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Antioxidant enzymes activity and lipid peroxidation processes in the blood of White Stork *Ciconia ciconia* chicks from W. Poland

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ABSTRACT: The aim of this study is to investigate the activity of most important antioxidant enzymes of blood of White Stork, altricial bird, during postnatal development. We also studied the degree of difference between the two environments: suburbs areas of Zielona Góra and polluted villages of Głogów Manufacture (SW Poland). We have taken under a consideration the content of thiobarbituric acid-reactive substances TBARS, superoxide dismutase, catalase, and ceruloplasmine diurnal and nocturnal activity, in polluted and suburban environments. We also analyzed the processes of lipid peroxidation in the blood of young storks and their age dependencies. Researches are leading on nestlings White Stork having jacks in villages of Czarna and Czarnowo in a village fence Zielona Góra. In total 11 nestlings of White Stork from 5 jacks have been surveyed. The age of birds changed from 19 up to 41 days from an output from an egg. The examination was started at 10 and ended at 12 am. At night blood simples were taken over in 22–24 pm.
Enzymatic activity, which has been studied in this paper, occurs to be various with age, and depends on the type of environment and the period of day and night. These changes are not significant, but they simultaneously have significant meaning of physiological point and enzymatic economy of individuals. The content of thiobarbituric acid-reactive substances TBARS ($nM \times l^{-1}$) in blood of White Stork chicks in suburbs tend to decreases during postnatal development, both during a day and at night. This phenomenon has not been so regular during a day in polluted areas.

The activity of superoxide dismutase increases during a day in suburbs, but these relationships seems to be significantly different. Similar relationships concerning the night have a reversible course. In polluted areas these dependences have also a decreasing tendency with age during a day. Catalase activity in blood of young White Storks from suburbs near Zielona Góra have irregular changes with age, but decreasing tendency during a day and at night. Its activity during a day is rather independent of age in polluted areas and slowly decreases. Ceruloplasmine activity in blood of White Stork chicks decreases with age in suburban areas during a day and night. These dependencies shows insignificant changes during a day in polluted environments but tends to increase.
It must be emphasize the statistically significant interactions between enzymes we investigated in this paper. Ecophysiological significance from physiological point of view have increasing dependencies of various enzymes interactions, especially those among TBARS, catalase and ceruloplasmine. Their mutual rates modifies an enzymatic activity of growing cells (cellular phone) and activates the lipid peroxidation processes. We can thus conclude about various environmental factors deals with heavy metal accumulation in the neighbouring environment (Głogów Copper Manufacture) which caused changes of biological peroxidation processes in blood cells of young storks. These can reflect by the production and utilization of reactive oxygen products in blood of birds.

KEY WORDS: *Ciconia ciconia*, chicks, thiobarbituric acid-reactive substances TBARS, superoxide dismutase, catalase, ceruloplasmine, lipid peroxidation, blood, age dependencies, polluted environments, W Poland

Introduction

White Stork chicks, living along a pollution gradient might differ in their compensatory capacity for effective elimination of pro-oxidative toxicity of metals and many organic pollutants. Metals are known to influence the oxidative status of organisms, and antioxidant enzymes have been often proposed as biomarkers of effect. They are also responsible for enhanced production of reactive oxygen species and for inhibition of antioxidant activity (Valko et al. 2005).

Organisms inhabiting chronically polluted environments regulate additional expenditure of energy for supplementary detoxification (Wilczek et al. 2004). Such chronic exposure extending over multiple generation may led to dramatic shifts as a result of genetic drift and mutations (Belfore, Anderson 1998). These changers may takes place in many vertebrate and invertebrate populations in trophic circuits of a feed from areas from heavy metal pollution. This means that young White Stork chicks accordingly, living along a pollution gradient might differ in their compensatory capacity for effective elimination of pro-oxidative toxicity of metals and many organic pollutants.

Metals are known to influence the oxidative status of organisms, and antioxidant enzymes have been often proposed as biomarkers of effect. They are also responsible for enhanced production of reactive oxygen species and for inhibition of antioxidant activity. Lead and cadmium affect the structure of lipids and enhance lipid peroxidation (Viarengo 1989, Łaszczyca 2000). Copper and cadmium act both directly and indirectly, causing and increase in cellular iron level, inhibition of antioxidant activity, depletion of cellular glutathione or inhibition of glutathione-related enzymes (Kang 1997, Lagadic 1999). Various studies have confirmed that

metals activate signalling pathways and the carcinogenic effect of metals has been related to activation of mainly redox-sensitive transcription factors, involving NF-kappaB, AP-1 and p53 (Cruz-Rodrigues, Chu 2002, Rudolf et al. 2005, Valko et al. 2005).

Above ecophysiological regularities have their own source in various relationships between physio-biochemical processes of adaptations at cellular level (pro--antioxidant balance, lipid peroxidation processes intensity) and the possibilities of population reactions White Stork chicks upon environmental stress.

The aim of this study is to investigate the activity of most important antioxidant enzymes of blood of White Stork, altricial bird, during postnatal development. We have taken under a consideration the content of thiobarbituric acid-reactive substances TBARS, superoxide dismutase, catalase, and ceruloplasmine diurnal and nocturnal activity, in polluted (Głogów Manufacture) and suburban environments (Zielona Góra). We also analyzed the processes of pro-antioxidant balance in the blood of young storks and their age dependencies and type of the environment.

Material and methods

Study area

Blood samples for analyses were collected from young storks developing in relatively pure and suburban environment (Czarna and Czarnowo, villages distanced of about 20 km from Zielona Góra (100. thousand inhabitants, SW Poland) and in small distance from Głogów, where copper manufacture is placed.

In total of 27 of White Stork chicks from 11 jacks which have been numbered accordingly from 1 up to 27 have been surveyed. The age of birds changed from 19 up to 54 days from an output from an egg. In the case of samples from Zielona Góra they were collected at distance of several kilometers from a city. For elimination of diurnal rhythm changes all examinations were started at 10 and ended at 12 am.

Polluted area, Głogów Copper Manufacture, produced copper and lead from lead fields. "Głogów" plant copper leads an active proecological activity. Green fields consists about 50% of protective areas of this manufacture complex. The forests presents on about 900 ha, i.e. 32% of this area. Acid soils are subjected by calcification. One of numerous proecological ventures of the manufacture was desulphuring installation and modernize of sulphur acid manufacture. These innovations have contributed towards rapid decrease of sulphur dioxide. Now the process of modernization of lead department is continued. It is the last significant proecological investment in plant. Main inspector of environmental protection has crossed out hawthorns from list of oppressive plant (bet) for environment conditionally.

Enzyme assays and MDA content

Erythrocyte haemolysate was used for superoxide dismutase (SOD) activity. The SOD activity was estimated from the extent of inhibition of superoxide (O_2^-) de-

pendent quercetine transformation, according to the method of Kostiuk et al. (1990). The catalase (CAT) activity was determined in the plasma blood by the amount of H_2O_2 consumed x minute^{-1} with molibdate ammonium (Koroliuk et al. 1988). The ceruloplasmin (CP) activity was estimated using p-phenilenodiamine method of Kolb and Kamyshnikov (1982).

Samples of investigated wing venous blood take for investigation a level of processes oxidative stress by measuring malondialdehyde (MDA), the last product of lipid breakdown caused by oxidative stress. Lipid peroxidation process will be measured by the thiobarbituric acid-reactive substances (TBARS) method (Timirbulatov & Selezniev 1981).

Statistical analysis

The values are expressed as mean ± S.D. for the animals in light and dark time of day separately. Significant differences among the means were measured using a multiple range test at min. $P < 0.05$. Data not having a normal distribution were log-transformed. Correlations were calculated between enzymes activity in the blood of chicks and their age (linear regression). Significance of these correlations (regression coefficients) was examined using ANOVA for correlation test. Relationships between areas and diurnal time were examined using correlation analysis, and differences in correlation coefficients between two habitats were tested by using the normal Z-statistic (Blalock 1977).

Results

Antioxidant enzymes (catalase – CAT, superoxidedismutase – SOD, glutathione peroxidase – GPx, glutathione reductase – GR, reduced, and oxidized glutathione ratio) play a vital role in protecting cellular damage from harmful effects of reactive oxygen species ROS. The level of changes which have come in the environment can be tested by means of definition of changes in system of production of active forms of oxygen and antioxidant enzymes activity.

Enzymatic activity, which has been studied in this paper, occurs to be various with age, and depends on the type of environment and the period of day and night. These changes are not significant, but they simultaneously have significant meaning of physiological point and enzymatic economy of individuals. The content of thiobarbituric acid-reactive substances TBARS ($nM \times l^{-1}$) in blood of White Stork chicks in suburban areas near Zielona Góra tend to decreases in postnatal development, both during a day ($y = –0.056x + 0.7298$) and at night ($y = –0.0071x + 0.5576$), as show Table 1. This phenomenon has not been so regular during a day in polluted areas ($y = –0.0019x + 0.7692$), as Table 1 illustrates.

The activity of superoxide dismutase ($U \times min \times l^{-1}$) increases during a day in suburban environments ($y = 0.2989x + 78.2440$) as show Table 2, but these relationships seems to be significantly different. Similar relationships concerning the night have a reversible course ($y = –2.700x + 153.450$); Table 2. In polluted areas these dependences have also a decreasing tendency with age during a day ($y = –0.3487x + 71.0870$), as Table 2 illustrates.

Table 1. Thiobarbituric acid-reactive substances TBARS (nM/l) content in blood of White Stork *Ciconia ciconia* chicks in not polluted (control) and polluted areas near Zielona Góra (Poland)

Suburbs				Polluted		
age (days)	10–12 am	age (days)	10–12 am	age (days)	day of life	day
Czarna	19	2.81	1.83	Kotla I	22	2.35
Czarnowo	26	3.35	1.93	Skidnów	22	2.93
Czarnowo	27	2.21	1.50	Skidnów	24	2.03
Czarnowo	27	2.13	1.71	Kotla I	25	2.21
Czarnowo	31	2.73	1.86	Kotla I	27	1.93
Czarnowo	34	2.86	1.61	Skidnów	27	2.79
Czarnowo	34	2.01	1.38	Chociemyśl	32	3.14
Zoo	±35	1.66	1.01	Moszowice	34	2.62
Czarna	41	3.31	1.63	Chociemyśl	34	2.99
Czarna	41	3.28	1.28	Pękoszów	34	2.41
Czarna	41	2.89	1.51	Moszowice	36	3.47
				Pękoszów	36	2.19
				Moszowice	40	1.75
				Grodziec Mały	49	1.74
				Grodziec Mały	51	1.63
				Grodziec Mały	54	1.91

Table 2. Superoxide dismutase (U/min/ml) activity in blood of White Stork *Ciconia ciconia* chicks in not polluted (control) and polluted areas near Zielona Góra (Poland)

Suburbs				Polluted		
age (days)	10–12 am	age (days)	10–12 am	age (days)	day of life	day
Czarna	19	301.57	444.13	Kotla I	22	161.24
Czarnowo	26	365.88	608.95	Skidnów	22	213.54
Czarnowo	27	354.30	611.29	Skidnów	24	505.41
Czarnowo	27	393.64	102.6	Kotla I	25	138.23
Czarnowo	31	461.17	294.15	Skidnów	27	209.17
Czarnowo	34	392.61	265.11	Kotla I	27	101.98
Czarnowo	34	485.50	311.17	Chociemyśl	32	169.46
Zoo	± 35	235.92	172.74	Moszowice	34	189.87
Czarna	41	726.27	295.01	Chociemyśl	34	85.84
Czarna	41	269.74	112.40	Pękoszów	34	248.38
Czarna	41	778.39	191.94	Moszowice	36	135.33
				Pękoszów	36	338.48
				Moszowice	40	290.56
				Grodziec Mały	49	109.33
				Grodziec Mały	51	160.96
				Grodziec Mały	54	151.13

Table 3. Catalase (mM/min/l) activity in blood of White Stork *Ciconia ciconia* chicks in not polluted (control) and polluted areas near Zielona Góra (Poland)

Not polluted				Polluted		
location	day of life	day	night	location	day of life	day
Czarna	19	10.12	4.98	Kotla I	22	12.06
Czarnowo	26	19.02	26.1	Skidnów	22	10.08
Czarnowo	27	20.01	29.34	Skidnów	24	13.52
Czarnowo	27	24.4	14.58	Kotla I	25	10.08
Czarnowo	31	5.84	6.66	Kotla I	27	15.88
Czarnowo	34	9.31	32.76	Skidnów	27	15.66
Czarnowo	34	8.75	7.38	Chociemyśl	32	8.04
Zoo	±35	10.12	21.96	Moszowice	34	12.34
Czarna	41	11.34	10.56	Chociemyśl	34	8.42
Czarna	41	8.68	21.42	Pękoszów	34	14.17
Czarna	41	24.48	5.58	Moszowice	36	8.06
				Pękoszów	36	11.34
				Moszowice	40	11.52
				Grodziec Mały	49	9.36
				Grodziec Mały	51	7.38
				Grodziec Mały	54	9.18

Table 4. Ceruloplasmine (U/ml) activity in blood of White Stork *Ciconia ciconia* chicks in not polluted (control) and polluted areas near Zielona Góra (Poland)

Not polluted				Polluted		
location	day of life	day	night	location	day of life	day
Czarna	19	3.45	3.15	Kotla I	22	6.91
Czarnowo	26	7.51	3.61	Skidnów	22	5.33
Czarnowo	27	6.72	7.01	Skidnów	24	7.61
Czarnowo	27	6.31	4.22	Kotla I	25	8.12
Czarnowo	31	7.02	3.23	Kotla I	27	4.28
Czarnowo	34	5.61	5.03	Skidnów	27	7.12
Czarnowo	34	6.14	4.95	Chociemyśl	32	5.42
Zoo	±35	5.28	4.63	Moszowice	34	6.21
Czarna	41	5.95	4.55	Chociemyśl	34	8.31
Czarna	41	5.25	5.16	Pękoszów	34	10.15
Czarna	41	8.38	2.62	Moszowice	36	–
				Pękoszów	36	10.12
				Moszowice	40	–
				Grodziec Mały	49	7.51
				Grodziec Mały	51	7.08
				Grodziec Mały	54	9.13

Table 5. Correlation coefficients (r) of enzymes activity interactions in blood of White Stork *Ciconia ciconia* chicks (p < ,05000, n=54) in control (not polluted NP) and polluted (P); environments near Zielona Góra (SW Poland)

	Age	T – NP d	T – NP n	T – P d	SD – NP d	SD – NP n	SD – P d	Ca – NP d	Ca – NP n	Ca – P d	Ce – NP d	Ce – NP n	Ce – P d
Age	1.0000	–.0448	–.0969	–.0148	.0138	–.1613	–.0284	–.0762	–.0599	–.0293	–.0369	–.0538	.0675
	p= –	p=.748	p=.486	p=.915	p=.921	p=.244	p=.838	p=.584	p=.667	P=.833	p=.791	p=.699	p=.628
T – NP d	–.0448	1.0000	.9788	–.3087	.9227	.8502	–.2730	.8570	.8117	–.3077	.9557	.9136	–.2819
	p=.748	p= –	p=0.00	p=.023	p=0.00	p=.000	p=.046	p=.000	p=.000	P=.024	p=0.00	p=0.00	p=.039
T – NP n	–.0969	.9788	1.0000	–.3115	.9168	.8829	–.2754	.8852	.7999	–.3105	.9655	.9191	–.2845
	p=.486	p=0.00	p= –	p=.022	0	0	p=.044	0	0	p=.022	0	0	p=.037
T – P d	–.0148	–.3087	–.3115	1.0000	–.2910	–.2702	.7970	–.2806	–.2641	.9168	–.3090	–.3037	.8230
	p=.915	p=.023	p=.022	p= –	p=.033	p=.048	p=.000	p=.040	p=.054	p=0.00	p=.023	p=.026	p=.000
SD – NP d	.0138	.9227	.9168	–.2910	1.0000	.7581	–.2573	.8667	.6568	–.2900	.9449	.8403	–.2657
	p=.921	0	0	p=.033	p= –	0	p=.060	0	0	p=.033	0	p=.000	p=.052
SD – NP n	–.1613	.8502	.8829	–.2702	.7581	1.0000	–.2389	.7825	.7849	–.2694	.8431	.8474	–.2468
	p=.244	p=.000	p=.000	p=.048	p=.000	p= –	p=.082	0	0	p=.049	p=.000	0	p=.072
SD – P d	–.0284	–.2730	–.2754	.7970	–.2573	–.2389	1.0000	–.2481	–.2335	.8717	–.2732	–.2685	.7799
	p=.838	p=.046	p=.044	p=.000	p=.060	p=.082	p= –	p=.070	p=.089	p=.000	p=.046	p=.050	0
Ca – NP d	–.0762	.8570	.8852	–.2806	.8667	.7825	–.2481	1.0000	.7588	–.2797	.9214	.8397	–.2563
	p=.584	0	0	p=.040	0	0	p=.070	p= –	0	p=.041	0	0	p=.061
Ca – NP n	–.0599	.8117	.7999	–.2641	.6568	.7849	–.2335	.7588	1.0000	–.2632	.8141	.9010	–.2412
	p=.667	0	0	p=.054	0	0	p=.089	0	p= –	p=.054	0	0	p=.079
Ca – P d	–.0293	–.3077	–.3105	.9168	–.2900	–.2694	.8717	–.2797	–.2632	1.0000	–.3080	–.3027	.8623
	p=.833	p=.024	0	p=0.00	0	p=.049	0	p=.041	0	p= –	0	0	0
Ce – NP d	–.0369	.9557	.9655	–.3090	.9449	.8431	–.2732	.9214	.8141	–.3080	1.0000	.9246	–.2822
	p=.791	0	p=0.00	0	0	0	0	p=0.00	0	p=.023	p= –	0	0
Ce – NP n	–.0538	.9136	.9191	–.3037	.8403	.8474	–.2685	.8397	.9010	–.3027	.9246	1.0000	–.2773
	p=.699	0	0	p=.026	0	0	p=.050	0	p=0.00	p=.026	0	p= –	P=.042
Ce – P d	.0675	–.2819	–.2845	.8230	–.2657	–.2468	.7799	–.2563	–.2412	.8623	–.2822	–.2773	1.0000
	p=.628	p=.039	p=.037	0	p=.052	p=.072	0	p=.061	p=.079	0	p=.039	p=.042	P= –

Catalase activity (mM × min × l^{-1}) in blood of young White Storks from Zielona Góra area have irregular changes with age, but decreasing tendency during a day (y = –0.0548x + 4.6485) and at night (y = –0.0546x + 5.1826). Its activity during a day is rather independent of age in polluted areas and slowly decreases (y = –0.0176x + 3.8682); Table 3.

Ceruloplasmine activity in blood of White Stork chicks decreases with age in suburban areas during a day (y = –0.0107x + 1.6102) and night (y = –0.0113x + 1.2704), as Table 4 presents. These dependencies shows statistically insignificant changes during a day in polluted environments but tends to increase (y = 0.0258x + 1.0501); Table 4.

It must be emphasize statistically significant interactions between enzymes we investigated in this paper (Kurhalyuk et al. in prep.). Thus particular significance from physiological point of view have increasing dependencies of various enzymes interactions, especially those among TBARS, catalase and ceruloplasmine (Table 5). Simultaneously, as Table 5 indicates, there are not statistically significant differences between both environments against TBARS content and the same concerning catalase (CAT) and ceruloplasmine (CP). The only significant differences occurs among both environments against SOD content in blood of White Stork chicks.

As Table 5 shows, the results of our investigations indicates that most of investigated enzymes participate in the course of statistically significant interactions, in both environments and diurnal period. However, it can be observed the almost lack of correlations among superoxide dismutase in both environments during a day and night. The same we can noted in the course of catalase and ceruloplasmine. On the other hand, interactions with TBARS products and other enzymes are always significant (Table 5).

Also, interactions between enzymes activity in blood and age of White Stork chicks principally not occurs. It shows, that environmental pollution does not have any impact on the interaction relationships between enzymes activity and age in blood of chicks (see Table 5).

We can thus conclude about various environmental factors deals with heavy metal accumulation in the neighbouring environment (Głogów Copper Manufacture) which caused changes of biological peroxidation processes in blood cells of young storks. These can reflect by the production and utilization of reactive oxygen products in blood of birds.

Discussion

The results of our investigations suggest that investigated enzymes participate in the course of statistically significant interactions, in both environments and the diurnal period (see Table 5). Simultaneously, interactions between pro-antioxidant balance in blood and age of White Stork chicks principally not occurs. Thus environmental pollution does probably not have any impact on the interaction relationships between particular enzymes activity in blood and age of chicks.

As so far stated, peroxidation of lipids or oxygen free radical generation in general is a physiological process important for cell metabolism, division and differenti-

ation and also for the biosynthesis of hormones and prostaglandins. On the other hand, free radicals generated through these processes are effectively scavenged by antioxidant defense system. Uncontrolled lipid oxidation caused by disturbances of that system may play a crucial role in some important bird diseases and toxicoses. The first route of lipid peroxide loading of the organism is via the feed, such as through oxidized lipids. Oxidized fatty acids are absorbed from the intestine mainly in the form of unsaturated ketocompounds and initiate lipid peroxidation in tissues (Łaszczyca 2000, Pinchuk & Lichtenberg, 2002, Mattson 2004).

Toxic heavy metals act antagonistically with physiological elements. The impact of anthropogenic activity upon the structure and functioning of ecological systems has been noted (Allen et al. 1984, Simmons et al. 1989, Masoud et al. 2003, Wilczek 2005). These studies indicate on the real impendence of biocenoses or whole ecosystems. It must be emphasized on the significance of employment of living organisms as bioindicators of environmental pollution (Marczak & Biedroń 1976, 1978, Minoranskij et al. 1990, Wojciechowski et al. 1991), because of the definite reactions (quality and quantity changes, physiological and domination structure changes); Eason, O'Halloran (2002). However, the typical symptom of structural and functional impoverished of fauna caused by environmental irreversible changes has been observed not before exceeding of definite threshold of environmental transformation (Zodl & Wittmann 2003). Moreover, not any investigations considered these problems as the toxins flow through the food chain including plants, phytophags, entomophags, and higher consumers (e.g. birds).

White Stork population at Dehesa de Abajo (Spain) is largely resident, and therefore, pollutants present in tissues and blood are mainly due to local exposure sources (Meharg et al. 2002). The White Stork chicks were still being exposed to accident derived toxicants over a year after the spill. Chicks born one year after the accident have lead isotope rations identical to the contaminated sludge, and distinct from the background park signature. This lead could have been transferred from female to an egg, and/or could have been derived from food collection in the contaminated zones and fed to the chicks. The sludge contained the know carcinogenesis, the aromatic amines but levels of this amines have not been reported in bird tissues following the accident. Data also shows that this population has very high levels of bill and leg deformities, corroborating the molecular analysis of genotoxic effects. It can be concluded from data of Smith et al. (2002) that temporal exposure to the different toxic metals through diet can be extrapolated for the young growing storks. It confirm according to results from biochemical, histopathological studies and skeletal deformations.

It belongs to underline, that the kinetic profile of peroxidation (Pinchuk & Lichtenberg 2002) is characterized by three major parameters: the lag preceding rapid oxidation, the maximal rate of oxidation (V_{max}) and the maximal accumulation of oxidation products (OD_{max}). Addition of antioxidants alters this pattern, affecting the kinetic parameters of oxidation. In particular, antioxidants may prolong the lag and/or decrease the V_{max} and/or decrease the OD_{max}. Such specific variation of the set of kinetic parameters may provide important information on the mechanism of the inhibitory action of a given antioxidant (scavenging free radicals,

metal-binding or other mechanisms). This may explain some of important changes and interactions between enzymes activity during nesting period of studied storks in this paper.

It also belongs to take under a consideration, that all organisms have evolved mechanisms to cope with a variety of stress situations. It results from our work also. One type of stress response is triggered by heavy metals, such as zinc, copper and cadmium (for convenience, the terms zinc, copper and cadmium are also used here to denote Zn^{2+}, Cu^{2+} and Cd^{2+}, respectively). On the other hand, necessity of carrying out of given researches caused by that external factors such as heavy metals, radiation, toxins, can lead to increased free radicals and other reactive oxygen species (ROS). It has been demonstrated that climatic stressors such as salinity, acidification, heavy metals, pesticides, modify population reactions upon environmental stress. Although low levels of ROS are essential in many biochemical processes, accumulations of ROS may damage biological macromolecules, that is, lipids, proteins, carbohydrates and DNA (Mates et al. 1999). It is important to note, that oxidative damage may be minimized by antioxidant defence mechanisms that protect cell against cellular oxidant and repair systems that prevent the accumulation of oxidatively damaged molecules (Kang 1997, Pinchuk & Lichtenberg 2002, Mattson 2004).

Thus cellular oxidative stress is due to the production of ROS, on the one hand, and weaknesses of antioxidative defence, on the other (Simmons et al. 1989). This is particularly true for cells with an active metabolism such as neurons and muscle cells, but it is also relevant for all other cell types. Hydrogen peroxide is an important member of ROS and is generated predominantly by mitochondria. In combination with reduced trace metals such as iron or copper, hydrogen peroxide is transformed into highly reactive hydroxyl radical which causes damage to virtually all macromolecules. Oxidation of nucleic acids results in mutations while protein denaturation leads to enzyme defects and impairment of cytoskeleton (Gosslau & Rensing 2002).

Lipid peroxidation in cell membranes is strongly involved in the perturbation of ion homeostasis, however. Because this cell damage ultimatively causes cell death, oxidative stress initiates several diseases. Mitochondria play a major role in this processes because they are the main source of ROS. Several strategies of antioxidative defence exist here: while transition metals can be inactivated by chelating proteins (e.g., ferritin), ROS can be reduced enzymatically (e.g., by the glutathione peroxidase) or non-enzymatically by antioxidants (e.g., by vitamins E and C, and glutathione) (Kang 1997, Kikugawa 2004). Simultaneously, stress proteins are implicated in the repair and transport of denatured proteins as well as in the inhibition of apoptosis (Masoud et al. 2003, Mattson 2004). It must be emphasized here, that these regularities or mechanisms are directly depend upon the concentration of iron and copper in cell environment. Increased iron in tissues and blood caused many disorders and has already been reported in mammalian species to oxidative stress (Britton et al. 2002).

Evidence is accumulating that free-radical production is increased in individuals with iron overload. Iron is an essential mineral for normal cellular physiology,

but an excess can result in cell injury. Iron in low-molecular-weight forms may play a catalytic role in the initiation of free radical reactions (Mattson 2004). The resulting oxyradicals have the potential to damage cellular lipids, nucleic acids, proteins, and carbohydrates; the result is wide-ranging impairment in cellular function and integrity. The rate of free radical production must overwhelm the cytoprotective defenses of cells before injury occurs. There is substantial evidence that iron overload in experimental animals can result in oxidative damage to lipids in vivo, once the concentration of iron exceeds a threshold level (Videla et al. 2003).

On the other hand, investigations by Vatassery (2004) show that iron uncouples oxidative phosphorylation whereas peroxynitrite and nitrite are inhibitors of oxidative phosphorylation. Oxidation of mitochondrial vitamin E is accompanied by generation of lipid peroxidation products, altered enzyme activity and electrical conductance etc., and result in inefficient oxidative phosphorylation. Iron can accumulate in neurons (globus pallidus and substantia nigra) pars reticulate and caused brain disorder with extrapyramidal dysfunction (Chiueh 2001). Studies of oxidant stress in Parkinsonian animal models suggest a linkage of iron overload to axonal dystrophy. Preconditioning induction of stress proteins (i.e., hemeoxygenase-1 and neuronal nitric oxide synthase) and hypothermia therapy suppress the generation of toxic reactive oxygen, lipid, and thiol species evoked by bioactive iron complexes in the brain (Vatassery 2004).

It can be concluded from our results, that living bodies may experience oxidative stress induced by reactive oxygen species and heavy metal ions (particularly Fe and Cu), which may damage components in the body and cause aging and disorders. Besides, in addition to the known defense systems against oxidative damage, Kikugawa (2004) describes new defense ones. Available cellular reductants, iron or copper, in low molecular weight forms may play a catalytic role in the initiation of free radical reactions (Britton 1996). According to available results, there may be a rate of free radical production that must be exceeded before cellular injury occurs. Evidence has now accumulated that iron or copper overload in experimental animals can result in oxidative damage to lipids in vivo, once the concentration of the metal exceeds a threshold level (Videla et al. 2003).

Besides, metal-induced toxicity and carcinogenicity, with an emphasis on the generation and role of reactive oxygen and nitrogen species, is observed by Valko et al. (2005). Metal-mediated formation of free radicals causes various modifications to DNA bases, enhanced lipid peroxidation, and altered calcium and sulfhydryl homeostasis. Lipid peroxides, formed by the attack of radicals on polyunsaturated fatty acid residues of phospholipids, can further react with redox metals finally producing mutagenic and carcinogenic malondialdehyde, 4-hydroxynonenal and other exocyclic DNA adducts (etheno and/or propano adducts). Whilst iron, copper, chromium, vanadium and cobalt undergo redox-cycling reactions, for a second group of metals, mercury, cadmium and nickel, the primary route for their toxicity is depletion of glutathione and bonding to sulfhydryl groups of proteins (Valko et al. 2005).

It is important to emphasize, that abovementioned regularities remains one of the sources of apoptosis and various neurodegenerative disorders and other dis-

eases. However, potential stimulants of apoptosis and diseases are factors that increase the risk of generating ROS (Videla 2003, Mattson 2004, Sato & Kondoh 2002). But, first of all, the most important are heavy metals (Wilczek 2005, Pulido & Parrish 2003, Rudolf 2005), pesticides (Masod et al. 2003) and other organic substances (Cruz-Rodrigues & Chu 2002). Moreover, antioxidants (both enzymatic and non-enzymatic) provide protection against deleterious metal-mediated free radical attacks. Vitamin E and melatonin can prevent the majority of metal-mediated (iron, copper, cadmium) damage both in vitro systems and in metal-loaded animals (Vatassery 2004). Toxicity studies involving chromium have shown e.g., that the protective effect of vitamin E against lipid peroxidation may be associated rather with the level of non-enzymatic antioxidants than the activity of enzymatic antioxidants. However, recent epidemiological study has shown that a daily intake of vitamin E of more than 400 IU increases the risk of death and should be avoided (Valko et al. 2005).

Researches lead by various studies allow to define a some neurodegenerative changes caused by influence of raised concentration of heavy metals and environmental stress. Many studies have proposed a deleterious pro-oxidant effect of vitamin C (ascorbate) in the presence of iron (or copper). Another results have shown that even in the presence of redox-active iron (or copper) and hydrogen peroxide, ascorbate acts as an antioxidant that prevents lipid peroxidation and does not promote protein oxidation in humans in vitro. Experimental results have also shown a link between vanadium and oxidative stress in the etiology of diabetes. On the other way, the impact of zinc on the immune system, the ability of Zn to act as an antioxidant in order to reduce oxidative stress and the neuroprotective and neurodegenerative role of zinc (and copper) in the etiology of Alzheimer's disease is also discussed (Rudolf 2005).

It may be supposed from our results, that novel preventative and therapeutic approaches for neurodegenerative disorders are emerging from basic research on the molecular and cellular actions of metals and membrane-associated oxidative stress in cells. This also have been stated by Mattson (2004). So, membrane lipid peroxidation and oxidative modification of various membrane and associated proteins (e.g., receptors, ion transporters and channels, and signal transduction and cytoskeletal proteins) occur in a range of neurodegenerative disorders. This membrane-associated oxidative stress (MAOS) is promoted by redox-active metals, most notably iron and copper. These mechanisms whereby different genetic and environmental factors initiate MAOS in specific neurological disorders are being elucidated (Valko et al. 2005).

The mechanism of cadmium-mediated acute hepatotoxicity, e.g., has been one of the subject of numerous investigations in toxic process (Rikans, Yamano 2000). Acute hepatotoxicity involves two pathways; one for the initial injury produced by direct effects of cadmium and the other for subsequent injury produced by inflammation. Primary injury appears to be caused by binding of Cd^{2+} to sulfhydryl groups on critical molecules in mitochondria. Thiol group inactivation causes oxidative stress, mitochondrial permeability transition, and mitochondrial dysfunction. Simultaneously, although cadmium may injure hepatocytes directly, there are

compelling reasons to believe that hepatocellular injury is produced in vivo as the result of ischemia caused by damage to endothelial cells. Secondary injury from acute cadmium exposure is thought to occur from the activation of Kupffer cells and a cascade of events involving several types of liver cells and a large number of inflammatory and cytotoxic mediators. In this regard, it is clear that Kupffer cell activation and neutrophil infiltration are important events in toxic process, and the involvement of proinflammatory cytokines and chemokines has also been implicated (Videla et al. 2003, Valko et al. 2005).

It must be emphasize statistically significant interactions between enzymes we investigated in this paper. Ecophysiological significance have increasing dependencies of various enzymes interactions, especially those among TBARS, catalase and ceruloplasmine, from physiological point of view. It shows about it, that their mutual rates modifies an enzymatic activity of growing cells (cellular phone) and activates lipid peroxidation processes.

Generally, it can be concluded from our research in this work about the necessity of search the particular mechanisms of dependencies between the concentrate on detailed concentration of heavy metals and other factors of stressful environments on the one hand and the mechanisms of ecophysiological answers of cell (cellular phones). Research of these types of reactions would be helpful towards differentiation of different type of organs and tissues (cellular phone).

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