals after multiple consecutive injections of copper sulfoxide in a dose of 1 mg/100 g body weight into such animals, and after multiple consecutive injections of tetrachloromethane in a dose of 0.1 mL of 50% oil solution per 100 g body weight into young (*c*) and old (*d*) animals: prooxidant content (LOOH-hydroperoxides in mitochondria (m LOOH), cytosol fraction (c LOOH) and blood serum (s LOOH), and aconitase activity in mitochondria mAco as well as activity of antioxidant enzymes (glutathione peroxidase (GPx), glutaredoxin (Gpx), glutathione reductase (GR), glucose-6-phosphate dehydrogenase (G6PDG), isocitrate dehydrogenase (ICDG), in mitochondria, cytosol and blood serum; all values of baseline levels (intact animals before the administration of hepatotoxic compounds were taken as 100%, i.e. induced changes were expressed as a percentage of baseline values, which were taken as 100%); n = 8, x ± SE; * – P < 0.05, ** P < 0.01, *** – P < 0.001 changes are statistically significant compared to control group of animals

These results indicate that the reactivity of the redox system in old animals against the background of the action of hepatotoxic compounds, regardless of their nature, is higher compared to that of young rats.

Discussion

The free-radical hypothesis of ageing contributed to the formation of one of the basic modern biomedical concepts – the concept of oxidative stress. Nowadays, oxidative stress is interpreted as one of the causes of the formation of most, if not all, pathologies and ageing of the organism (Araújo et al., 2016; Hajam et al., 2022). Due to its simplicity and elegance, this concept has become quite popular because: (1) changes in the redox status of the cell and the organism as a whole occur against the background of changes in both endogenous and a wide range of diverse exogenous factors, i.e, the redox system is extremely labile or "sensitive"; (2) theoretically, the products of free-radical reactions contribute to the disruption of the structure of lipids, proteins, and nucleic acids, and as a consequence, can be expected to disrupt their functions; (3) the commercialization of scientific research has contributed to the development of a wide range of tests to determine the products of free-radical reactions.

Along with this, with the accumulation of experimental data, and especially during the transition from *in vitro* to *in vivo* systems, it becomes necessary to supplement and even revise some positions of this concept. Perhaps the most convincing example of this is the work of Sies (1985), who was one of the first to formulate the concept of oxidative stress in 1985. According to this author: "Oxidative stress is a violation of the balance between pro-oxidants and antioxidants in the direction of a predominance of the former" (Sies, 1985, 1997). After 6 years, he made a "cautious" addition to this interpretation and proposed this clarification: "A disturbance of the balance between pro-oxidants and antioxidants toward a predominance of pro-oxidants may lead to disorders" (Sies, 1991), and in 2017, Sies clarified that "an imbalance between pro-oxidants and antioxidants, toward a predominance of pro-oxidants, results in impaired redox signaling and redox control and/or damage to molecules" (Sies et al., 2017). In this definition, Sies notes the role of oxidative stress in regulation and signaling, and most importantly, he points out that damage to molecules may not occur in the midst of the equilibrium shift toward pro-oxidants. On this basis, it remains unclear what factors determine the level and nature of potentially negative and unregulated (spontaneous) manifestations. Such evolution of Sies's views, as well as those of a large number of other researchers, is convincing evidence of the transition from mechanistic interpretations of the concept of oxidative stress to an understanding of the role of the redox system in the regulation of homeostasis and the ambiguity of the behavior of biological systems.

The results of the present work can be summarized in several statements. The initial characteristics of the studied indicators of the redox system in old animals differ from those of young animals, namely they are shifted towards antioxidants and not towards pro-oxidants, as one might expect.

Despite numerous studies of age-dependent features of the redox system, the available results are rather contradictory (Shields et al., 2021). Moreover, the physiological limits of oxidative stress indicators have not yet been established. This may be explained by the fact that the redox system "responds" not only to stressors, but also to physiological changes such as diet (Jakob & Reichmann, 2013), excessive exercise, and other physiological changes. In this context, it can be assumed that the redox potential in the cell and the organism as a whole is one of the most evolutionarily ancient and universal systems of metabolic regulation, and not only an indicator of the action of stress factors.

When considering possible mechanisms of homeostasis regulation at the level of the redox system, several significant aspects must be taken into account: (1) not only free radicals and their products, but also a wide arsenal of other compounds can act as oxidants in biological systems; (2) since the composition of redox components is different in different compart-

ments of the cell and probably in different tissues, these components form complex and completely unexplored hierarchical relationships at the level of the organism; (3) the increase in pro-oxidants is a signal for the induction of components of the antioxidant system, and first of all enzymes, violating the balance in the redox system, performs the function of signaling; (4) since enzymes are capable of performing not only one, but several different functions, i.e. they are usually polyfunctional, it can lead to the formation of new metabolic patterns, and as a result they can go into the self-sustaining mode, which can manifest as metabolic memory (Bozhkov et al., 2017).

Based on these provisions, the increase in activity of glutathione peroxidase in mitochondria and blood serum, as well as other antioxidant elements in old animals can be interpreted as an adaptive response of the organism to previous actions of various inducers. Such an increase in the number and activity of components of the antioxidant system can last for quite a long time in the case when such changes turn out to be "useful" for the newly formed homeostatic level.

As an example of differences in metabolic indices of young and old animals, along with antioxidant enzymes, we can cite data on the increased amount of such inducible proteins as metallothioneins (Bozhkov et al., 2021) and alcohol dehydrogenase in the liver (Bozhkov et al., 2024), which were elevated in old animals compared to young animals before the direct action of the corresponding inducers. These results and the data of the present work may indicate that adaptive changes may persist even after elimination of the inducing factor and/or the synthesis of these proteins may be induced by different (non-specific) inducers. And since old animals have inevitably experienced stress influences of different nature, including infectious influences, during a longer period of ontogenesis, they have formed different ratios in the redox system than young animals.

The second aspect of the present work is reduced to the fact that – multiple consecutive administrations to experimental animals of hepatotoxic compounds of different nature (copper sulfate and tetrachloromethane) shift the equilibrium in the redox system in the direction of prooxidants to the same extent, i.e. induce a "one-type" response at the level of the redox system. The fact that two different compounds, which differ in nature and properties, can cause similar quantitative and qualitative changes at the level of indicators of the redox system, indicates that the primary and probably non-specific response of the organism to various "perturbing " environmental factors is formed at the level of the redox system. Of course, quantitative changes in prooxidants and antioxidants depend on the dose, the nature of temporality of the metabolic system (Bozhkov et al., 2024) and the functional state of the organism at the moment of their influence, but this does not exclude the fact that the redox system can be considered as a universal evolutionary ancient system of metabolic regulation (Bozhkov et al., 2016).

Such "universalism" of the redox system may explain the fact that quite different pathological conditions are almost always associated with changes in the indices of the redox system, and changes in the feeding regime are accompanied by pronounced cyclic changes in the characterization of prooxidants and antioxidants (Bozhkov et al., 2021).

If this is indeed the case, then a fundamental question arises: "How and why does a non-specific response pass into a variety of nosological forms of manifestation?", i.e. acquire specificity.

An example of the legitimacy of such a question can be the results of studies on the effect of copper sulfoxide and tetrachloromethane not only on the indicators of the redox system, but also on the specificity of functional changes at the level of bone marrow and features of liver fibrosis formation (Ohiienko et al., 2019). Finding an answer to this extremely important and complex question requires specialized and in-depth studies.

We believe that the transition from "non-specificity" to "individual specificity" will depend at least on the functional features of the metabolic system of the organism at the moment of action of perturbing factors. Since it is methodologically impossible to determine and describe the functional state of the metabolic system at the moment of influence of exogenous factors, animals of different ages can be used as a model of different functional states of the organism. With this approach, it is possible to evaluate group characteristics rather than individual features of some metabolic indices, given that ageing is merely a temporal change in metabolism. If the above assumptions are correct, it can be expected that the response at the level of the redox system indicators to perturbing factors (copper sulfate and tetrachloromethane) in the group of young and old animals will differ. This was demonstrated in the present work.

The third position of the present work can be formulated as follows: "against the background of the action of hepatotoxic compounds, the redox system in old animals showed greater reactivity in comparison with young animals, which led to an "equalization" of the initially different indicators of the redox system in young and old animals".

Thus, if we take the studied indices of the redox system of young and old intact animals, i.e. their initial levels for 100%, then after three consecutive administrations of copper sulfoxide to these animals, a new configuration (pattern) of the redox system indices was formed in them, which were similar in young and old animals, in contrast to their initial levels (Fig. 2, 6a, 6b). Surprisingly similar patterns of the redox system were formed in young and old animals and after the action of tetrachloromethane on them (Fig. 6c, 6d).

Such "equalization" (Fig. 6) of the initially different quantitative characteristics of the redox system parameters (Fig. 2) in young and old animals indicates that the quantitative changes in prooxidants are not a spontaneous or uncontrolled process. It can be argued that the shift of equilibrium between prooxidants and antioxidants is a universal response of biological systems, which does not depend on the level of system organization to a variety of factors of both exogenous and endogenous nature. The uniform "changes" of redox system indicators as a primary non-specific response will be "transformed" into specific molecular-physiological manifestations, which will depend on individual age and temporal features of the biological system at the moment of formation of such signals.

Conclusions

Hepatotoxic compounds, which are of a different nature, induce the same type of response at the level of the redox system in both the liver and the organism as a whole. This response can be considered as a primary non-specific adaptive response of the organism to stress factors. The initial differences in the indicators of the redox system (in old animals the balance is shifted towards pro-oxidants) against the background of the effects of hepatotoxins will be completely eliminated, i.e. indicators of the redox system in young and old animals "even out," which may indicate the presence of a system for controlling the level of prooxidants. Further studies of the role of the redox system in the formation of physiological manifestations will contribute to the understanding of the transition of adaptive physiological processes to pathophysiological ones.

The authors declare no conflict of interests.

We would like to thank Dr. Bettina Kränzlin Präsidentin GV-SOLAS for advice and assistance in content and conducting experiments on laboratory animals.

References

Abdelazim, A. M., & Abomughaid, M. M. (2024). Oxidative stress: An overview of past research and future insights. All Life, 17(1), 2316092.

Alkadi, H. (2020). A review on free radicals and antioxidants. Infectious Disorders Drug Targets, 20(1), 16–26.

Asakawa, T., & Matsushita, S. (1980). Coloring conditions of thiobarbituric acid test for detecting lipid hydroperoxides. Lipids, 15(3), 137–140.

Averill-Bates, D. (2024). Reactive oxygen species and cell signaling. Review. Biochimica et Biophysica Acta. Molecular Cell Research, 1871(2), 119573.

Barja, G. (2014). The mitochondrial free radical theory of aging. In: Jeon, K. W. (Ed.). Progress in molecular biology and translational science. Elsevier, Amsterdam. Vol. 127. Pp. 1–27.

Bauman, D. E., Brown, R. E., & Davis, C. L. (1970). Pathways of fatty acid synthesis and reducing equivalent generation in mammary gland of rat, sow, and cow. Archives of Biochemistry and Biophysics, 140(1), 237–244.

Beckman, K. B., & Ames, B. N. (1998). The free radical theory of aging matures. Physiological Reviews, 78(2), 547–581.

Beinert, H., Kennedy, M. C., & Stout, C. D. (1996). Aconitase as ironminus signsulfur protein, enzyme, and iron-regulatory protein. Chemical Reviews, 96(7), 2335–2374.

Bozhkov, A. I., Akzhyhitov, R. A., Bilovetska, S. G., Ivanov, E. G., Dobrianska, N. I., & Bondar, A. Y. (2024). The effect of retinol acetate on liver fibrosis de-

pends on the temporal features of the development of pathology. Journal of Clinical and Experimental Hepatology, 14(3), 101338.
Bozhkov, A. I., Bozhkov, A. A., Ponomarenko, I. E., Kurguzova, N. I., Akzhyhitov, R. A., Goltvyanskii, A. V., Klimova, E. M., & Shapovalov, S. O. (2021). Elimination of the toxic effect of copper sulfate is accompanied by the normalization of liver function in fibrosis. Regulatory Mechanisms in Biosystems, 12(4), 655–663.
Bozhkov, A. I., Dobrianska, N. I., Akzhyhitov, R. A., Novikova, A. V., Haiovyi, S. S., Pankratova, A. O., Ivanova, O. O., Onipko, I. R., & Komburlei, Y. O. (2024). Young and old animals "use" different strategies for adapting to the chronic effects of ethanol. Translational Medicine of Aging, 8, 1–11.
Bozhkov, A. I., Klimova, E. M., Nikitchenko, Y. V., Davydov, V. V., Zvyagintseva, O. V., Kurguzova, N. I., Sidorov, V. I., & Naglov, A. V. (2014). Stem cells take part in regulation of prooxidant activity and immunity at liver fibrosis. American Journal of Biomedical and Life Sciences, 2(6), 5–12.
Bozhkov, A. I., Klimova, O. M., Nikitchenko, Y. V., Kurguzova, N. I., Linkevych, O. S., Lebid, K. M., Protsenko, O. S., Remneva, N. A., Al-Bahadly, A. M. M., & Al-Begai, M. A. Y. (2017). Ontogenetic approach to the study of mechanisms of copper-induced liver fibrosis. Advances in Aging Research, 6(3), 39–55.
Bozhkov, A. I., Nikitchenko, Y. V., Klimova, E. M., Linkevych, O. S., Lebid, K. M., Al-Bahadli, A. M. M., & Alsardia, M. M. A. (2017). Young and old rats have different strategies of metabolic adaptation to Cu-induced liver fibrosis. Advances in Gerontology, 7(1), 41–50.
Bozhkov, A. I., Nikitchenko, Y. V., Lebed', K. N., Linkevych, O. S., Kurguzova, N. I., Klimova, O. M., Begai, M. A. Y. A., Al-Bahadly, A. M. M., & Alsardia, M. M. A. (2016). The cyclic feeding regime induces decaying age-dependent oxidative stress and regulates the cell chain of the immunity. Advances in Aging Research, 5(6), 151–165.
Bozhkov, A. I., Sidorov, V. I., Alboqai, O. K., Akzhyhitov, R. A., Kurguzova, N. I., Malyshev, A. B., Albegai, M. A. Y., & Gromovoi, T. Y. (2021). The role of metallothioneins in the formation of hierarchical mechanisms of resistance to toxic compounds in young and old animals on the example of copper sulfate. Translational Medicine of Aging, 5, 62–74.
Brygadyrenko, V., & Ivanyshyn, V. (2015). Changes in the body mass of *Megaphyllum kievense* (Diplopoda, Julidae) and the granulometric composition of leaf litter subject to different concentrations of copper. Journal of Forest Science, 61(9), 369–376.
Dröge, W. (2002). Free radicals in the physiological control of cell function. Physiological Reviews, 82(1), 47–95.
Gallogly, M. M., Shelton, M. D., Qanungo, S., Pai, H. V., Starke, D. W., Hoppel, C. L., Lesnefsky, E. J., & Mieyal, J. J. (2010). Glutaredoxin regulates apoptosis in cardiomyocytes via NFkappaB targets Bcl-2 and Bcl-xL: Implications for cardiac aging. Antioxidants and Redox Signaling, 12(12), 1339–1353.
Gardner, P. R., Nguyen, D. D., & White, C. W. (1994). Aconitase is a sensitive and critical target of oxygen poisoning in cultured mammalian cells and in rat lungs. Proceedings of the National Academy of Sciences, 91(25), 12248.
Hajam, Y. A., Rani, R., Ganie, S. Y., Sheikh, T. A., Javaid, D., Qadri, S. S., Pramodh, S., Alsulimani, A., Alkhanani, M. F., Harakeh, S., Hussain, A., Haque, S., & Reshi, M. S. (2022). Oxidative stress in human pathology and aging: Molecular mechanisms and perspectives. Cells, 11(3), 552.
Halliwell, B., & Gutteridge, J. M. C. (2015). Free radicals in biology and medicine. Oxford University Press, Oxford.
Harman, D. (1956). Aging: A theory based on free radical and radiation chemistry. Journal of Gerontology, 11(3), 298–300.
Harman, D. (2006). Free radical theory of aging: An update: Increasing the functional life span. Annals of the New York Academy of Sciences, 1067, 10–21.
Ivanova, D. G., & Yankova, T. M. (2013). The free radical theory of aging in search of a strategy for increasing life span. Folia Medica, 55(1), 33–41.
Jomova, K., Raptova, R., Alomar, S. Y., Alwasel, S. H., Nepovimova, E., Kuca, K., & Valko, M. (2023). Reactive oxygen species, toxicity, oxidative stress, and antioxidants: Chronic diseases and aging. Archives of Toxicology, 97(10), 2499–2574.
Lushchak, V. I., & Storey, K. B. (2021). Oxidative stress concept updated: Definitions, classifications, and regulatory pathways implicated. Experimental and Clinical Sciences Journal, 20, 956–967.
Miller, G. (1959). Protein determination of large numbers of samples. Analytical Chemistry, 31(5), 964.
Ohiienko, S. L., Bondar, A. Y., Ivanov, E. G., & Bozhkov, A. I. (2019). Liver fibrosis has a different effect on the "lifespan" of lymphocytes and neutrophils in the *in vitro* system isolated from the bone marrow of young and old rats. Gerontology and Geriatrics, 4(1), 36–40.
Ohkawa, H., Ohishi, N., & Yagi, K. (1979). Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Analytical Biochemistry, 95(2), 351–358.
Paglia, D. E., & Valentine, W. N. (1967). Studies on the quantitative and qualitative characterization of erythrocyte glutathione peroxidase. The Journal of Laboratory and Clinical Medicine, 70(1), 158–169.
Raghavachari, N., & Lou, M. F. (1996). Evidence for the presence of thioltransferase in the lens. Experimental Eye Research, 63(4), 433–441.
Shields, H. J., Traa, A., & Van Raamsdonk, J. M. (2021). Beneficial and detrimental effects of reactive oxygen species on lifespan: A comprehensive review of comparative and experimental studies. Frontiers in Cell and Developmental Biology, 9, 628157.
Shostya, A., & Siabro, A. (2022). Effects of copper citrate on physiological-biochemical parameters of ejaculate of sire boars. Regulatory Mechanisms in Biosystems, 13(2), 121–129.
Sies, H. (1985). Oxidative stress: Introductory remarks. In: Sies, H. (Ed.). Oxidative stress. Academic Press, London. Pp. 1–8.
Sies, H. (1991). Oxidative stress: From basic research to clinical application. The American Journal of Medicine, 91(3), 31–38.
Sies, H. (2020). Oxidative stress: Concept and some practical aspects. Antioxidants, 9(9), 852.
Slauch, J. M. (2011). How does the oxidative burst of macrophages kill bacteria? Still an open question. Molecular Microbiology, 80(3), 580–583.
Varghese, S., Tang, Y., & ImLay, J. A. (2003). Contrasting sensitivities of *Escherichia coli* aconitases A and B to oxidation and iron depletion. Journal of Bacteriology, 185(1), 221–230.
Yoshikawa, T., & You, F. (2024). Oxidative stress and bio-regulation. International Journal of Molecular Sciences, 25(6), 3360.
Zaheer, N., Tewari, K. K., & Krishnan, P. S. (1967). Mitochondrial forms of glucose 6-phosphate dehydrogenase and 6-phosphogluconic acid dehydrogenase in rat liver. Archives of Biochemistry and Biophysics, 120(1), 22–34.