Piotr Łaszczyca

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Wydawnictwo Uniwersytetu Śląskiego Katowice 1999

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Contents

1. Abstract

The aim of this study is to determine whether there are typical interactions and relationships among activities of indicative enzymes of free-radical scavenger system under the influence of environmental stressors. Animals representing various taxonomic groups: laboratory mice, rats, frogs, slugs of genus *Agrolimax* and *Arion*, earthworms *Dendrobaena* and *Lumbricus*, as well as Madagascar cockroach *Gromphadorhina* were treated with agents known for their prooxidative or antioxidative activity, such as: cadmium, paraquat, carbon tetrachloride, selenite and vitamin E. Diversified time schedules and ways of treatment with these agents were used in order to produce alterations of enzymatic activities and to assess whether there are essential relationships and signs of compensation among indices of antioxidative processes. The activity of superoxide dismutase, glutathione peroxidases, glutathione reductase, glutathione S-transferase, catalase as well as iron-ascorbate-stimulated lipid peroxidation and the content of thiobarbituric acid reactive substances were measured.

When various doses of paraquat and cadmium were applied to mice, frog or invertebrate species the examined enzymes responded in a biphasic manner, characterised by an increase after lower and a decrease after higher doses. In some cases inverted biphasic pattern was also observed. The complex, "mosaic" alterations of enzyme activity within various organs, characterised by opposite changes of examined parameters were observed and explained as resulting from a diverse distribution of applied compounds within organism as well as from specific susceptibility of particular organs and enzymes. Additionally, the "mosaic" response pattern may reflect another aspect of biphasic dose-response relationships in the examined organs.

Since a compensation of altered activity of peroxidase related pathway of free radical scavenger system by opposite alteration of superoxide dismutase and catalase activity was observed in several cases, the hypothesis on the mutual compensatory relations within antioxidative system was drawn and summarised as follows. An inhibition of Se-dependent glutathione peroxidase related pathway of free radical scavenger system in the organs of prooxidant-treated animals may provoke a stimulation of catalase and superoxide dismutase activity, which compensates

for this inhibition. This mechanism possibly acts also in the opposite direction, compensating decreased activity of catalase with glutathione peroxidase or glutathione S-transferase activity. Additionally, some evidence was obtained for the compensation of decreased glutathione peroxidases activity by increasing reaction rate of these enzymes due to enhanced flux and availability of reduced glutathione, on condition that activity of glutathione reductase will rise sufficiently and the pool of reduced nicotinamide dinucleotide phosphate (NADPH) is maintained.

The general frame of mutual compensatory mechanism within the antioxidative system postulated here seems to be common and universal, at least in the examined vertebrate species, insects and earthworms.

Other observations and conclusions were also drawn on the basis of presented results.

2. Abbreviations

BLPC	– background lipid peroxide content as assessed by thiobarbituric acid test
BM	– body mass of experimental animals
CAT	– catalase (activity)
GGTP	– gamma-glutamyltranspeptidase (activity)
GPX, Se-GPX, nonSe-GPX	– glutathione peroxidase or its main isoenzymes (activity)
GR	– glutathione reductase (activity)
GSH	– reduced glutathione
GSSG	– oxidised glutathione
GST	– glutathione S-transferase (activity)
4-HNE	– 4-hydroxynonenal – a product of lipid peroxidation
LP, FeAsc-LP	– iron-ascorbate-stimulated lipid peroxidation as assessed by thiobarbituric acid test
MDA	– malondialdehyde – a product of lipid peroxidation
MT	– metallothionein
ODU; mODU	– conventional units (milliunits) of absorbance or "optical density"
O_2^{-*}	– superoxide anion radical
OH^*	– hydroxyl radical
PL-ase-A_2	– phospholipase A_2
PQ	– paraquat (1,1'-dimethyl-4,4'-bipyridinum dichloride) applied as an experimental agent
Prot, Prt	– protein content
PUFAs	– polyunsaturated fatty acids
ROS	– reactive oxygen species, among them: oxygen free-radicals
SOD	– superoxide dismutase (activity)
α-T	– alpha-tocopherol (also as vitamin E: VE)
TBA	– thiobarbituric acid as reactant
TBARS	– content of thiobarbituric acid reactive substances as assessed by thiobarbituric acid test

TC – tetrachloromethane = carbon tetrachloride (CCl_4) applied as a prooxidative agent

VE – vitamin E (also tocopherol: α-T) applied as an experimental agent

Standard abbreviations were used for chemical agents, unless other abbreviations are indicated in the text.

3. Introduction

Numerous environmental factors may affect biochemical processes of organisms and reduce their ability to grow and reproduce. Since 70. there has been a growing evidence of the crucial role of free-radical chemistry in these phenomena. Free-radical processes are considered as the factors involved in the action of nearly each stressor or noxious stimulus. These comprise accidental events which are induced by a variety of xenobiotics, side effects of common metabolic reactions as well as indispensable, endogenous reactions converting toxic substances. The mechanism of free radical generation consists mostly in a partial reduction of molecular oxygen during mitochondrial or microsomal reactions. These phenomena may be classified into:

– respiratory processes where electron leakeage from mitochondrial electron transporting chain at the ubiquinone step (mitochondrial complex I) generates oxygen free radicals,

– microsomal reaction of hydroxylation catalysed by flavoproteide enzymes and cytochrome P450,

– nonenzymatic, oxygen and transitional metal ion-dependent reactions of oxidation-reduction, including autooxidation of catecholamines, flavins and reduced ferridoxins,

– pathological processes initiated by ischemia – reperfusion and involving production of xanthine from degraded adenine polyphosphonucleotides and proteolytic activation of xanthine oxidase within the cell,

– "respiratory burst" of leucocytes in the course of inflammation which consists of reactions catalysed by NADPH oxidase followed by hemoprotein peroxidase and hydrogen peroxide dependent formation of cytotoxic oxidants (Grisham and McCord 1986; Southorn and Powis 1988; Halliwell and Gutteridge 1990).

An impact of toxic environmental agents upon animal organism frequently involves free-radical phenomena. Generation of reactive oxygen species and the resulting cellular damage may be mediated either directly by a toxic compound and its metabolites or by alterations of metabolic links which indirectly increase the process of free-radical generation. The former case may be exemplified by effects of poisoning with ozone, paraquat or carbon tetrachloride (Liczmański 1988 a; Southorn and Powis

1988; Soudamini et al. 1992; Poli 1993; Bartosz 1995), while the latter may be attributed to the action of cadmium or specific enzyme inhibitors (Sunderman 1987; Halliwell and Gutteridge 1990; Hassoun and Stohs 1996). Oxygen free radicals or their precursors being primary factors of free-radical damage are frequently called as ROS – reactive oxygen species. Cell injury-related activation of "dormant" enzymes and release of iron ions, playing catalytic role in oxidation, are supposed to account for lipid peroxidation within *in vivo* and *in vitro* systems (Herold and Spiteller 1996; Spiteller 1996). Activation of lipooxygenases and esterases, among them of phospholipase A_2, may result in an increase of fatty acid release and an accumulation of lipid peroxides (Kuijk et al. 1987; Farooqui et al. 1997).

3.1. Reactive oxygen species in organs of animals subjected to environmental stress

The main representatives of free-radical species of the ROS family are: excited, singlet dioxygen ($^1O_2^*$), the product of univalent reduction of O_2 – superoxide anion radical (O_2^{-*}), its protonated form – hydroperoxyl radical (HO_2^*) dominating in acidic environment, hydrogen peroxide (H_2O_2) – the product of two-electron reduction of O_2 or dismutation of O_2^{-*}, and hydroxyl radical (OH^*) which is generated during the transitional metal ion-catalysed reactions of O_2^{-*} and H_2O_2 known as the reactions of Fenton and Haber-Weiss (Grisham and McCord 1986).

Hydroxyl radical (HO^*) is the main factor of free-radical toxicity. Being electrically neutral and lipophilic hydroxyl radical may penetrate lipid membranes. It is able to initialise noxious reactions with virtually any organic compound and to abstract methylene hydrogen from polyunsaturated fatty acids (PUFAs). The latter process initiates lipid peroxidation in the cells or in the *in vitro* systems. Lipid hydroperoxides, once formed, may undergo transitional metal-catalysed reactions producing highly reactive aldehydes and ketones, i.e., malondialdehyde (MDA) and 4-hydroxynonenal (HNE). These compounds and their derivates are called thiobarbituric acid reactive substances (TBARS), since they may be detected as a pink adduct to thiobarbituric acid (TBA) formed during *in vitro* reaction. Mechanisms of these processes are reviewed elsewhere (Grisham and McCord 1986; Gutteridge 1988; Southorn and Powis 1988; Halliwell and Gutteridge 1990; Porter et al. 1995).

Products of peroxidation generate new free-radicals, inactivate enzymes, they destroy antioxidants and cause cross-linking of proteins or DNA (Gebicki 1997; Requena et al. 1997). These effects modify or even disrupt cell structures, such as mitochondrial and endoplasmic membranes, which in turn impair calcium transport

and calcium homeostasis (Racay et al. 1997; Xu et al. 1997). Membrane disruption may disorganise respiratory chain and cause further enhancement of free-radicals production (Vercesi et al. 1997).

3.2. Indicative constituents of systems protecting against free-radical pathology

All organisms possess adequate enzymatic and nonenzymatic defensive mechanisms which mitigate harmful effects of free-radicals. Despite species-related differences activities of these antioxidative mechanisms may be considered as a measure of biological response to environmental factors and a measure of adaptation (Liczmański 1988 b; Walker et al. 1996).

The first line of defence are cytosolic and mitochondrial superoxide dismutases (Cu, Zn-SOD and Mn-SOD; EC 1.15.1.1.) which catalyse the dismutation of superoxide anion radical to hydrogen peroxide (Misra and Fridovich 1972; Southorn and Powis 1988). Prooxidant factors may induce an increased expression of both eucariotic SODs (Krall et al. 1988; Stevens et al. 1988; Niwa et al. 1993). Hydrogen peroxide is then removed by either several isoenzymes of glutathione peroxidase (GPX; EC 1.11.1.9.) or by catalase (CAT; EC 1.11.1.6.) found in the cytosol and mitochondria of most tissues. CAT activity becomes important at higher concentrations of hydrogen peroxide, at which the enzyme decomposes most of this compound (Grisham and McCord 1986; Liczmański 1988 a; Southorn and Powis 1988; Gaetani et al. 1996). This makes the role of CAT distinct from the role of GPXs, which is more active at lower H_2O_2 concentrations. Moreover, CAT cannot metabolise organic hydroperoxides and its activity is limited to the hydrophilic compartment of cell (Grisham and McCord 1986). Cytosolic (Se-GPX1), blood plasma specific (Se-GPX-P) and gastrointestinal isoenzyme (GI-Se-GPX) are the main forms of Se-dependent glutathione peroxidase (Paglia and Valentine 1967; Tappel 1976; Chu et al. 1993; Dreher et al. 1997). Reactions of GPXs need reduced glutathione and thus glutathione reductase (GR; EC 1.6.4.2.) and glucose-6-phosphate dehydrogenase (G6PD; EC 1.1.1.49.) are involved, providing reduced glutathione and NADPH (Williams and Arscott 1970). In the invertebrates, which display only trace activities of GPXs (Ahmad et al. 1989; Mathews et al. 1997) a newly recognised ascorbate peroxidase decomposes hydrogen peroxide at the expense of ascorbic acid (Mathews et al. 1997). Products of lipid peroxidation (MDA, HNE, acrolein, crotonaldehyde, hydroxyhexanal) may be removed either by phospholipid hydroperoxide specific Se-dependent glutathione peroxidase (PH-Se-GPX; EC 1.11.1.12) described in the testes, heart, liver and

blastocyst (Sunde et al. 1993; Brigelius-Flohe et al. 1994; Codeas et al. 1996; Dreher et al. 1997) or conjugated with glutathione by several isoenzymes of glutathione S-transferase (GST; EC 2.5.1.18.) to form nontoxic mercapturates (Habig and Jakoby 1981 a; Pickett and Lu 1989). Most of these isoenzymes may be distinguished and classified on the basis of their substrate specificity (Simins and Van der Jagt 1981; Mannervik and Guthenberg 1981; Habig and Jakoby 1981 a, b; Mannervik 1988), tissue specificity and subunit composition (Mikstacka 1988; Pickett and Lu 1989; Lenartova et al. 1996). Cytosolic "α" or "A" isoform of GST possesses glutathione peroxidase activity against lipid hydroperoxides and is mentioned as selenium-independent peroxidase (nonSe-GPX) (Sun et al. 1996). In the cultured rat liver cells α,β-unsaturated aldehydes cause induction of GSTs (Fukuda et al. 1997). Distinct forms of GST were determined in insects (Simmons et al. 1989), molluscs, nematodes (Borgeraas et al. 1996) and earthworms (Stokke and Sternsen 1993; Borgeraas et al. 1996).

Peroxidised fatty acids may be excised selectively from membranes by phospholipase A_2 (PL-ase-A_2; EC 3.1.1.4). This reaction is an initial step to the fatty acid substitution and a repair of damaged membranes (Kuijk et al. 1987). Several isoenzymes of different phospholipases are involved in a membrane repair, remodeling, exocytosis, neurotransmitter release, phospholipid turnover and detoxification of phospholipid peroxides. Membrane-repairing system contains additionally lisophospholipase (LPL-ase; EC 3.1.1.5) and acyl-CoA-lisophosphatidilcholine acyltransferase (ACLAT; EC 2.3.1.23). The system responds to induction or inhibition with chemical and physical prooxidative (i.e. UV-B irradiation) and antioxidative (i.e., vitamin E) factors (Grataroli et al. 1993; Kuo et al. 1995; Chen et al. 1996; Tran et al. 1996).

Numerous stress factors, such as poisoning with heavy metals (i.e. cadmium) or prooxidants (i.e. paraquat, carbon tetrachloride, menadione), oxidative stress, transient ischemia or heat exposure induce an integrated synthesis of proteinaceous components of acute phase response system. The system consists of a family of "heat shock proteins" (hsp's), enzymes of free radical scavenger system: SODs, GPXs, GR, GSTs, CAT and metallothioneins (MTs) (Cigliano et al. 1996; Iszard et al. 1995; Stammler and Volm 1996; Vanremmen et al. 1996; Wiegant et al. 1997; Yamashita et al. 1997). Under stress conditions the hepatic content of MT and other indices of oxidative damage were correlated with an increased cortisol level in the blood (Tort et al. 1996). The direct effect of dexamethasone injected to adult rats was an increased synthesis of CAT, GPX and SOD in their lungs (Jose et al. 1997). Some isoforms of metallothionein are also induced by glucocorticoids (Richards et al. 1984; Searle et al. 1984; Varshney et al. 1986; Nath et al. 1987; Kershaw and Klaassen 1992), although the induction of MT depends on nonsteroid hormones, growth factors (TNF, interleukins) or directly on the action of $OH^{\bullet}$ (Sato and Bremner 1993).

The ubiquitous presence of antioxidative enzymes and their universal role in the organs of animals, which was characterised above, as well as responsiveness to prooxidative and antioxidative agents allow using their activity as convenient indices of physiological response to oxidative stress.

Ceruloplasmin, transferin and ferritins also participate in sequestration of transitional metal ions (Cu, Fe), which prevents generating free-radicals from Fenton-like reactions (Cutler 1986; Halliwell and Gutteridge 1990). Protection against prooxidative effects of cadmium and mercury may be offered by the complexation of these ions with glutathione (GSH). This reaction *in vitro* leads to the formation of Cd(GSH) and $Cd_2(GSH)_2$ complexes (Diaz-Cruz et al. 1997).

Low molecular compounds form a second line of the defence and inhibit free-radical reactions in a nonenzymatic way. These are α-tocopherol and its derivates (Grisham and McCord 1986; Niki et al. 1995; Kamal-Eldin and Appelquist 1996), carotenoids and their derivates (Buettner 1993; Sies and Stahl 1995), ascorbate at high concentrations (Buettner 1993; Sies and Stahl 1995; Buettner and Jurkiewicz 1996), sulphydryl and thioether compounds, uric acid (Cutler 1986; Liczmański 1988 b), and even some unsaturated phospholipids (Lambelet et al. 1994; Pamplona et al. 1996; Tirosh et al. 1997).

These properties of low molecular antioxidants allow one to use them as natural, dietary additives or supplements which enhance antioxidative defence and to prevent prooxidative action of xenobiotics.

3.3. Experimental factors inducing free-radical related alterations in animals

3.3.1. Cadmium as an inducer of prooxidative damage

Cadmium can be used as a model heavy metal able to induce free-radical related pathological processes *in vivo* and *in vitro* due to a destruction of membranal PUFAs (Halliwell and Gutteridge 1990; Hassoun and Stohs 1996). The content of lipid peroxidation products (lipofuscin and TBARS) in organs of rodents increases after a single, short term or prolonged administration of cadmium either by injection (Caisova and Eybl 1986; Andersen and Andersen 1988), or subcutaneous implants of cadmium salt crystals (Jamall and Smith 1985 a; Jamall and Sprowls 1987; Jamall et al. 1989), gastric gavage (Sharma et al. 1991; Rana and Boora 1992), or feeding

(Olsson 1986; Hussain et al. 1987; Shukla et al. 1987; Shukla et al. 1988 a, b, c; Hudecova and Ginter 1992; Wiśniewska-Knypl and Wrońska-Nofer 1994). The mechanism of prooxidative cadmium action is not clear as Cd itself is neither a strong electron donor nor an acceptor. Cadmium-induced acidification of cytoplasm was proposed as a triggering event for a damage of plasma membranes, increase of their permeability and further peroxidation (Koizumi et al. 1996). Alterations of the cellular content and mobility of transitional metal ions (Cu, Fe, Zn) and selenium may also be involved. Fenton-like reactions and membrane destabilisation are proposed as the next step by which hydroperoxides, ketones, epoxides and hydroxyderivates of fatty acids are generated and act as secondary free-radical toxins (Sunderman 1987). Casalino et al. (1997) conclude that prooxidative action of cadmium is mediated by iron ions released from biological membranes, but an involvement of reactive oxygen species is excluded, since Cd-induced prooxidant state in the rat liver mitochondria and microsomes is unaffected by exogenous SOD, CAT or mannitol, whereas it is completely blocked by vitamin E. On the contrary, Cd-induced generation of superoxide anion radical *in vivo* has been demonstrated in the thyroid gland of pigeons (Prakash et al. 1997). Typically, inhibition of antioxidative enzymes (SOD, GPX, GR, CAT) by Cd ions has been reported in the organs of animals (Gill et al. 1989; Sharma et al. 1991; Pal et al. 1993) but some investigators observed an increase of TBARS content accompanied by increased activity of GPX and GR in the isolated intestine subjected to Cd toxicity *in vitro* (Szymańska and Laskowska-Klita 1993) or an increase of SOD, CAT, GPX, GR and GST activity in the organs of rodents fed with cadmium containing diet (Lee and Oh 1981; Caisova and Eybl 1986; Chung and Maines 1987; Sugawara et al. 1989; Rana and Boora 1992; Kostic et al. 1993). Contrasting results were obtained in rats fed high cadmium diet for a month in which renal activity of both SOD isozymes and of Se-GPX were reduced. At the same time, GST activity and vitamin E concentration were increased, whereas kidney CAT activity, ascorbate content and levels GSH and GSSG in red blood cells were not influenced (Stajn et al. 1997).

An increase of Fe^{2+}-ascorbate stimulated lipid peroxidation was also observed in the rat organs under combined treatment with cadmium and selenite (Laszczyca et al. 1993). At the same time the background content of lipid peroxides (BLPC) increased in the liver and brain after the treatment with cadmium and selenium yeast. Cadmium intoxication (Łaszczyca et al. 1993; Kawka-Serwecińska and Łaszczyca 1992) induced an increase of SOD activity in the kidney, brain and testes of treated rats. It also increased renal CAT activity, but GGTP activity was reduced by cadmium in the liver and increased in the brain. Moreover, the experiments with cadmium and ozone (Łaszczyca et al. 1996 c) demonstrate that Cd inhibited GPX activity in the liver, kidney and brain, increased GST activity in the liver, kidney and heart, CAT activity in the kidney, and decreased CAT activity in the brain. These results suggest a mutual compensation among enzymes of free radical scavenger system.

3.3.2. Paraquat and carbon tetrachloride-induced prooxidative effects in animals

Paraquat (1,1'-dimethyl-4,4'-bipyridinum dichloride) is a water-soluble, nonselective herbicide, which due to its high toxicity is nowadays used only as an experimental prooxidative factor (Yasaka et al. 1981; Liczmański 1988 a, b; Bagchi et al. 1993; Piotrowski et al. 1996; Tawara et al. 1996). Similarly to other prooxidants (i.e. menadione, phenazine, dioxins, indometacin, bleomycin) paraquat undergoes univalent microsomal reduction and interferes with electron transporting system of mitochondria generating free-radicals as toxic byproducts (Liczmański 1988 a; Southorn and Powis 1988; Soudamini et al. 1992; Poli 1993; Bartosz 1995). In cultured cell lines as well as in house-flies and other animals the activities of Mn-SOD and Cu,Zn-SOD were significantly increased by paraquat (Allen et al. 1984; Kerr et al. 1988; Krall et al. 1988; Stevens et al. 1988; Niwa et al. 1993). GPX and DT-diaphorase were also induced, while CAT and G6PD were not (Niwa et al. 1993; Brogaard and Clausen 1997). However, other investigators obtained opposite results – an increase of CAT and an inhibition of GPX activity after the treatment with paraquat (Stevens et al. 1988).

Carbon tetrachloride (CCl_4), being a lipid-soluble hepatotoxin for mammals, undergoes a single electron reduction in the presence of NADPH and microsomal NADPH: cytochrome P450 reductase. Byproducts of these reaction induce oxygen radicals (Bartosz 1995) which, reacting with membranal PUFAs, initiate and propagate further free-radical processes (Bagchi et al. 1993). CCl_4-treated rats exhaled increased amount of acetone and ethane, while, in contrast to CCl_4-treated mice, there were no differences of hepatic TBARS content (Letteron et al. 1990; Min et al. 1992; Dennis et al. 1993; Nakagawa 1993). A decrease of hepatic but an increase of serum GST activity (Nakagawa 1993), an induction of MT synthesis in the liver and alterations of hepatic GSH content were other observed results of CCl_4 treatment (Min et al. 1992).

3.4. Agents protecting the organism against prooxidative damage

Selenium and zinc compounds, tocopherol and ascorbic acid are considered as the main antioxidative agents and have been extensively used for this purpose in

the experimental and therapeutic treatment. The idea of "selenium and/or zinc related protection" against heavy metal toxicity is frequently tested experimentally, however excessive intake of these compounds may be vulnerable.

3.4.1. Selenium as a protective agent against peroxidative damage

Studies of Weiss and Sunde (1997) carried on cultured cells indicate that selenium plays a dual role, being a regulator of gene expression for Se-GPX and an essential part of active centre of this enzyme. Adequate dietary level of selenium for rodents and other mammals is 0.1 μg/g of dry weight and the dose – effect relationship among dietary selenium and activity of Se-dependent GPX isoenzyme is sigmoidal with a breakpoint at this value (Lane et al. 1991; Buckman et al. 1993 a; Weiss et al. 1997). The evidence that 0.2 mg Se/kg of diet is required to support full expression of three Se-dependent GPXs (cellular, plasma and phospholipid hydroperoxide specific isoenzyme) was obtained recently in young pigs (Lei et al. 1998). As much as 4 mg Se/kg diet is necessary to increase GPX activity in the rat brain (Sun et al. 1998).

Selenium deficiency reduces Se-GPX activity to less than 1% of normal value (Chow and Tappel 1974; Burk et al. 1978; Olsson 1986; Ji et al. 1988; 1992; Olsson et al. 1993; Weiss et al. 1997), and consequently, enhances spontaneous lipid peroxidation (Castano et al. 1993; Wang et al. 1993) as well as Cd-induced peroxidation in the organs of rodents (Jamall and Smith 1985 a, b; Olsson 1986; Jamall 1987). Typically, no changes or even a decrease of nonSe-GPX, G6PD and GR, GST and CAT activity in the liver and muscle were reported (Chow and Tappel 1974; Burk et al. 1978; Ji et al. 1988), but in some experiments a compensatory activity increase of cardiac Mn-SOD, hepatic CAT (even to 244% of control) and cytoplasmic-GST were observed in Se deficient animals (Burk et al. 1978; Olsson 1986; Ji et al. 1992; Olsson et al. 1993).

Selenium supplemented to healthy rats affected neither the content of TBARS in the blood and selected organs, nor cardiac mitochondrial and cytosolic activity of Se-GPX, SODs, nor the content of vitamin A and E. However, erythrocyte Se-GPX activity was increased (Coudray et al. 1996), while repetitive injections of selenium compounds increased Se-GPX activity in the liver, kidney, but not in the heart (Lane et al. 1991). Protective effects of dietary supplementation with Se at doses of 0.5÷1 μg/g range against cadmium toxicity were evidenced as restored activities of GPXs, GR and GST and decreased TBARS content in the liver, kidney, heart and testes of cadmium poisoned rodents (Lee and Oh 1981; Meyer et al. 1982; Jamall and Smith 1985 a, b; Chung and Maines 1987; Jamall 1987; Sugawara

et al. 1989; Rana and Boora 1992) as well as improved blood indices of hepatic damage (Flora et al. 1982) and prevention of testicular necrosis (Sugawara et al. 1989). Se-dependent protection against the toxicity of other heavy metals has been postulated as well (Gabor et al. 1983). However, excessive dietary selenium (2.05 and 4.05 μg Se/g dry weight) for 8 weeks caused a decrease of Zn,Cu-SOD, CAT and GPX activity, and of retinol content in the rat liver (Albrecht et al. 1994).

In cadmium-poisoned rats twelve weeks of *per os* treatment with selenite or selenium-enriched yeast caused a decrease of SOD activity in the liver. At the same time, in the kidney and testes selenite prevented SOD activity against Cd-induced increase (Kawka-Serwecińska and Łaszczyca 1992; Łaszczyca et al. 1993), but in the brain SOD activity was increased by selenium-enriched yeast treatment (Łaszczyca et al. 1993). Either selenite or selenium enriched yeast reversed cadmium-induced increase of renal CAT activity (Łaszczyca et al. 1993), and caused a reduction of CAT activity in the liver and kidney, irrespectively of cadmium intoxication. Although, the alterations of SOD and CAT activity in the brain tended to be opposite to those in the liver and kidney, mutual compensation of GPX and CAT activity may be postulated on the basis of these results.

Antioxidative effects of selenium supplementation can not be limited to the increase of Se-GPX activity since the increase of organ Se content occurs 2 hours after the injection of selenium compounds, while GPX activity increases after 12 hours, reaching its maximal value 3÷4 days later. Moreover, increased Se supply inhibits lipid peroxidation in the nuclear and microsomal fractions, which do not contain Se-GPX (Guseinov et al. 1990). Chemical complexation of heavy metals with selenium together with either glutathione or low-molecular weight proteins may offer another protective mechanism of selenium supplementation (Mochizuki et al. 1982; Naganuma et al. 1982; Nath et al. 1984).

3.4.2. Antioxidant properties of vitamin E and vitamin C

Tocopherols (vitamin E), ascorbic acid (vitamin C) and carotenoids constitute an intracellular system of low-molecular weight antioxidants. The system prevents propagation of free radicals, notably: peroxyl radicals and singlet oxygen, and protects intracellular and membranal lipids against peroxidative damage. Tocopherols, which act as an initial link of the system, undergo oxidation and are regenerated by reduced glutathione, dihydrolipoate or ascorbate. Metabolic pools of these reducing compounds are in turn maintained at the expense of NADH or NADPH (Buettner 1993; Sies and Stahl 1995). Vitamin E is an antioxidant that partitions into lipid phase of cellular membranes, converts oxygen free-radicals (Southorn and Powis 1988; Niki et al. 1995; Kamal-Eldin and Appelquist 1996)

and ceases propagation of lipid alkyl and lipid peroxyl radicals (Grisham and McCord 1986). Oxidised tocopherol constitutes tocopheroxyl radical which must be reduced by the enzymatic reaction with glutathione (Grisham and McCord 1986) or ascorbate (Liczmański 1988 b; Mulder et al. 1995). The latter is then oxidised to dehydroascorbate which has to be reduced at the expense of GSH or α-lipoic acid (Liczmański 1988 b; Xu and Wells 1996).

Increased content of TBARS and lipid hydroperoxides was observed in the organs of vitamin E-deficient rats and cattle (Buckingham 1985; Walsh et al. 1993; Tokumaru et al. 1997). Ascorbate stimulated lipid peroxidation was also enhanced in these conditions (Walsh et al. 1993). In the homogenates of liver and kidney, subjected to 1 hour of nonstimulated lipid peroxidation, the content of TBARS, conjugated dienes, hexanal and the release of volatile hydrocarbons were inversely proportional to the logarithm of vitamin E dose supplied with the diet to the experimental rats (Hu et al. 1989). Protection against both enzymatic (NADPH dependent) and nonenzymatic (Fe^{2+}-ascorbate driven) lipid peroxidation was increased at any of tested levels of vitamin E supplementation (Hassan et al. 1985; Rojas et al. 1996; Tirmenstein et al. 1997). Complex, synergistic antioxidative action of tocopherol and ascorbate was demonstrated *in vitro* in unilamellar liposomes, in which the lipid phase was enriched with tocopherol while an internal space was loaded with ascorbate (Waters et al. 1997).

Vitamin E applied to cadmium poisoned rats caused a reversal of the Cd-increased lipid peroxidation in the liver, prevented Cd-related increase of SOD activity but increased activity of GPX and CAT (Krajcovicova-Kudlackova et al. 1995). Antioxidative action of tocopherol was described by Shukla et al. (1988 a, c) in the brain of subchronically cadmium poisoned and tocopherol treated rats. Vitamin E prevented testicular toxicity of hydroxyl radical in mice injected repetitively with cadmium salts (Shen and Sangiah 1995) as well as hepatotoxic, prooxidative effects of menadione or CCl_4 injected to mice (Min et al. 1992) and rats (Tirmenstein et al. 1997). Modulation of phospholipase A_2 activity by vitamin E may provide another protective mechanism against peroxidative damage, however the final inhibitory or stimulatory effect depends on vitamin E concentration in a biphasic manner and varies for isozymes of PL-ase A_2 (Tran et al. 1996; Mukherjee et al. 1997).

Vitamin C (ascorbate) is the most important nonenzymatic antioxidant which acts in the water soluble phases. It can scavenge superoxide, hydrogen peroxide, peroxyl radicals, hypochlorite and singlet oxygen (Buettner 1993; Sies and Stahl 1995). Ascorbate is involved in antioxidative processes in at least two different ways: as the nonenzymatic antioxidative agent cooperating with tocopherols and as the substrate for ascorbate peroxidase – the antioxidative enzyme of invertebrates (Mathews et al. 1997). Ascorbic acid is particularly needed for protection of microsomal membranes against cytochrome P450-mediated lipid peroxidation and protein oxidation, where SOD is ineffective. Moreover, an inverse relationship

between the activity of L-gulonolactone oxidase (LGO – a terminal enzyme of ascorbic acid synthesis) and SOD activity was discovered and correlated with the progress of evolution of terrestrial tetrapods (Nandi et al. 1997). The role of ascorbate in free-radical defence, however, is ambiguous. At low tissue or *in vitro* concentrations, in the presence of transitional metal ions (Fe, Cu) and some chelators, ascorbate acts as the prooxidative agent initiating ascorbate driven lipid peroxidation (Halliwell and Gutteridge 1990; Łaszczyca et al. 1995; Buettner and Jurkiewicz 1996). The critical concentration of ascorbate at which its prooxidative action is replaced by antioxidative activity was found to be about 0.2 mmol/l in microsomal preparations from rat testes and heart (Melin et al. 1997). *In vitro* reaction based on this mechanism is used as a measure of tissue susceptibility to lipid peroxidation (Łaszczyca et al. 1995). Another postulated function of ascorbate is to maintain metal ions in the active site of metalloenzymes in the reduced state (Padh 1991).

3.5. Homeostasis under the action of noxious environmental factors

3.5.1. Concepts of homeostasis and compensation in animals under environmental stress

The concepts of homeostasis, stress-induced physiological compensation and disadaptation are the central ideas applied to the impact of ecotoxins on organisms (Walker et al. 1996). According to these concepts, organisms subjected to the environmental stress or intoxication tend to maintain some of their vital function unchanged at the cost of the impairment of (temporarily) less important functions (the principle of compartmentation). Moreover, impaired processes may be compensated (replaced or substituted) by other processes playing similar role for homeostasis. Any "semistable" level of physiological response may be obtained in several ways and may manifest itself by characteristic biochemical alterations – biomarkers reflecting an intensity of the noxious stimulus (Walker et al. 1996). The values of biomarker indices observed actually depend on the temporary balance between inductive, progressive processes of restitution, recovery or repair, and inhibiting, regressive processes of damage, degradation or blockade (a principle of the antagonistic processes). The partitioning of these processes leads to dose dependent effects called hormesis (Luckey et al. 1975; Hopkin 1989) or paradoxical

and oligodynamic effects (Gołubiew et al. 1978). The best illustration of hormetic effect is the bell-like or U-shaped curve of parameter changes over the intensity range of acting factor. The changes caused by environmental factors should be governed by a kind of economy described mainly in terms of energy allocation – optimisation of catabolic processes – and the so-called "scope for growth" (Leung 1991; Depledge and Fossi 1994; Straalen 1994; Walker et al. 1996; Migula et al. 1997).

The major problem to be solved is to detect the most important link in the chain of observed processes, while being aware that the complete network of physiological relationships cannot be reduced to and fully described by the observation of any more or less arbitrarily selected set of parameters.

3.5.2. Are the compensatory mechanisms universal among animals from various taxa?

The choice of adequate indices is still complicated since each animal species responds differently to the particular, noxious stimulus. The ratio between the no-effect-level of the most sensitive and the least sensitive species could amount to 10 000–100 000. In addition, there are no universally sensitive species, as the most sensitive species was different for various chemicals (Lagadic et al. 1994), i.e., earthworm *Lumbricus terrestris* appeared more resistant to 2-choloroacetamide but more susceptible to cadmium nitrate than *Eisenia fetida* (Fitzpatrick et al. 1996). Terrestrial gastropods possess unique high tolerance to heavy metals due to metallothionein dependent heavy metal binding in the midgut gland, which protects other organs from metal toxicity (Berger et al. 1995; Dallinger 1996). In general, snail and slug species can be regarded as heavy metal "macroconcentrators" (Rabitsch 1996; Laskowski and Hopkin 1996 a). Specific, cadmium induced, cadmium and copper binding, cysteine rich, non-metallothionein protein (CRP) was discovered in the earthworms (Willuhn et al. 1996 a), and its presence makes impossible the comparisons of the defensive systems within various species.

Other physiological parameters may also vary among species in 3 or even more orders of magnitude, as for example activity of selenium dependent glutathione peroxidase (Se-GPX) against H_2O_2 in vertebrate and invertebrate species (Ahmad et al. 1989). In earthworms (*Lumbricus terrestris*) and squid (*Loligo opalescens*) similarly low but detectable activity of both H_2O_2 and tert-butylhydroperoxide (t-BuOOH) specific glutathione peroxidase (Se-GPX and nonSe-GPX, respectively) were detected. The activity of nonSe-GPX, being the isoenzyme of glutathione S-transferase (GST), and the activity of catalase (CAT) compensate for trace activities of Se-GPX in the insects, earthworms and molluscs. Moreover, in the cells of

invertebrates the activity of CAT is not restricted to peroxisomes but it is also present in cytoplasmic and reticular compartment (Ahmad et al. 1989). On the other hand, the diversity of GST isoenzymes in earthworms of the genus *Eisenia* is similar to that known for vertebrates; however, the enzyme does not respond to typical inducers, while molluscan and nematode tissues contain isoforms of GST absent in other taxa (Stokke and Sternsen 1993; Borgeraas et al. 1996).

Specific adaptations, unusual among mammals, exist in animals which experience variation in oxygen availability due to environmental oxygen lack, breath-hold diving (anoxia-tolerant turtles), extracellular freezing (freeze-tolerant snakes and frogs) or apnoeic breathing patterns in arrested metabolic states (estivating snails). The induction of antioxidative enzymes (CAT, GST and GPX, but not of SOD and GR) during the hypoxic states is the strategy developed by amphibians for minimalizing the damage caused by reoxygenation (Storey 1996; Hermes-Lima and Storey 1996). Other facultative anaerobes, such as freshwater turtles, deal with the oxidative stress during the anoxic-aerobic transition by maintaining constitutively high levels of antioxidants, which are comparable to those found in mammals (Storey 1996).

What seems to be curious in the reviewed material is that frequently in similar noxious conditions the responses of enzymatic antioxidative systems are different, exhibiting either a strong inhibition or – conversely – a marked increase in activity, induction or activation of particular enzymes (Rana and Boora 1992; Kostic et al. 1993; Szymańska and Laskowska-Klita 1993). Some aspects of these reactions are unclear when two or more enzymes have similar substrate specificity, as i.e., Se-GPXs and CAT, which both are able to decompose H_2O_2; however, at the expense of different electron donors. Taking into account that the reaction catalysed by CAT is "metabolically cheap" – it does not need reduced nucleotides – while GPXs reduce hydroperoxides at the cost of NADPH, the explanation based on enzyme compartmentation appeared to be at least partial and inexhaustible. This point of view is reinforced by results in which a kind of mutual compensation of decreased GPXs activity by CAT or GST was observed (Burk et al. 1978; Olsson 1986; Ji et al. 1992; Olsson et al. 1993; Lin et al. 1993). Opposite activity changes of SOD and GPX or CAT are also confusing (Buckman et al. 1993 b). Compensatory mechanism may exist in the chain of reactions providing NADPH for reduction of oxidised glutathione (GSSG) and thus regulating the activity of GPXs by the local concentration of GSH (Kawate and Suzuki 1983; Allen et al. 1985) or by opposite changes of GPX and GR activities (Chung and Maines 1987; Lee and Oh 1981). Possible compensation would involve also free-radical quenchers and nonenzymatic scavengers, which stop free-radical processes on the level of early propagation. The cellular activity of these compounds may, in turn, depend on the activity of appropriate regenerating systems.

3.6. The aims of the study

On the basis of the data reviewed above, the following aims of the present study have been formulated:

1. To determine what kind of relationships exist among the activities of indicative enzymes of free-radical scavenger system and the indices of peroxidation under the action of selected environmental stressors.
2. To assess whether these relationships are universal among selected representatives of major animal taxa.
3. To investigate, whether there is a relation or mutual compensation among the activities of particular constituents of the antioxidative system, characterised by similar physiological role as, for example, among GPX, GST and CAT. Particularly:
   - Do there exist inverse relations between the activity of GPX and CAT, which both have similar substrate specificity against hydrogen peroxide, or between GPX and GST, where isoenzymes of GST possess peroxidase activity?
   - Is there a difference among the levels and alterations of GPX and CAT activity, which can be considered: the former enzyme as a regulatory one, and the latter as a nonregulatory enzyme?
   - Is there any cooperation, mutual compensation or parallel behaviour in respect of the observed alterations of the activities of GPX and GR, which both function in a complementary, sequential system?

4. Materials and methods

4.1. Animals

Selected indices of antioxidative system were analysed in animals from different phyla: three month old male Wistar rats, 6÷12 weeks old male laboratory mice from the line B6, frogs *Rana "esculenta" L.* (Berger and Michałowski 1963; Młynarski 1987), slugs of the species: *Agrolimax agrestis L. (= Limax agrestis L. = Deroceras agreste L.)*, *Arion rufus L.* (Urbański (1959), earthworms: *Lumbricus terrestris L.*, *Dendrobaena rubida (Savigny 1826)* according to Plisko (1973) and laboratory reared Madagascar hissing cockroach *Gromphadorhina portentosa*. Wild animals were caught in their natural habitat in the vicinity of Katowice and maintained in laboratory conditions (18÷21°C or 13÷15°C, daylight regime: 12D/12L) for 7÷14 days before biochemical determinations.

An interesting feature of frog physiology is rapid induction of antioxidative enzymes in their organs (Hermes-Lima and Storey 1996; Perez-Campo et al. 1993). This, combined with common use of frogs for laboratory purposes, was the reason to conduct presented experiments on this species. However, it is necessary to mention that Berger and Michałowski (1963) stated that *Rana esculenta* is a bastard of *Rana ridibunda* (Pallas) and *Rana lessonae* (Camerano). Particular characteristic of slug and snail physiology is their high tolerance to heavy metal toxicity due to metallothionein-dependent metal binding in the midgut gland (Dallinger 1996; Rabitsch 1996; Laskowski and Hopkin 1996 a; Berger et al. 1995). Earthworms, as other invertebrates, display opposite activity pattern of CAT and GPX than that in vertebrates, which was the reason to choose these species. The role in the environmental food chains and availability in the environment were other reasons. Madagascar cockroach *Gromphadorhina* has been used in our laboratory for ecotoxicological studies for several years. Easy rearing and preparation of organs, relatively high body mass – up to 15 g, simple way of intoxication via oral route, high toxicological resistance and well known physiology make this species a convenient model for studies (Nakonieczny 1993).

4.2. Standard treatment and housing of animals

Standard treatment of mammals and laboratory reared cockroaches comprise feeding adequate commercial diet (Murigran) *ad libitum* and allowing them free access to tap water. Daylight regime was 12 hours of light and 12 hours of darkness. Mice were housed in groups of 4÷6 animals in steel or plastic cages at a temperature ranging between 18÷21°C. Cockroaches *Gromphadorhina portentosa*, as termophilic animals, were maintained at 30°C. During pharmacological treatment animals were kept in separate cages to allow individual dosage or measurements of contaminated-food consumption.

Frogs were kept at 13÷15°C room temperature, up to 6÷10 individuals in plastic cages, with a shallow layer of water at the bottom. During treatment lasting for four days each frog was placed in a single glass cylinder (15 cm of diameter) filled with tap water up to the depth of 1.5÷2 cm.

Slugs and earthworms were housed in the same conditions as frogs. Slugs were kept in groups of 4÷5 individuals in glass pots and fed with fresh lettuce leaves. During the treatment each slug was put into a separate pot. The humidity was near 100%, as evidenced by condensing water. Earthworms were housed in a garden soil mixed with clear sand and carefully watered. Treated individuals were placed separately in pots filled with humid filtering paper.

4.3. Experimental treatment

Treatment of animals consisted in subcutaneous or intraperitoneal injections of cadmium acetate, sodium selenite, paraquat, vitamin E or hydrocortisone solutions (Tab. 1.). All agents were dissolved in an adequate physiological saline. Disposable syringes were used for each individual. During one experimental trial mice were gavaged with cadmium acetate and sodium selenite with an adapted bulb-ended needle of syringe. Slugs and earthworms were injected with microsyringe allowing dosage of microliter quantities of liquids. Slugs were treated into the rear part of their body near the edge of the "coat". Earthworms were injected into body cavity rostrally to the clitellum, since otherwise the injection provoked authotomy of the injected segments. Approximately 0.2 g slices of fresh banana (for 10 g of animal BM) were successfully used as a carrier of applied paraquat doses for the cockroach *Gromphadorhina portentosa*. Saline treatment was applied to control groups.

Rats were watered for 12 weeks with water containing 50 mg Cd^{2+}/l *(0.45 mmol Cd/l)* introduced as cadmium acetate, and gavaged once a week with 0.05 μg α-tocopherol per 1 g BM (0.03% vitamin E – Polfa). Mice were treated with experimental factors either *per os* or by subcutaneous injection twice during two consecutive days and then killed on the third day. The doses of 2.5 nmol Se per 1 g BM and 200 nmol Cd per 1 g BM were gavaged in 10 μl volume per 1 g BM. The doses injected to mice amounted to 5 nmol Se and 15 nmol Cd per 1 g BM and were applied in 8 μl of solution per 1 g BM. Paraquat dose equivalent to 4.75 μg PQ per 1 g BM *(18.5 nmol PQ/g BM)* was injected subcutaneously in the volume of 5 μl per 1 g BM. The dose of vitamin E, injected as α-tocopherol acetate in arachidonic acid (Polfa), amounted to 75 μg VE per 1 g BM, which corresponds to 2.5 μl of solution per 1 g BM. To assess a nonspecific "stress" effects hydrocortisone (HCS – as hydrocortisone hemisuccinate, Polfa) was injected subcutaneously at the dose of 40 μg HCS per 1 g BM of mice, which corresponds to 8 μl per 1 g BM. Some animals obtained combinations of two factors but each of them was applied in a separate portion. In another experiment, mice were injected subcutaneously with 1.9; 4.7 or 11.8 μg of paraquat per 1 g BM twice during four consecutive days and then sacrificed on the fifth day.

Frogs were injected into lymphatic sacks two times, on the first and third day of treatment and sacrificed on the fifth day. Cadmium was applied as one of three gradually increasing doses of 0.45; 1.80; 7.19 μg Cd/g BM *(4; 16 and 64 nmol Cd per 1g BM)*. Paraquat doses were 1.19; 4.75 and 19 μg per 1 g of animal BM *(4.7; 18.5; 73.9 nmol PQ/g BM)*. Reference frogs were injected with 0.65% saline. The volume of injected solutions amounted to 10 μl per 1 g of animal BM.

The same protocol of cadmium dosage (0.45; 1.80; 7.19 μg Cd/g BM on the first and third day) was adopted for slugs *Arion* and earthworms *Dendrobaena*, but additionally 0.79 μg Se per 1g BM (*10 nmol Se/g BM* as sodium selenite solution) was injected. In another experiment, slugs *Agrolimax* and earthworms *Lumbricus* were treated with carbon tetrachloride or paraquat. Two doses of carbon tetrachloride: 31 or 150 nl CCl_4/g BM were injected as 3 μl of 1% or 5% mixture of CCl_4 in the olive oil applied per 1 g of animal BM. Paraquat was injected at the doses of 2.5; 5 and 10 μg per 1 g BM of animal at a volume of 2 μl per 1 g BM.

Five doses of paraquat were applied by gastric route to the cockroach *Gromphadorhina portentosa* in the main experiment and three doses in an additional trial. These were: 12; 24; 47; 95 and 190 μg PQ per 1 g BM and 12; 47 and 190 μg PQ per 1 g BM, respectively *(47; 93; 183; 369; 739 nmol PQ/1g BM)*. 5 μl of PQ solution was used per 1 g of cockroach mass.

Chemicals were purchased from Sigma Chemical Company, Merck Chemical Company (nucleotides, specific substrates for enzymatic reactions, coomasine, Tris) or Polish Chemical Reagents Company (components of buffers, saline and hydrogen peroxide). Paraquat, as Gramoxone – a commercial, 19% w/v water solution of active substance – 1,1'-dimethyl-4,4'-dichlorodipiridine, was obtained from The

Institute of Organic Industry (IPO) in Pszczyna. Vitamin E and hydrocortisone were produced by Polfa pharmaceutical company and distributed by CEFARM.

4.4. Sample preparation

Vertebrates were decapitated under ether anaesthesia. Moderate freezing (4°C for several minutes) was applied to invertebrates for anaesthesia and immobilisation. Samples were taken out from liver, kidney, heart, initial part of small intestine, brain (left hemisphere with brain stem) of rats, mice and frogs; from hepatopancreas, a middle part of intestine and foot of the slugs; from body wall and the intestine of earthworms; and a next to the gut part of the cockroach intestine. Dissected samples were cleaned out of connective tissue and fat, washed with the ice cold physiological solution, dried on filtering paper, cut into small pieces and homogenised by means of a mechanically driven Elvenhjem-Potter glass homogenizer (1:11 w/v; 150 mmol/l KCl, 10 mmol/l phosphate buffer pH 7.4; 4°C). Postnuclear fraction was separated by differential centrifugation (1000g, 10 min; 4°C) in a frozen Janetzki K-24 centrifuge with an angle rotor. Crude homogenates were used for determination of lipid peroxidation and lipid peroxide content while the activity of the enzymes was assayed in appropriately diluted postnuclear fractions.

4.5. Biochemical determinations

The **content of thiobarbituric acid reactive substances** (TBARS) was determined spectrophotometrically according to Ohkawa et al. (1979) in organ homogenates (1:11 w/v) boiled with thiobarbituric acid (TBA) for an hour. **Iron-ascorbate-stimulated lipid peroxidation** *in vitro* was conducted for 30 minutes at 37°C and subsequently interrupted with trichloroacetic acid, developed by boiling with TBA and measured spectrophotometrically (Goran-Anneren and Epstein 1987; Boehme et al. 1977). The yield of TBARS was expressed either in relation to weight of fresh tissue or in relation to tissue protein content. Activity of **superoxide dismutase** (SOD; EC 1.15.1.1.) was measured in postnuclear fractions by means of adrenaline method according to Misra and Fridovich (1972) with modifications of Matkovics et

al. (1977). The method is based upon autooxidation of epinephrine which produces both superoxide radical anion and indicative byproduct – adrenochrome, detected spectrophotometrically at wavelength $\lambda = 480$ nm. Under the conditions of test the conventional unit of SOD activity is defined as the amount of enzyme which causes a half-inhibition of spontaneous autooxidation (Misra and Fridovich 1972). The changes of optical absorbance were recorded with the Carl-Zeiss (Jena) K-100 graphic recorder coupled with Eskalab Alfa spectrophotometer (Smith Kline). The maximal slope of S-shaped curve was a basis for further calculations. The determinations were calibrated with a commercial preparation of the enzyme (from bovine liver) obtained from Sigma Company. The activity of **selenium-dependent glutathione peroxidase** (GPX; EC 1.11.1.9.) against H_2O_2 or **selenium-independent isoenzyme** with cumene hydroperoxide as a substrate were assayed according to Paglia and Valentine (1967) and Tappel (1976). Determination of **glutathione reductase** activity (GR; EC 1.6.4.2.) was conducted according to the description of Racker (1955) with modifications of Colman (1970), Barja et al. (1990) and Wilkinson (1976). Activity of **glutathione S-transferase** (GST; EC 2.5.1.18.) was assayed with 1-chloro-2,4-dinitrobenzene (CDNB) as the substrate (Habig and Jakoby 1981 a, b; Mannervik and Guthenberg 1981; Simins and Van der Jagt 1981). A background NADPH consuming activity (for GPXs and GR) or noncatalysed CDNB conjugation with GSH (GST) were determined and subtracted from the individual measurements. Changes of absorbance were read every 15 second. The initial 90÷105 second from about 2.5÷3 minute readings, characterised by linear changes of absorbance, were taken as the basis for calculations. Care was taken to avoid absorbance changes exceeding 0.050 ODU/min, and the material was diluted when necessary. The activity of **catalase** (CAT; EC 1.11.1.6.) against 10 mmol/l H_2O_2 was determined spectrophotometrically at 230 nm in a postnuclear supernatant (Orr 1970) at 25±2°C. Temperature activity constant (Q_{10}) for CAT is of the order $Q_{10} = 1.1$ (Aebi 1974). The changes of absorbance were recorded graphically and a maximal slope of the obtained curve was calculated. The method was calibrated in respect of extinction coefficient by titration of H_2O_2 according to the routine method described in the manuals (Minczewski and Marczenko 1973).

Activity of endogenous serine proteases was inhibited when necessary by addition of phenylmethyl sulfonyl fluoride (0.5 mmol/l in homogenate) according to the method of Fahrney and Grold (1963).

To test the efficiency of cadmium administration to mice **cadmium and selenium tissue concentrations** were determined in a part (approx. 0.2 g) of dissected liver and muscle of mice. Samples were dried in 120°C and mineralised with 2 ml of perchloric acid in the pyrex flask covered loosely with pyrex tear-like bulbs as covers. Dry residues were dissolved in demineralised water and analysed for cadmium and selenium content with atomic absorption spectrophotometer by means of graphite furnace micromethod ("Solaar" Unicam 939 Atomic Absorption Spectrophotometer).

Table 1

Summary of experimental design

Tables	Animal species	Factors	Mode of treatment	Doses	Time and schedule of treatment		Codes for groups	Determined
Fig.5	Rat – Wistar /male/	Placebo * Cadmium Tocopherol	in water to drink in water to drink *per os* – gavage	50 ppm Cd^{2+} 0.05 μg $\alpha T/g_{BM}$	12 weeks 12 weeks 12 weeks	each day each day once a wk	N, Cd, VE, Cd+VE	LP
2–5 & 3–4	Mice – B6 /male/	Placebo * Cadmium Selenium	*per os* – gavage	22.5 μg Cd^{2+}/g_{BM} 0.20 μg Se/g_{BM}	2 days	each day	N, Cd, Se, Cd+Se	SOD, GPX, GR,
6–9	Mice – B6 /male/	Placebo * Cadmium Selenium Paraquat Tocopherol H-cortisone	i.p. injection	1.7 μg Cd^{2+}/g_{BM} 0.4 μg Se/g_{BM} 4.75 μg PQ/g_{BM} 75 μg $\alpha T/g_{BM}$ 40 μg HC/g_{BM}	2 days	each day	N, Cd, Se, Cd+Se PQ, VE, VE+PQ HCS	GST, CAT, LP $[Cd^{2+}]$ [Se]
10–13	Mice – B6 /male/	Placebo * Paraquat	i.p. injection	1.9; 4.7 or 11.8 μg PQ/g_{BM}	4 days	each 2 days	N, PQ02, PQ05, PQ12	GPXs, GR, GST, CAT
14–16	Frogs	Placebo * Cadmium Paraquat	i.p. injection	0.45; 1.80; 7,19 μg Cd/g_{BM} 1.19; 4.75 and 19 μg PQ/g_{BM}	4 days	each 2 days	N, Cd04, Cd16, Cd64, PQ01, PQ05, PQ19	
17–19 & 20–21	Slugs	Placebo * Cadmium Selenium,	i.c. injection	0.45; 1.80; 7,19 μg Cd/g_{BM} 0.79 μg Se/g_{BM}	4 days	each 2 days	N, Cd04, Cd16, Cd64, Se × (Cd04Se/ Cd16/Cd64)	SOD, GPXs, GR,
22–23 & 24–25	Earth-worms	CCl_4, Paraquat		31; 150 nl CCl_4/g_{BM} 2.5; 5; 10 μg PQ/g_{BM}			TC01, TC05 PQ02, PQ05, PQ10	GST, CAT TBRS
26–27	Cock-roaches	Placebo * Paraquat	*per os*	12; 24; 47; 95; 190 μg PQ/g_{BM}	4 days	each 2 days	N, PQ12, PQ24, PQ47, PQ95, PQ190	

Abbreviations: Tables – numbers of tables which contain corresponding results; * Placebo – an adequate solution used to dissolve test factor, i.e. 0.65% saline for frogs; i.p. – intraperitoneal injection; s.c. – subcutaneous injection; i.c. – injection to the cavity of the body; g_{BM} – gram of animal body mass, to determine the dose of applied experimental factor; N, Cd, Se, PQ, CCl_4 (TC), αT (VE), HC – codes for control (normal) and experimental treatment: cadmium, selenium, paraquat, carbon tetrachloride, vitamin E and hydrocortisone, respectively; SOD, GPX, GR, GST, CAT, LP, $[Cd^{2+}]$, [Se], LP, TBRS – abbreviations for determined parameters

Protein content was determined by means of the cumasine method of Bradford (1976). 50 µl of the analysed fraction was added to 2.5 ml of solution containing 100 µg/ml of Coomasie Brilliant Blue. Readings at 595 nm were calibrated against standard solutions of bovine serum albumin (BSA) and calculated by means of mean square linear regression method as described in manuals. The temperature of determination was held beneath 25°C to avoid the effect of increased temperature upon the process of dye adsorption.

4.6. Statistical elaboration of results

Statistical routine computer analyses comprise one-way and multifactor analysis of variance and correlation analysis. Either Statgraphics 5.1 or Statistica 4.5 software were applied. The results were elaborated by means of the tests of Bartlett or of Levene for variance homogeneity. The test of Fisher was used to detect the differences of means, while either the test of Duncan or Least Significant Difference (LSD) test was used for post hoc evaluation of particular mean values for probability: $p < 0,05$.

5. Results

5.1. Effects of prooxidants and antioxidants on indices of peroxidation

Cadmium gavage to **male B6 mice** caused about 50-fold increase of liver Cd content and about 4÷7 fold increase of Cd content in skeletal muscle (Fig. 1–2). The effects of injected cadmium acetate were similar in respect to the liver and muscle content of cadmium. At the same time selenite applied either orally or parenterally to mice produced more than 10-fold increase of hepatic Se content and about 2÷4-fold increase of muscular Se content indicating that both ways of treatment were effective and nearly equivalent in a quantitative respect (Fig. 1–2). No significant interactions among Cd and Se in respect to their tissue content were found under concomitant oral or subcutaneous treatment with both elements.

5.1.1. Increasing cadmium doses cause biphasic alterations of the antioxidative system but inhibition predominates

A typical result of either oral or parenteral cadmium treatment was inhibition of SOD activity. This effect was observed in the liver and kidney of **mice** (Tab. 2–5; Tab. 6–9) and kidney and intestine of **frogs** (Tab. 15A–16A). However, in the hepatopancreas and intestine of **slugs** ***Arion*** (Tab. 20–21) and in the intestine of **earthworms** ***Dendrobaena*** (Tab. 25) SOD activity was unchanged or tended to rise insignificantly after the treatment with increasing doses of Cd.

Se-GPX activity was decreased by cadmium treatment in the liver and kidney of **mice** (Tab. 2–5; Tab. 6–9), but heart enzyme was affected only under oral treatment. In the kidney of **frogs** (Tab. 15A) and body wall of **earthworms**

Dendrobaena (Tab. 24) cadmium doses equal or higher than this used in mice caused decrease of Se-GPX, too. However, in the **frogs'** liver and intestine increasing doses of Cd produced biphasic alterations of Se-GPX (Tab. 14A–16A), which were characterised by an increase with the lower doses and a decrease to the control value with the higher doses. Similarly, the intermediate dose of Cd increased Se-GPX activity in the hepatopancreas of **slugs** ***Arion*** (Tab. 20) and intestine of **earthworms** ***Dendrobaena*** (Tab. 25) while doses of Cd lower or higher than used for mice tended to decrease Se-GPX activity.

A significant increase of GST activity was indicated as an effect of Cd-treatment in the liver of **mice**, as well as an effect of increasing doses of Cd in the **frog** liver.

Glutathione reductase (GR) was inhibited by cadmium in the liver, kidney and brain of orally poisoned **mice** (Tab. 2–5) and in the brain of Cd-injected mice (Tab. 9), but in the heart an increase of GR activity has occurred following Cd gavage. In the **frogs** injected with Cd a dose dependent increase of GR activity was observed in the liver, with no change in other organs (Tab. 14A–16A). Similar doses of Cd caused an increase of GR activity in the body wall of **earthworms** irrespective of selenite dosage, while in the gut a decrease followed by an increase occurred with the increasing Cd doses (Tab. 24–25). However, the latter effect was significant only when treated groups had been compared, with no significant effects in comparison to control. No significant changes of GR activity occurred in organs of **slugs**.

Catalase (CAT) activity increased in the kidney of **mice** following oral cadmium treatment (Tab. 3) as well as in the liver and kidney of parenterally treated animals (Tab. 6–7). A biphasic alteration of CAT activity – an increase followed by a decrease – which was provoked by increasing Cd doses in the **frog** liver appeared insignificant (Tab. 14B). At the same time, increasing doses of Cd caused a progressive decrease of CAT activity in the kidney of frogs (Tab. 15B). No change of CAT activity in the hepatopancreas and intestine of **slugs** and in the body wall and intestine of **earthworms** has occurred (Tab. 20–21; 24–25).

The inhibition of SOD-GPX-GR system in the liver and brain of **mice** orally poisoned with cadmium was accompanied by an increase of stimulated lipid peroxidation (LP), while in kidneys the LP value remained unchanged, which coexisted with the increase of CAT activity in this organ. Parenteral Cd treatment increased hepatic, renal and cerebral LP, however, the latter alteration was less pronounced than those in the liver and kidney. In **male Wistar rats** poisoned with cadmium containing water for 12 weeks (Fig. 3) similar effects of cadmium were evidenced by an increase of stimulated lipid peroxidation (LP) in the liver, kidney and brain. The same parameter can not be assessed in invertebrates because of physiological reasons discussed in the following chapter.

5.1.2. Effects of paraquat are also dose dependent and biphasic but diverse in invertebrates

Paraquat stimulates SOD activity in the liver, kidney, heart and brain of **mice**, however this effect was observed only in the separate trial with medium paraquat dose (Tab. 6–9). Stimulatory effects appeared insignificant in the **frog** organs (Tab. 14B–16B) and in the intestine of **earthworms *Lumbricus*** (Tab. 22), but they were pronounced and significant in the hepatopancreas and foot muscle of **slugs *Agrolimax*** (Tab. 17–19), as well as in the body wall of **earthworms *Lumbricus*** (Tab. 23). Low dose of paraquat stimulated enzyme activity in the intestine of **cockroach *Gromphadorhina***, but higher dose did not (Tab. 27). Biphasic variations of the effect with the increase of paraquat dose were also observed in the **slugs**.

The highest among the three doses of paraquat injected to **mice** (Tab. 10–13) appeared inhibitory for hepatic and renal Se-GPX, when compared to control values or values obtained in mice treated with lower doses of paraquat. Moreover, in a separate trial (Tab. 6–9) medium paraquat dose caused an increase of Se-GPX activity in the liver and heart of mice. This suggests complex, biphasic response. Biphasic response was also obtained in the liver of **frogs** (Tab. 14B–16B), in the hepatopancreas and (insignificant) in the intestine of **slug *Agrolimax*** (Tab. 17–19), where lower doses increased while the highest dose decreased the activity of Se-GPX. A biphasic response – an increase under low dosage of paraquat followed by a decrease under high dosage – occurred also in the intestine of **cockroach *Gromphadorhina*** (Tab. 26). On the contrary, in the intestine of **frogs** higher doses of paraquat gradually increased the activity of Se-GPX (Tab. 16). No simple, well defined dose – effect response of Se-GPX activity to PQ treatment was obtained in the body wall and intestine of **earthworms**.

The highest dose of paraquat inhibited hepatic and renal nonSe-GPX in **mice** when compared to the control values or the values obtained under treatment with lower doses (Tab. 10–13). Biphasic effects characterised by an increase followed by a decrease with increasing paraquat doses were observed for nonSe-GPX in the intestine of **mice** (Tab. 12), hepatopancreas and intestine of **slugs** (Tab. 17–19), and for the intestine of **cockroach** (Tab. 26). In the foot muscle of **slugs** increasing doses of paraquat gradually increased the activity of enzyme (Tab. 19).

Biphasic effects of increasing paraquat doses with initial stimulation were observed for GST activity in the kidney and intestine of **mice** as well as in the intestine of the **slug** (Tab. 19). Liver and kidney of **frogs** (Tab. 14B–15B) and intestine of **earthworms** (Tab. 22) responded to the paraquat treatment with stimulation of the enzyme activity.

GR activity in the intestine and brain of **mice** (Tab. 12–13) increased (or was unchanged) in animals treated with lower doses but declined under the treatment

with the highest dose of paraquat. At the same time, only the highest doses caused significant increase of GR activity in the **frog** liver and kidney (Tab. 14B–15B) as well as in the intestine of ***Gromphadorhina*** (Tab. 26).

CAT activity in the organs of **mice** as well as in other examined species did not show any regular alterations under the treatment with paraquat, except for dose dependent decrease in the liver of **mice** (Tab. 11) and intestine of **cockroach** (Tab. 26–27). However, the intermediate paraquat dose in the **frog** liver (Tab. 14B) and the highest dose in the **earthworm** intestine (Tab. 22) caused an increase of CAT activity suggesting biphasic alteration.

These alterations of antioxidative enzyme activity did not prevent paraquat-induced increase of LP in the liver, kidney and brain of **mice** (Tab. 6–9). Paradoxically, paraquat did not elevate the level of TBARS in the organs of **earthworms** and **slugs**, which even significantly decreased in the intestine of earthworms (Tab. 22–23) and in the foot muscle of slugs (Tab. 19).

5.1.3. Effects of carbon tetrachloride in invertebrates are paradoxical

A lower dose of carbon tetrachloride (TC) applied to the **slugs** ***Agrolimax*** (Tab. 18–19) caused a marked increase of SOD activity in their intestine and foot muscle, while in the intestine of **earthworms** ***Lumbricus*** (Tab. 22) SOD activity was increased only by the highest dose of TC.

No significant effects of TC on nonSe-GPX, GST and CAT activity were demonstrated in the slugs, but at the same time GST activity increased in the body wall of **earthworms** treated with the lower dose of TC.

Paradoxically, the level of TBARS decreased in the hepatopancreas, but increased in the intestine of **slugs** (Tab. 17–19) treated with the higher dose of TC. No significant effects of TC upon TBARS content were detected in the organs of earthworms.

5.1.4. Antioxidative action of selenite is restricted to glutathione peroxidase and not so obvious

Either oral or parenteral treatment with selenite did not change SOD activity in organs of **mice**, except for its decrease in the liver and heart (Tab. 2–5; Tab. 6–9).

However, selenite applied in combination with cadmium prevented cadmium-induced decrease of SOD activity in the liver and kidney. No effects of selenite on SOD activity were observed in the organs of **earthworms *Dendrobaena*** (Tab. 24–25) and **slugs *Arion*** (Tab. 20–21), although, when compared to the effects of the lowest and intermediate dose of Cd, Se administration produced a decrease of SOD activity in the hepatopancreas of **slugs**.

In contrast to an oral treatment, injections of selenite increased the activity of hepatic and renal Se-GPX in **mice** (Tab. 2–5; Tab. 6–9) with no effects in slugs and earthworms. Moreover, selenite counteracted effects of cadmium in the organs of **mice** restoring the activity of Se-GPX. Both ways of treatment with selenite caused increase of glutathione S-transferase (GST) activity in the liver of **mice**.

Glutathione reductase activity was unchanged or decreased in the organ of **mice** following selenite treatment, except for the liver in which injected selenite caused an increase of GR activity (Tab. 2–5; Tab. 6–9). Only negligible alterations of GR activity were observed also in earthworms and slugs.

Selenite decreased CAT activity in the liver, heart and brain of **mice**, in spite of the differences between the effects of oral and parenteral treatment (Tab. 2–5; Tab. 6–9). However, similar effects of injected selenite on CAT activity in the hepatopancreas of slug and body wall of earthworms appeared insignificant.

Stimulated lipid peroxidation (LP) decreased in the liver of **mice** irrespective of the mode of treatment with selenite, while renal LP was decreased only under parenteral treatment.

As a general effect, selenite counteracted action of Cd upon hepatic SOD, Se-GPX, GST and LP, renal SOD and CAT, cardiac SOD, CAT and GR as well as cerebral SOD and GST in **mice**, although the joint action of Cd and Se inhibited hepatic CAT (Tab. 2–5; Tab. 6–9). A resultant decrease of LP from the values increased by Cd above the control to the values lower or near to the control was also observed in the liver, kidney and brain of mice. No significant and clearly defined effects of combined treatment with selenite and cadmium were observed in **slugs *Arion*** (Tab. 20–21). In the **earthworms *Dendrobaena*** (Tab. 25) all the combinations of injected Se with Cd caused a decrease of SOD activity in the intestine. Se treatment prevented Cd-induced decrease of Se-GPX activity in the body wall and alterations of intestinal Se-GPX. These were, however, significant in comparison with the effects of higher Cd doses. Intestinal CAT activity decreased under the combined action of the highest Cd dose and Se (Tab. 24–25).

Vitamin E applied alone decreased LP in the liver and heart of **mice** (Tab. 6–9), while applied together with PQ it prevented PQ-induced alterations of LP in the liver, kidney and brain, as well as protected cardiac and renal SOD, hepatic CAT, renal GST, hepatic and cardiac Se-GPX and cardiac GR against PQ-induced alterations. In an additional trial, the application of vitamin E had no effect on control rats, but in cadmium poisoned animals (Fig. 3) it led to a partial reversal of

the Cd-increased LP in the liver, kidney and brain homogenates. However, the LP values in these organs were still higher than in the control group.

5.1.5. Response to corticosteroids in mice does not mimic antioxidative defence

Hydrocortisone (HCS) injected subcutaneously to mice according to two-times for two-day schedule (Tab. 6–9), produced an increase of GR activity in their liver and kidney. GST activity in the liver and heart was also increased. In the heart HCS slightly decreased CAT activity, increased Se-GPX activity and caused an increase of cardiac LP. No effects of hydrocortisone were observed in the brain.

5.2. Alterations of activity are positively correlated for most of defensive enzymes

Correlation analysis of the results obtained in examined species under treatment with selected prooxidants and antioxidants revealed positive correlations among activities of SOD and Se-GPX in the liver, kidney, heart and brain of laboratory **mice**, in the **frogs**' intestine, hepatopancreas and intestine of **slugs** ***Agrolimax*** as well as in the body wall and intestine of **earthworms** ***Dendrobaena*** (Tab. 28–29). SOD activity was also positively correlated with the activity of GR in the kidney and heart of **mice**, tested organs of **frogs**, intestine of **slugs** ***Arion***, body wall and intestine of **earthworms** ***Dendrobaena***.

Diversified pattern of relationships occurred among SOD activity and GST activity which appeared to be inversely related in the liver of **mice** and in the **frogs**' intestine, while positively correlated in the frogs' liver and kidney, hepatopancreas of **slugs** ***Agrolimax*** and intestine of **cockroaches** ***Gromphadorhina***. SOD activity was negatively correlated with CAT activity in the foot of **slugs** ***Agrolimax***, but positively correlated in the **frogs**' intestine, hepatopancreas of **slugs** ***Arion*** and body wall of ***Dendrobaena***. Inverse relation, characterised by negative value of correlation was detected among SOD activity and FeAsc-LP in the kidney of **mice** and among SOD activity and content of TBARS in the foot of **slugs** ***Arion***. However, in the heart of **mice** SOD activity and LP were correlated positively.

Se-GPX activity was positively correlated with activity of nonSe-GPX and GST in the organs of laboratory **mice**, **frogs'** intestine, hepatopancreas and intestine of **slugs** ***Agrolimax*** and intestine of **earthworms** ***Lumbricus*** and **cockroaches** ***Gromphadorhina***. Se-GPX activity was also positively correlated with the activity of CAT in the liver of **mice**, intestine of **frogs**, hepatopancreas of **slugs** ***Arion*** and organs of both examined species of **earthworms**. However, in the kidney and intestine of **frogs** Se-GPX activity and CAT activity appeared to be correlated negatively.

Negative correlation of Se-GPX activity and LP was detected only in the organs of **mice**, since LP was not determined in other species.

Inverse relation characterised by negative correlation coefficient was detected for GST and GR activity in the kidney, heart and brain of **mice**, while in the liver of **mice** and organs of **frogs** GST and GR activity were correlated positively. Exhibiting similar irregularity, **frogs'** intestinal GST activity appeared inversely related to CAT activity, but positive correlation occurred among these enzymes in hepatopancreas of **slugs** ***Agrolimax*** and body wall of **earthworms** ***Lumbricus***.

The activity of GR was positively correlated with that of CAT in the liver, kidney and heart of **mice**, intestine of **slugs** ***Agrolimax***, ***Arion*** and **earthworms** ***Dendrobaena***.

Similarly, positive correlation appeared among CAT activity and LP in the liver of **mice**, and CAT activity with TBARS content in the foot muscle of **slugs** ***Agrolimax***. Only the intestine of **earthworms** ***Lumbricus*** developed opposite relation among CAT activity and content of TBARS.

To summarise these relations, what is surprising is the positive correlation between the activity of Se-GPX and CAT observed in most cases, except for the negative correlation in the frogs' kidney and intestine. Inverse relation among activity of GST and GR in organs of mice is also worth noticing.

6. Discussion

6.1. Prooxidative agents produce more complex effects on indices of oxidative status than those commonly described

6.1.1. Doses of prooxidants usually applied by investigators vary by orders of magnitude

The oral cadmium dose established in these experiments for mice at 200 μmol Cd/kg BM corresponds to about 40% of acute oral lethal dose (LD_{50} = 500 μmol Cd/kg BM) according to the review of Andersen (1989). Metal absorption from a single dose was estimated at 1÷6 μmol Cd/kg BM with the intestinal absorption rate of 0.5÷3% (Andersen 1989). Similar protocol of acute cadmium dosage (89÷893 μmol Cd/kg BM) was applied by Lehman and Klaassen (1986), Shimizu with Morita (1990) and Sharma et al. (1991).

Cadmium dose of 15 μmol Cd/kg BM, injected subcutaneously to mice, amounted about a half of parenteral lethal dose (LD_{50} = 32 μmol Cd/kg BM: Jones et al. 1979; Andersen 1989). Single or repeated parenteral dose of cadmium ranged from 0.01 up to 26.7 μmol Cd/kg BM (Horio et al. 1981; Flora et al. 1982; Chmielnicka et al. 1983; Lehman and Klaassen 1986; Hussain et al. 1987; Shukla et al. 1987; Andersen and Nielsen 1988; Manca et al. 1991). However, doses higher than LD_{50}, up to 89 μmol Cd/kg BM, had been injected to mice as well (Hasegawa and Ogata 1982; Sato et al. 1983; Caisova and Eybl 1986; Onosaka et al. 1986; Andersen 1989; Kojima et al. 1990 b). In the present experiments frogs receiving two injections of cadmium at the doses of 4; 16 or 64 μmol Cd/kg BM manifested signs of lethal toxicity after the highest dose, which caused that 3 among 12 individuals died.

The same protocol of cadmium dosage was adopted for snails *(Arion)* and earthworms *(Dendrobaena)*. Brown garden snails *Helix aspersa* survived on an

artificial diet contaminated with up to 1290 µmol Cd/kg of dry matter, which – with the assimilation rate of Cd estimated at 68% – produced average daily Cd intake of about 7 µmol Cd/g BM (Laskowski and Hopkin 1996 a, b). In Roman snail *Helix pomatia* two days of feeding an artificial diet with 2450 µmol Cd/kg dry mass resulted in the total uptake of 0.7÷1 µmol Cd per adult individual (71÷82% of the dose recovery in soft tissues) and did not cause mortality (Berger et al. 1995). Earthworms *Eisenia foetida* and *Lumbricus terrestris* exposed to 44÷8890 µmol Cd/kg dry mass of artificial soil survived for weeks accumulating large amounts of cadmium (Honeycutt et al.

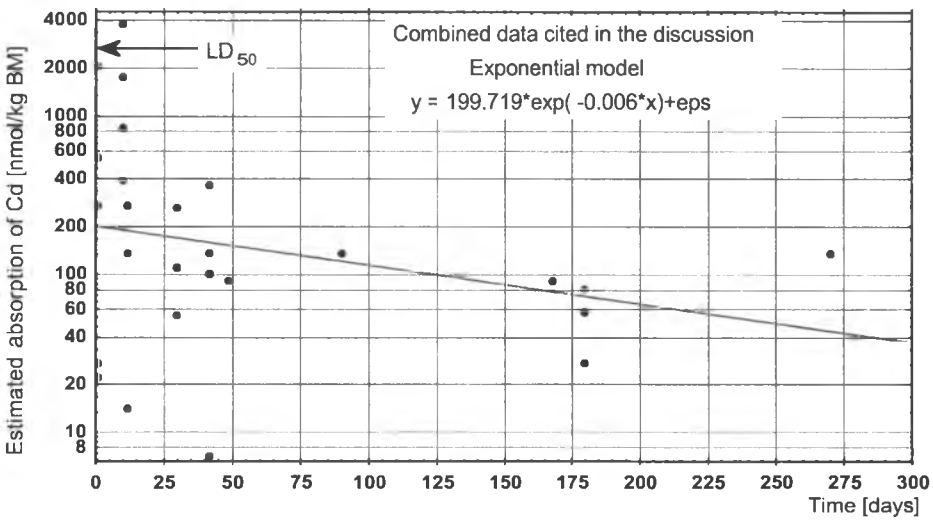

Fig. 1. Typical oral dosage of Cd to rodents

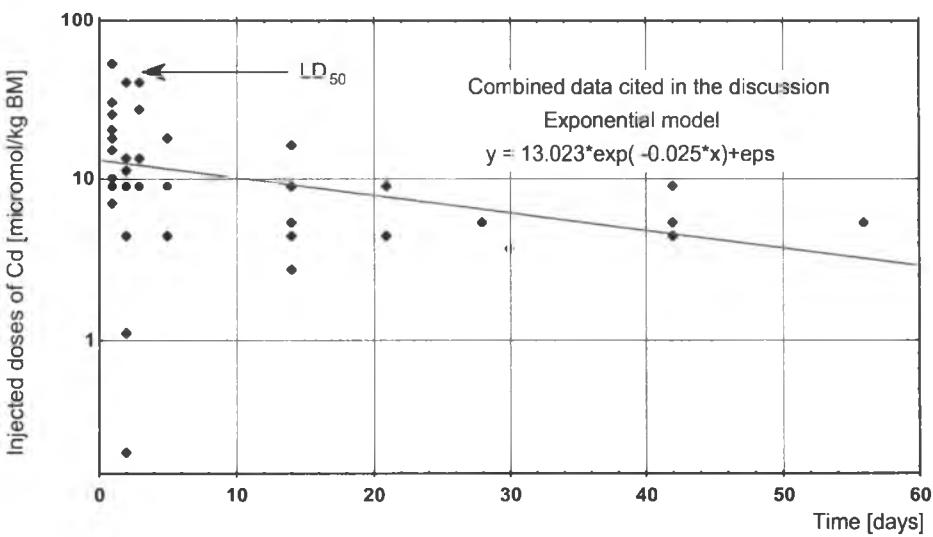

Fig. 2. Typical parenteral dosage of Cd to rodents

1995; Fitzpatrick et al. 1996). The daily uptake of Cd under these conditions ranged from 17 to 270 μmol Cd/kg tissue (Honeycutt et al. 1995).

In the present experiments selenium was gavaged to mice at a dose of 2.5 μmol Se/kg BM or injected subcutaneously at a dose of 5 μmol Se/kg BM. According to the data reviewed by Alderman and Bergin (1986) and assuming 80÷90% absorption of ingested selenite (Mason and Weaver 1986) these doses exceeded several-fold the daily requirement for Se but were below a threshold of acute toxicity (6.3 μmol Se/kg BM). The lethal dose has been estimated at 32 μmol Se/kg BM for most animal species (Alderman and Bergin 1986) and does not show large variability with respect to either oral or parenteral way of poisoning (Miatani and Suzuki 1982; Seńczuk 1990; Sohn et al. 1991; Willhite et al. 1992). In several reviewed experiments the doses of injected Se ranged from 0.038 (Lane et al. 1991) to 30 μmol Se/kg BM (Chunk et al. 1982; Flora et al. 1982; Miatani and Suzuki 1982; Chung and Maines 1987; Sugawara et al. 1989; Thompson and Clement 1991). Slugs *(Arion)* and earthworms *(Dendrobaena)* were injected with 10 μmol Se/kg BM, which was twice as large a dose of selenite as that applied to mice. A lower dose ingested with a diet containing 10 μmol Se/kg dry mass caused no macroscopic signs of toxicity in the cockroach *Gromphadorhina portentosa* (Nakonieczny 1993), while in larvae of other insects (*Tribolium sp.* and *Tenebrio sp.)* dietary concentrations up to 31÷127 μmol Se/kg dry mass inhibited development but did not cause mortality (Hogan and Cole 1988; Hogan and Razniak 1991).

The highest dose of paraquat (74 μmol PQ/kg BM) injected to mice, frogs, slugs and earthworms was similar to a single parenteral lethal dose (LD_{50}) for rats and humans, which is estimated at range between 58 and 155 μmol PQ/kg BM (Seńczuk 1990; Wang et al. 1992; Widdowson et al. 1996 a, b). The doses applied parenterally in most of acute experiments on rodents ranged between 78 (Hoffer et al. 1992; Matsubara et al. 1996) and 310 μmol PQ/kg BM (Hara et al. 1993; Melchiorri et al. 1996). A dose of 580 μmol PQ/kg BM is regarded as oral LD_{50} for rats (Shara et al. 1992; Bagchi et al. 1993), assuming 20% absorption of paraquat from the gastrointestinal tract. In this respect no macroscopic signs of acute lethal toxicity were discovered in the cockroach *Gromphadorhina portentosa* treated by gastric route with five distinct doses of paraquat: 47; 93; 183; 369; 739 μmol PQ/kg BM.

Treatment of mice with vitamin E (α-T) consisted of two injections of 75 mg α-T/kg BM (160 μmol α-T/kg BM). The doses of 150÷1500 μg α-T/kg BM are usually injected intramuscularly in clinical practice (Podlewski and Chwalibóg-Podlewska 1986), while in cadmium poisoned rats Shukla et al. (1988 a) had applied by the same way 5 mg α-T/kg BM 15 times for 30 days. In experiments conducted in our laboratory vitamin E was gavaged to adult rats in the repeated dose of 50 μg α-tocopherol/kg BM for 3 months.

The doses of carbon tetrachloride injected to slugs *(Agrolimax)* and earthworms *(Lumbricus)* in this study (31 and 150 μl CCl_4/kg BM – 290 and 1410 μmol CCl_4/kg BM) did not exceed acute human and rat oral lethal dose

LD_{50} of 28÷47 mmol CCl_4/kg BM (Seńczuk 1990; Bagchi et al. 1993). Several investigators applied 28 mmol CCl_4/kg BM orally to rats or mice (Dennis et al. 1993), while intraperitoneal doses ranged between 0.17 and 1880 µmol CCl_4/kg BM (Villarruel et al. 1990; Huang 1991; Ferreyra et al. 1992, 1993; Nakagawa 1993).

The dose of hydrocortisone (HCS) injected to mice to assess nonspecific "stress" effects, was established at 40 mg HCS/kg BM (82 µmol HCS/kg BM). The usual dosage amounts: for dexamethasone – 0.14 mg/kg/day in humans and 0.14÷3 mg/kg BM daily for 1÷7 days in rats (Akdemir et al. 1992; Elwood et al. 1992; Tomas et al. 1992), while for corticosterone in rats – 4÷10 mg/kg BM daily for several weeks (Qulati and Crabb 1992; Tucker and Torres 1992).

6.1.2. Dose – effect response to prooxidants is biphasic in most observed cases

6.1.2.1. Prooxidants increase the rate of lipid peroxidation, but this effect is not universal

Common intuitive presumption that indices which characterise the rate of lipid peroxidation rise progressively with the increasing dosage of prooxidative factor should be revised following to the results presented by Manca et al. (1991). According to these results, nonstimulated lipid peroxidation was enhanced in a dose dependent manner by cadmium salts admixed to the homogenate of rat organs. Nevertheless, gradually increased doses of cadmium injected repetitively to rats caused biphasic alterations of nonstimulated lipid peroxidation, characterised by an initial increase followed by a decrease (even below the control value) despite progressively raising organ content of cadmium (Manca et al. 1991).

In the present experiments an increase of iron-ascorbate-stimulated lipid peroxidation (FeAsc-LP) was demonstrated in the liver, kidney and brain of mice after either oral or parenteral acute cadmium treatment, Higher FeAsc-LP were also observed in the liver, kidney and brain of rats poisoned with cadmium as well as treated with both cadmium and selenite together for 12 weeks (Łaszczyca et al. 1993). An administration of paraquat (PQ) to male B6 mice caused an increase of FeAsc-LP in their liver, kidney and brain. Paradoxically, administration of paraquat or carbon tetrachloride did not enhance, but even tended to decrease the content of TBARS in the organs of slugs *Agrolimax* and earthworms *Lumbricus*. A similar effect, a paradoxical decrease of TBARS content in paraquat-poisoned house flies, was also observed by Allen et al. (1984). Only the higher dose of carbon tetrachloride did increase the content of TBARS in the intestine of slugs. Moreover, the alterations of cerebral FeAsc-LP in mice were less pronounced than those in the liver, while in

the kidney FeAsc-LP value increased only after parenteral Cd treatment. These results were opposite to relationships reported by Manca et al. (1991) in respect of nonstimulated peroxidation in the brain of cadmium injected rats. At the same time, a direct index of oxidative-antioxidative balance – a background content of lipid peroxidation products – increased in the liver and brain of rats subjected to the subchronic treatment with cadmium and selenium yeast and after the treatment with cadmium alone (Łaszczyca et al. 1993).

Numerous investigators have reported that cadmium exerts enhancing effect on the indices of lipid peroxidation in mammals (Shukla et al. 1988 a, b, c; Andersen and Andersen 1988; Jamall et al. 1989; Rana and Boora 1992; Hudecova and Ginter 1992). Prooxidative action of paraquat has been documented as increasing the content of intermediates and end-products of peroxidation in the mammalian organs as well (Yasaka et al. 1981; Bagchi et al. 1993; Piotrowski et al. 1996). However, in contrast to such indices as content of peroxides, conjugated dienes, lipofuscins and directly determined free-radicals, the rate of lipid peroxidation needs further comments. The rate of lipid peroxidation reflects temporary balance between induction and inhibition of the membrane repairing enzymes, since the iron-ascorbate-stimulated LP should be rather used as an index of the activity of membrane repairing system related to phospholipases than as the measure of lipid peroxide content (Kuijk et al. 1987; Łaszczyca et al. 1995; Chen et al. 1996; Burgess and Kuo 1996; Herold and Spiteller 1996). The activation of specific oxygenases and hydrolases as well as the release of iron ions may account for the determined intensity of peroxidation (Kuijk et al. 1987; Herold and Spiteller 1996; Spiteller 1996; Farooqui et al. 1997). Moreover, in organs of invertebrates such indices as stimulated (FeAsc-LP) or nonstimulated lipid peroxidation and TBARS content appeared incompatible with corresponding parameters in mammals and probably inadequate (Łaszczyca et al. 1995; 1996 b, d). For these reasons FeAsc-LP was not determined in organs of invertebrates in the present study.

6.1.2.2. Superoxide dismutase displays biphasic response to increased doses of prooxidants

In the kidney, brain and testes of rats fed cadmium containing diet for 12 weeks the activity of SOD was significantly higher than in the reference group (Łaszczyca et al. 1993). On the contrary, short-lasting, either intragastral or parenteral cadmium treatment in mice as well as various doses of Cd injected to frogs produced a decrease of SOD activity in their organs. However, similar doses of injected Cd did not produce significant alterations of SOD activity in slugs *Arion* and earthworms *Dendrobaena*. Observed variability of cadmium effects in respect to the magnitude and the organ being affected may be attributed, at least in part, to the kinetics and time course of metal distribution within particular organs and species.

In paraquat (PQ) administered mice SOD activity increased in their liver, kidney and heart. In the intestine of frogs treated with three various doses of PQ SOD activity decreased and an increase caused by the intermediate dose of PQ in the frogs' liver appeared insignificant. A biphasic response (an increase followed by a decrease) of SOD activity to the treatment with different doses of PQ was observed in the hepatopancreas of PQ-injected slugs *Agrolimax* and in the intestine of Madagascar cockroach *Gromphadorhina* fed a diet with PQ. Administration of carbon tetrachloride (TC) caused biphasic response of SOD in the intestine and foot of slugs *Agrolimax* as well. Treatment of earthworms *Lumbricus* with the increasing doses of paraquat and carbon tetrachloride resulted in a progressive increase of SOD activity.

These results indicate that cadmium induces delayed compensatory increase of SOD activity, despite the immediate inhibition of this enzyme. Moreover, different effects of intragastral and parenteral Cd treatment in mice demonstrate that the way of administration affects toxic action of this metal, probably due to intestinal and hepatic mechanisms of cadmium inactivation (Nath et al. 1984; Dolężych 1994). The lack of the Cd effects upon SOD activity in snails and earthworms may be a result of their high resistance to cadmium toxicity, which was suggested by several authors as being related to protein-dependent Cd binding (Dallinger 1996; Berger et al. 1995; Rabitsch 1996; Laskowski and Hopkin 1996 a; Willuhn et al. 1996 a, b).

Signs of paraquat toxicity in rats, reported by Tsuchiya et al. (1996), did not involve alteration of SOD activity. Neither alteration of SOD activity was demonstrated in the experiment of Allen et al. (1984), who had exposed house flies to drinking water contaminated with 1 mmol PQ per litre (260 ppm PQ), which should produce an average PQ intake lower by about an order of magnitude than those in this study. Nevertheless, in cultured fibroblasts, lymphocytes and neutrophils the activity of both Mn-SOD and Cu,Zn-SOD was induced by paraquat (Kerr et al. 1988; Krall et al. 1988; Stevens et al. 1988; Niwa et al. 1993).

The inhibitory action of short time treatment with cadmium on SOD activity is contrasting with inductive effects of paraquat and probably indicates a direct action of heavy metal ions on enzymatic protein and an inductive (at least in certain doses), indirect, free-radicals-dependent effect of paraquat. The results suggest also that both tested factors exert dose dependent, biphasic effect upon SOD activity in animal organs.

6.1.2.3. Biphasic dose – response of glutathione peroxidase is typical while glutathione reductase tends to rise

In the liver and intestine of frogs, hepatopancreas of slugs *Arion* and intestine of cockroaches *Gromphadorhina* increasing doses of either Cd or PQ caused biphasic alterations of Se-GPX activity, characterised by an increase at low dose

treatment followed by a decrease under the highest dose. Injections of graded paraquat (PQ) doses to male B6 mice caused similar biphasic dose – response of activity of Se-GPX in their liver and heart as well. An inverted biphasic pattern was shown for either Cd-induced or PQ-induced alterations of Se-GPX activity in the body wall and intestine of earthworms *Dendrobaena* and *Lumbricus*. In contrast to these results, the effect of PQ in frog intestine was characterised by a progressive increase of Se-GPX activity with increasing PQ doses while in the kidney of frog increasing doses of Cd progressively depressed Se-GPX activity. An inhibition of Se-GPX activity was also observed in the organs of mice after short time, single-level cadmium exposure or in rats after prolonged dietary cadmium poisoning (Łaszczyca et al. 1996 c). The activity of nonSe-GPX in the liver and intestine of mice, hepatopancreas and intestine of slugs *Agrolimax*, intestine of earthworms *Lumbricus* and of cockroaches *Gromphadorhina* underwent a similar pattern of increase followed by a decrease after treatment with increasing doses of PQ. Biphasic pattern of GST activity alteration was also observed in the intestine of cockroach *Gromphadorhina*, as well as in the slugs *Agrolimax* and earthworms *Lumbricus* treated with graded doses of PQ. However, at the same time, hepatic and renal activities of GST increased progressively in the frogs with increased doses of PQ.

Observed alterations of glutathione reductase (GR) activity followed the pattern of Se-GPX alterations. These were the cases of decreased GR activity in the liver, kidney and brain of mice subjected to acute oral treatment with Cd, biphasic alteration of GR activity in the intestine and heart of mice treated with graded doses of PQ and inverted biphasic pattern for Cd-induced alterations of GR activity in the body wall and intestine of earthworm *Dendrobaena*. In contrast, in the liver and kidney of frogs and intestine of cockroach *Gromphadorhina* treated with increasing doses of PQ activity of GR increased progressively with increasing doses of PQ. These changes correspond to biphasic alterations of Se-GPX activity in the tested organs. Since neither increased mortality nor a decrease of apparent viability of frogs and cockroaches were observed, these opposite alterations of GPX and GR activity may be regarded as a mutual, compensatory mechanism under high PQ doses. What should be mentioned, however, is that no alterations were reported for GR activity in house flies poisoned with low dose of PQ, which caused signs of oxidative damage (Allen et al. 1984).

The results indicating inhibitory action of Cd on the system of glutathione metabolising enzymes are in agreement with observations described by other authors (Meyer et al. 1982; Olsson 1985; Jamall and Smith 1985 a, b; Jamall et al. 1989; Shukla et al. 1989; Sharma et al. 1991; Pal et al. 1993; Wiśniewska-Knypl and Wrońska-Nofer 1994; Stajn et al. 1997). Despite well known inhibition of enzymes by Cd ions, the effects of cadmium poisoning on enzyme activity in mammals are characterised by large ambiguity. Szymańska and Laskowska-Klita (1993) observed an immediate increase of GPX and GR activity after *in vitro* incubation of the

inverted intestinal sack in cadmium containing media. A great stimulation of GR and GPX activity occurred after 24 h in the liver and kidney of rats intoxicated with 22.2 μmol Cd/kg BM (Casalino et al. 1997). An increased activity of GPX and GR in red blood cell was reported in rats after 30 days of feeding with cadmium containing diet (Rana and Boora 1992; Kostic et al. 1993). Similarly, an increase of Se-GPX and GST, but a decrease of GR activity was observed in the testes, liver, kidney and blood of cadmium treated rodents (Lee and Oh 1981; Chung and Maines 1987). Such effects and diversified, in respect of direction and magnitude, alterations of enzyme activity in rodent organs may reflect the metabolic fate, distribution kinetics and tissue affinity of cadmium, with kidney as a target organ to which absorbed metal is transferred from its primary, hepatic depots (Nordberg 1984; Nath et al. 1984; Goyer et al. 1989; Kadrabova et al. 1992).

6.1.2.4. Alterations of catalase activity are more complex, but show biphasic pattern as well

An intriguing event observed in the previous experiment, an increase of CAT activity in the kidney of rats fed with Cd for 12 weeks (Łaszczyca et al. 1993, 1996 a), appeared similar to Cd-induced increase of CAT activity in the liver and kidney found in laboratory mice subjected to repetitive injections of Cd. At the same time an increase of CAT activity in the liver, heart and brain of rodents gavaged with cadmium appeared significant only when compared to decreased CAT activity under selenite treatment. In the kidney of mice short lasting gavage of cadmium resulted, however, in a decrease of CAT activity. Similarly, in the Cd-injected frogs renal CAT activity decreased progressively with the increasing Cd dose.

A biphasic dose – response relationship was revealed for CAT activity in the liver and heart of mice, in the liver of frogs treated with different doses of paraquat, in the hepatopancreas of slugs *Agrolimax* treated with either paraquat or carbon tetrachloride, and body wall of earthworms *Lumbricus* treated with carbon tetrachloride. Similar pattern of alterations in the slug intestine appeared insignificant. In contrast to these results, increasing doses of PQ progressively depressed renal CAT activity in frogs and intestinal CAT activity in cockroaches *Gromphadorhina*, while in earthworms *Lumbricus* they resulted in a progressive increase of intestinal CAT activity.

Obtained results indicate that short lasting parenteral treatment with Cd caused more pronounced effects upon the activity of CAT and other indices of peroxidative processes (LP, Se-GPX) than an oral treatment equivalent in respect of absorbed dose. Reported reaction of CAT to cadmium poisoning varies depending on additional conditions. For example, dietary Cd was shown to decrease while subcutaneous implants of cadmium salts crystals increased CAT activity (Jamall

and Sprowls 1987). A fall of CAT activity in the liver of mice due to dietary Cd administration has been reported (Shukla et al. 1989), but other authors observed this effect only under concomitant undernourishment or coadministration of alcohol (Gill et al. 1989; Sharma et al. 1991; Pal et al. 1993). On the other hand, cadmium-induced increase of CAT activity in the kidney and red blood cells of rodents was described in several papers (Kojima et al. 1990 a; Rana and Boora 1992; Kostic et al. 1993). Similarly, cadmium combined with physical effort and ozone injections deepened the increase of CAT activity produced in the heart and kidney by ozone treatment (Łaszczyca et al. 1996 c). An increase of CAT activity as a sign of paraquat toxicity was reported in the erythrocytes and liver of rats by Tsuchiya et al. (1996) and in the house fly by Allen et al. (1984).

6.1.2.5. Complex dose – response relationships in animals treated with prooxidants

The biphasic alterations of hepatic and intestinal Se-GPX and CAT activity in the frog as well as of SOD and Se-GPX activity in the organs of slugs and earthworms conform to the principle of hormesis (Luckey et al. 1975; Hopkin 1989). The results which correspond to biphasic response pattern were also obtained by Kędziorski (1993) in house cricket *Acheta domestica* fed the diet contaminated with different doses of Cd (100÷200÷400 μmol Cd/kg) for 46÷90 days. In the gut and Malpighian tubules, where the burdens of cadmium were the highest, the activity of carboxylesterases and GST decreased after the highest dose, while in the fat body of these insects – the organ accumulating the least amount of metals – the activity of carboxylesterases and GST was higher than in the control. At the same time, lower doses of cadmium caused a compensatory increase of carboxylesterase activity in examined organs, proving complex, biphasic reaction to poisoning as well (Kędziorski 1993). Interestingly, low nontoxic concentrations of cadmium (about 1 μmol/l) stimulate DNA synthesis and cell proliferation in various cultured cell lines, whereas higher concentrations are inhibitory. Cadmium, at micromolar concentrations, enhances the expression of immediate early genes, tumor suppressor gene p53 and genes of protective molecules, including MTs, GSH, and some heat shock proteins. The mechanisms underlying the modulation of gene activity by cadmium are possibly related to its interference with cellular signalling at the level of cellular receptors, calcium and zinc homeostasis, protein phosphorylation and modification of transcription factors (Beyersmann and Hechtenberg 1997). Diversified, "mosaic" reactions of antioxidative enzymes to Cd poisoning were observed by numerous investigators, who also found an increase of GPX, CAT or SOD after either acute or subchronic Cd treatment in the organs of rodents (Lee and Oh 1981; Olsson 1985; Chung and Maines 1987; Kojima et al. 1990 a; Kostic et al. 1993). These results may

surely correspond to certain chosen points on the bell-like or inverted U-shaped curve describing the dose – response relationship.

The biphasic dose – response of enzyme activity observed in the present study reflects variability of the threshold level for the induction of particular enzymes and a complexity of balance among induction and inhibition or other deletorious effects upon enzyme activity (i.e., cellular protein leakeage). Moreover, the results demonstrate that none single dose of tested prooxidant factors allows to observe similar effects in all the links of physiological processes and that a change in one link does not have to be of the same direction as in the others. Such a dose – effect relationship does not allow anticipation of the effect of a particular dose in different species as well as in various organs.

6.1.3. Some indices display miscellaneous and paradoxical deviations

The rate of iron-ascorbate stimulated lipid peroxidation (FeAsc-LP) in organs of insects, earthworms and slugs was lower by order or two orders of magnitude, or even undetectable, when compared to that in vertebrates. At the same time TBARS content was similar to that in vertebrates. Moreover, homogenates of examined slug organs showed an unrecognised, denaturable activity, which inhibited iron-ascorbate stimulated lipid peroxidation when admixed to the homogenates of mammalian liver (Łaszczyca et al. 1996 b, d). A possible reason for these differences among vertebrate and invertebrate species may depend on the presence of ascorbate peroxidase in the organs of insects and other invertebrates. The enzyme removes products of lipid peroxidation with the concurrent oxidation of ascorbate (Mathews et al. 1997). An excessive amount of ascorbate used for iron-ascorbate-driven lipid peroxidation may exert an undesirable stimulating effect upon the activity of ascorbate peroxidase making a measurement of lipid peroxidation lacking sense. For these reasons, the commonly used indices of lipid peroxidation, such as FeAsc-LP, NADPH-LP and TBARS content appeared incompatible in the organs of invertebrates with the corresponding parameters in mammals and they are methodologically inadequate (Łaszczyca et al. 1995; 1996 b, d). The complex nature of lipid peroxidation is still accomplished for ambiguous role of phospholipases in this process. An increased activity of Ca-independent PL-ase-A_2 was demonstrated to be correlated with a content of MDA in organs of selenium and vitamin E deficient rats (Burgess and Kuo 1996). Phospholipase A_2 activity is mentioned to exert protective role when its activity increases prior to oxidative stress, but its activation following the oxidative stress is thought as deletorious. The final effect is related to the ratio of removed TBARS and remaining conjugated dienes (Kuo et al. 1995).

A paradoxical effect of either paraquat or carbon tetrachloride administration, which did not enhance the level of TBARS in the organs of slugs and earthworms was similar to the effect of paraquat upon house fly observed by Allen et al. (1984). A content of TBARS in intestine of slugs increased only after a higher dose of carbon tetrachloride. These effects may be related to the enzymatic mechanisms discussed above, but other – unknown – reasons and relationships are possible as well.

Some of paradoxical effects may result from a misbalance between the activities of the enzymes of antioxidative system. Particularly, the activity of SOD should not exceed the activity of CAT, since otherwise unbalanced flux of hydrogen peroxide may initiate or facilitate Haber-Weiss reaction within the cell. Excessible activity of SOD may also interfere with reactions of lipid free-radical termination, which need the presence of superoxide anion radical (Michiels et al. 1994).

Brain of mice and rats seems to be less endangered to prooxidative action of cadmium than liver, which was observed also in experiment with other noxious factors as ozone (Łaszczyca et al. 1996 c) or lead (Dąbrowska-Bouta et al. 1996). High cerebral content of polyunsaturated fatty acids and iron together with low activity of antiperoxidative enzymes and high ascorbate content might be a factor promoting peroxidation (Bondy and LeBell 1991). The integrity of haematoencephalic barrier and a high content of GSH seems to be the most important antioxidative defence (Romero et al. 1991). Nevertheless, Manca et al. (1991) suggest that these relationships are not so evident, since they observed the highest rate of nonstimulated peroxidation in the brain and lung of cadmium poisoned rats.

6.2. There are no simple conclusions on compensatory physiological response nor on antagonism among noxious and protective agents

6.2.1. Not only protective effects of antioxidant supplementation occurred in the system

An experimental model of supplementation of xenobiotic-poisoned animals with "universal antidota" is frequently applied in toxicological studies. Selenium or zinc compounds as well as tocopherols, ascorbate, retinol or carotenoids are commonly used because of their role in cellular defence. Some investigators had

reported preventive effects of supplementary selenium or zinc on the intestinal absorption or organ deposition of metal ions (Meyer et al. 1982; Chmielnicka et al. 1983; Blazka et al. 1988); however, unchanged or elevated deposition of toxic ions may be masked by a supplementary element which forms inactive complexes with toxic species (Mochizuki et al. 1982; Naganuma et al. 1982; Nath et al. 1984).

In the presented experiments no significant interactions in respect of Cd and Se accumulation were found in organs of mice as a result of joint application of Cd with Se, irrespective of the mode of treatment. These results are consistent with the reports of Meyer et al. (1982) and Blazka et al. (1988).

6.2.1.1. Protective effects of antioxidants in prooxidant treated animals appear stereotyped

Either acute or subchronic supplementation with selenium compounds by oral or parenteral route prevented several Cd-induced alterations in organs of experimental animals, among these: an enhancement of hepatic, renal and cerebral FeAsc-LP in mice; an inhibition of hepatic, renal, cardiac and cerebral SOD and GPX-GR system activity in mice; biphasic alterations of Se-GPX activity in the hepatopancreas of slugs *Arion*, decreased activity of Se-GPX in the body wall of earthworms *Dendrobaena*; and an enhancement of hepatic and renal CAT activity in mice (only under parenteral treatment) as well as in subchronically treated rats (Łaszczyca et al. 1993).

Oral supplementation with vitamin E (VE) had no effect on FeAsc-LP in control rats, but it caused a partial reversal of Cd-induced increase of FeAsc-LP when applied to rats subchronically poisoned with cadmium. In mice, injections of vitamin E, had no effect on the activity of most enzymes, but prevented paraquat-induced increase of FeAsc-LP as well as an increase of GPX, GR, GST and CAT activity. However, PQ-induced increase of SOD activity was not prevented by VE. Applied alone, vitamin E decreased FeAsc-LP in the liver and heart and decreased CAT activity in the heart (as well as in a lesser degree in other organs except for the brain).

The effects of supplementation with selenium, alone or combined with cadmium, appeared to be typical, when compared to those described in numerous papers, and characterised by an increase of Se-GPX activity with a concomitant decrease of lipid peroxidation (Chow and Tappel 1974; Lee and Oh 1981; Flora et al. 1982; Meyer et al. 1982; Gabor et al. 1983; Jamall and Smith 1985 b, c; Olsson 1986; Chung and Maines 1987; Sugawara et al. 1989; Lane et al. 1991; Rana and Boora 1992). Nevertheless, many authors claim that Se supplementation stimulates activity of Se-GPX and GST only in Se-deficient animals (Buckman et al. 1993 a; Davidson and Kennedy 1993). An excessive Se intake may exert a toxic action (El Begearmi and Combs 1982; Early and Schnell 1982; Alderman and Bergin 1986; Koller and

Exon 1986). Unless used doses of selenium were within the toxic range, an inhibition of GR activity observed in the brain of orally treated mice could be regarded as a sign of marginal overdosing of selenium. This decrease of GR activity, interfering with GPX activity (which needs GSH – the product of reaction catalysed by GR), may tend to increase LP in the brain. Observed interactions among Cd and Se may suggest the existence of a protective mechanism which was described as based upon Se-dependent complexation of Cd (Mochizuki et al. 1982; Naganuma et al. 1982; Nath et al. 1984). In the present experiments on mice, these relationships may serve as an explanation for the lack of alterations of enzyme activity despite unchanged Cd accumulation in Se supplemented animals, which was observed under combined treatment with Cd and Se in the presented experiments.

The results of vitamin E supplementation in mice are similar to the observations of Shukla et al. (1987), despite differences of the methods by means of which lipid peroxidation was measured (Łaszczyca et al. 1995). The mechanism of vitamin E interaction with cadmium is not clear, as prooxidative action of cadmium is not understood in details and vitamin E prevention is directed rather towards final signs (removal of peroxidized lipids or lipid radicals) than towards causal mechanism of prooxidative injury (Buckingham 1985; Grisham and McCord 1986; Clemens and Waller 1987; Hu et al. 1989; Min et al. 1992). A modulation of phospholipase A_2 activity by vitamin E may provide a protective mechanism against peroxidative damage; however, the enzyme plays an ambiguous role in the process of lipid peroxidation (Burgess and Kuo 1996; Tran et al. 1996). Final inhibitory or stimulatory effects depend upon vitamin E concentration in a biphasic manner and vary for different isoenzymes of PL-ase (Tran et al. 1996; Mukherjee et al. 1997). Burgess and Kuo (1996) found that vitamin E and selenium deficient rats showed a 5-fold increase of Ca-independent PL-ase-A_2 activity which had been followed by an increased content of MDA in their organs. At the same time, Tran et al. (1996) reported increased activity of PL-ase-A_2 in cultures of heart myoblasts supplemented with vitamin E. Thus, even a partial reversal of the Cd-increased LP by vitamin E, which was observed in the liver, kidney and brain of rats, might be interpreted either as a result of a decreased generation of peroxides or as inhibited activity of membrane-repairing system. On the other hand, protective effects of vitamin E, which are characterised by a reversal of increased tissue content of TBARS and inhibition of iron-ascorbate dependent, as well as microsomal, lipid peroxidation, have been well documented in the vitamin E-deficient rats and cattle (Buckingham 1985; Hassan et al. 1985; Krajcovicova-Kudlackova et al. 1995; Hu et al. 1989; Rojas et al. 1996; Tokumaru et al. 1997). Despite lowered lipid peroxidation, GPX activity was not changed in rats supplemented with vitamin E (Hassan et al. 1985). On the other hand, vitamin E protected SOD activity in the blood against a decrease induced by high cholesterol diet fed to guinea pigs in which increased lipid peroxidation has occurred (Panczenko-Kresowska and Ziemlański 1994).

6.2.1.2. The effect of supplementary factor need not be beneficial or neutral by itself

However, sole supplementation with selenium compounds was not inactive, as it has provoked: a decrease of FeAsc-LP in the liver of mice treated by either oral or parenteral route; a decrease of SOD activity in their organs, a decrease of GR activity in the brain of gavaged mice and in the organs of slugs *Arion* and intestine of earthworms *Dendrobaena* (demonstrated with Multivariate Analysis of Variance); an enhancement of Se-GPX and GST activity in the liver and kidney of injected mice; an increase in the kidney but decrease of GST activity in the heart and brain of orally treated animals as well as a "mosaic" decrease of CAT activity (in the heart and brain after the injection and in the liver after the gavage). A reduction of CAT activity by sole selenium supplementation was also observed previously in the liver and kidney of rats, while selenium enriched yeast increased SOD activity in the rat brain (Łaszczyca et al. 1993). In both discussed cases a possibility of toxic effects due to overdosing should be rejected on the basis of reviewed models of dosage applied by other authors (Early and Schnell 1982; Alderman and Bergin 1986; Koller and Exon 1986).

6.2.2. Prooxidants combined with antioxidants may act additively

Coadministration of cadmium with selenite produced (in comparison to control) a decrease of SOD activity in the intestine of slugs *Arion* and earthworms *Dendrobaena* and decreased CAT activity in the intestine of earthworms. Both effects may not be regarded as an obvious sign of compensation or protection. Similarly, combined doses of Cd and Se given *per os* to mice did not revert Se-induced decrease of CAT activity in their liver and heart. Moreover, in the rats subjected to joint treatment with cadmium and selenite, FeAsc-LP was higher and above the values of control and Cd-treated animals, while background lipid peroxide content (BLPC) increased in the liver and brain after combined treatment with cadmium and selenium enriched yeast (Łaszczyca et al. 1993). Either GR activity in kidney or CAT activity in the brain of mice were altered by paraquat combined with vitamin E more than by any of these two factors acting alone. Additive action of factors, believed to be antagonistic, are known but not common. For example, Gabor et al. (1983) showed additive enhancing effect of lead and selenium on the lipid peroxide content in kidney of rats, while in guinea pigs ascorbate combined with cadmium depressed hepatic cholesterol content and serum GGTP activity as well as increased serum alanine and asparagine aminotransferase activity more than any of these factors alone (Nagyova et al. 1994 a, b).

6.2.3. No single, simple mechanism of protection or compensation may be expected

A complex mosaic pattern of inhibition and restoration of enzyme activity, which is described in the present study, was also observed by Nakonieczny (1993) in the digestive tract of the cockroach *Gromphadorhina portentosa* treated with dietary cadmium and/or selenium. No recovery of inhibited ATP-ases and phosphatases occurred in this experiment, but Cd-dependent inhibition of some digestive hydrolases was prevented by applied selenite (Nakonieczny 1993). The receptor concept, developed by Ehrlich and Clark in the beginnings of twentieth century, which describes the mode of toxin action, allows one to suppose that supplementary agent may exert protective action competing with toxin for one receptor site and at the same time it may cause opposite effects in another receptor site. Such a possibility should be considered for selenium, which is characterised by a narrow range of physiological tolerance (Early and Schnell 1982; Alderman and Bergin 1986; Koller and Exon 1986).

The antiperoxidative role of selenium, which constitutes an active site of selenium dependent glutathione peroxidases, is well recognised at present (Chow and Tappel 1974; Lane et al. 1991; Chu et al. 1993; Sunde et al. 1993; Brigelius-Flohe et al. 1994). Protection against toxic effects of Cd, which is reached by Se supplementation is also well documented (Lee and Oh 1981; Flora et al. 1982; Meyer et al. 1982; Gabor et al. 1983; Jamall and Smith 1985 b, c; Olsson 1986; Chung and Maines 1987; Sugawara et al. 1989; Rana and Boora 1992). The mechanism of protective selenium action on heavy metal poisoning in animals may depend, at least in part, on the formation of macromolecular complexes of selenium with proteins, which together immobilise foreign toxic metal ions (Mochizuki et al. 1982; Naganuma et al. 1982; Nath et al. 1984; Badiello et al. 1996). The combined effects of vitamin E and vitamin C on the rate of lipid peroxidation and activity of involved enzymes were already discussed in the previous chapters.

Adaptative reactions to stress consist in an induction of proteins of acute phase response. Antioxidative enzymes: SOD, GPX, GR, CAT and metallothioneins (MTs) are the examples of these proteins (Liczmański 1988 a; Sato and Bremner 1993). Although the acute phase response involves many regulatory factors (i.e., interleukins and low molecular weight alarmons), the role of adrenal steroid hormones cannot be overlooked (Quionones and Cousins 1984; Richards et al. 1984; Varshney et al. 1986). In order to test this possibility, hydrocortisone was injected to mice, and a resulting increase of GR activity in their liver and kidney was observed. GST activity in the liver and heart was also increased, CAT activity decreased in the heart, but Se-GPX activity and Fe,Asc-LP increased. Reviewed results of glucocorticoids administration demonstrate that, despite the fact that

these hormones may affect the activity of antioxidative enzymes (SODs, GPXs, GST or CAT) and the rate of lipid peroxidation, the final effect depends on other factors. The age of cells and whole organisms (Arahuetes et al. 1993; Town et al. 1993; Hamasaki et al. 1994), the exposition to additional stress factors (Hidalgo et al. 1991) and the level of other hormones and metabolites (Dougall and Nick 1991; Aoki et al. 1993; Boyce and Mantle 1993; Hamasaki et al. 1994) modify the response to glucocorticoids. These results suggest that reaction to prooxidative factors can not consist in stress response, especially in those processes which are regulated by steroid hormones of adrenals.

6.2.4. The response of organism as a whole system seems to be only decisive

General signs of animal status, such as life expectancy, fertility, or the scope for growth have been proposed on the basis of the reviews of Hopkin (1986, 1989) and Depledge and Fossi (1994), as the best indices of a way in which organisms cope with environmental stress. This point of view was applied by Laskowski and Hopkin (1996 b) to assess the effects of environmental poisoning in snails. On the other hand, the assessment of these holistic and general indices is a prolonged process which requires an extensive period of time to conclude, which – by comparison – makes instantaneous determination of enzyme activity particularly useful.

The results of this study demonstrate that the predominant way in which treatment with antioxidant factors helps to maintain homeostasis in animals affected by prooxidants is a restoration of depressed antioxidant activity or an induction of compensatory activity of a complementary system. Opposite alterations of GPX and CAT or GPX and GR activity might serve as examples of these mechanisms and are in agreement with those described in the literature (Burk et al. 1978; Allen et al. 1985; Martins et al. 1991; Lin et al. 1993). An increased activity of GST, which was observed in mice, slugs, earthworms and insects treated with prooxidants, may offer another compensative mechanism. However, supplementation of an individual with preventive agent seems to be applicable only to limited, well defined conditions. Limitations arise from the dose – reaction relationships and time dependence of reaction. Moreover, animal models do not allow for precise prediction of effects and estimation of the effective dose for another species (Lagadic et al. 1994). Biphasic dose – effect response, demonstrated in systems examined in presented experiments, shows that each particular link of metabolic processes is characterised by another threshold of compensatory reactions, reversible injury or irreversible damage. The susceptibility of different elements of defensive systems

must not overlap with each other (Walker et al. 1996). This may be a cue for naturally occurring agents, such as heavy metals, but does not have to be for artificial agents, as for example carbon tetrachloride. The alterations observed in this study seem to prove this supposition, since signs of anomalous reactions or inverted biphasic dose – response have been obtained under the treatment with carbon tetrachloride in slugs and earthworms.

6.3. Components of the antioxidative system respond to stressors in concordance despite species and enzyme related differences

6.3.1. General pattern of response to prooxidants is similar in different taxa

Biphasic response of SOD activity to the treatment with paraquat is characteristic for all the examined species, while acute Se injections tended to the decrease activity of this enzyme. Opposite responses of SOD activity to Cd-treatment – a decrease in organs of vertebrate but an increase in invertebrates – should be reexamined, since they may be a sign of a higher resistance to Cd-poisoning of slugs and earthworms than that of examined vertebrates. This possibility does not exclude a biphasic response to Cd-treatment, despite differences of the threshold level for a particular reaction and species.

In general, a pattern of Cd-induced alterations of Se-GPX activity is biphasic too, but in mice the response of Se-GPX activity varies with respect to a dose and mode of treatment. Paraquat treatment produces also biphasic effects on activity of Se-GPX, nonSe-GPX, GR and GST in examined species. Variations of effects in respect of the dose and mode of treatment in mice, as well as the lack of significant effects of PQ in earthworms might suggest that in the warm-blooded animals an effective dose of PQ is lower, but the action of PQ more pronounced. Similarly, invertebrates appeared less sensitive to Se-treatment, which is reflected as no effect on Se-GPX and GST activity, in contrast to increased activity of Se-GPX and GST in Se-injected mice. Biphasic alterations of GR activity in the intestine and brain of mice treated with different doses of PQ and predominant inhibitory response of GR activity in organs of mice injected with either cadmium or selenium are opposite to the stimulatory effect of PQ and biphasic effect of Cd on GR activity in frogs or

lack of a response in snails and earthworms. These might prove a different threshold of response to the treatment with Cd, Se or PQ in these species.

CAT has also displayed signs of biphasic alterations in animals treated with Cd, however, invertebrates seem to be less sensitive and there was no regularity in the response of frogs and earthworms to PQ treatment.

FeAsc-LP varies depending on tested organs, species and mode of treatment, but in mice and rats an increase of LP was a predominant effect. Incompatibility of FeAsc-LP and TBARS does not allow for comparisons making evident these differences among mammals, other vertebrates and invertebrate species.

Moreover, under the treatment with tested stressors the relationships among the activity of particular enzymes were concordant in respect of a direction of induced changes and sign of correlation coefficients. Positive correlation among activities of SOD and GPX, SOD and GR, nonSe-GPX and GST, Se-GPX and CAT as well as negative correlation among SOD and LP or TBARS may serve as examples.

Diversified pattern of relationships was demonstrated in the case of activity of SOD and GST which had been positively correlated in the liver and kidney of frogs, hepatopancreas of *Agrolimax* and intestine of cockroach *Gromphadorhina*, but negatively correlated in the liver of mice and in the intestine of frogs. The activity of SOD and CAT was positively correlated in the frogs' intestine, hepatopancreas of slugs *Arion* and body wall of earthworms *Dendrobaena*, but negatively correlated in the foot muscle of *Agrolimax*. Discordant relations appeared also among slugs *Agrolimax* with earthworms *Lumbricus* and frogs in respect of the correlations of CAT activity to GST activity, as well as among GST and GR activity in mice and frogs.

The differences of antioxidative system compositions and mechanisms of adaptation to oxidative stress seem to increase with taxonomic distance of tested species. There is an evidence for: (i) a diversity of mammalian, oligochaetan and molluscan isoenzymic pattern of GST (Borgeraas et al. 1996; Stokke and Sternsen 1993); (ii) diverse levels of activity and subcellular localisation of CAT (Ahmad et al. 1989) as well as (iii) a specific, cysteine rich, non-metallothionein protein in earthworms, playing the same role as vertebrate metallothioneins (Willuhn et al. 1996 a, b). Moreover, (iv) since insects (and other invertebrates) apparently lack significant activity of Se-GPX and nonSe-GPX (Ahmad et al. 1989), and CAT has a low affinity for H_2O_2, ascorbate peroxidase was proposed as the important member of insect antiperoxidative system which catalyses the oxidation of ascorbic acid with the concurrent reduction of hydrogen peroxide or lipid peroxides (Mathews et al. 1997). Similarly, (v) an inverse relationship between L-gulonolactone oxidase (LGO – a terminal enzyme of ascorbic acid biosynthesis) and SOD was discovered as related to the progress of evolution of terrestrial tetrapods. SOD is not induced in the early tetrapods, while guinea pigs, bats, monkeys and man are the species lacking LGO (Nandi et al. 1997). Additionally, (vi) uric acid – the end product of

purine degradation – capable to protect cell membranes against peroxidation (Cutler 1986; Liczmański 1988 b), is present in large concentration in uricotelic animals (insects or terrestrial snails), while nearly absent in ureotelic mammals.

Only minor effects of treatment with Cd and Se in slugs *Arion* and positive correlations between activity of SOD and CAT, Se-GPX and GR, Se-GPX and CAT as well as GR and CAT in their hepatopancreas and intestine, may reflect higher resistance of this species to heavy metal poisoning, suggested also in other snail species by Berger et al. (1995) and Laskowski with Hopkin (1996 a, b). Moreover, the hormetic type reactions, observed only for SOD and Se-GPX activity in hepatopancreas, were insignificant and less pronounced than in other species, which may again prove higher resistance of snails to heavy metal toxicity (lack of a breakpoint of reaction, if stimulus is weaker than threshold for inversed alteration).

6.3.2. Activity of most enzymes changes in a concordant way

Alterations of the determined enzymes were positively correlated in most of the observed cases of various treatments and examined species. A decrease of enzyme activity is usually regarded as a sign of damage and disadaptation, while an increase as a positive, adaptative response. However, this point of view need not be true, since enzyme induction is metabolically expensive, while an induced activity of enzyme is rather a sign of "a battle" but not of "a victory". The deletorious effects of imbalance between activity of SOD and CAT were mentioned earlier. Commonly known increase of parathion toxicity after its partial biotransformation serves as another example. Both effects, either inductory or deletorious, were described for reactive oxygen species as well as for the factors which have been used in the present experiments (acute phase response – see: Introduction).

Positively correlated alterations were characteristic for activity of SOD and Se-GPX, SOD and GR or GR with CAT in organs of mice, frogs, slugs and earthworms, and Se-GPX with nonSe-GPX or GST in these species and in cockroaches. Moreover, unless in particular cases alterations of activity of a given enzyme were discordant, following alterations of activity appeared parallel and positively correlated:

– SOD and GST in the liver and kidney of frogs, hepatopancreas of slugs and intestine of cockroach;
– SOD and CAT in the intestine of frogs, hepatopancreas of snails and body wall of earthworms;
– Se-GPX and CAT in the liver of mice, intestine of frogs, hepatopancreas of slugs and organs of earthworms;
– GST and GR in the liver of mice and organs of frogs;
– CAT and GST in hepatopancreas of slugs and body wall of earthworms.

These positive correlations should be interpreted as an effect of similar threshold level of regulatory mechanisms for enzyme induction or a similar mechanism of enzyme inactivation by noxious factors.

Negative correlations, which may indicate compensatory mechanism, were observed for the activity of: GST and SOD in the liver, GST and GR in the kidney, heart and brain of mice, GST and both: SOD and CAT in the intestine of frogs, Se-GPX and CAT in the kidney and intestine of frogs. The activity of Se-GPX and CAT underwent opposite alteration in kidney of mice treated with Cd and in the hepatopancreas and intestine of snails *Agrolimax* treated with paraquat and carbon tetrachloride. A similar pattern of reaction was previously reported for Cd-poisoned rats (Łaszczyca et al. 1993).

Positive correlation of (i) CAT activity and either FeAsc-LP in the liver of mice, or TBARS content in the foot muscle of slugs *Agrolimax*, (ii) negative correlation of FeAsc-LP and both: SOD and Se-GPX activity in the organs of mice, or (iii) correlations between TBARS content and either CAT activity in the intestine of earthworms *Lumbricus* or SOD activity in the foot of slugs *Arion* may reflect different stages of adaptative response. In this respect, inverse relation among SOD and CAT activity in the foot of slugs *Agrolimax* may be a sign of temporary failing of adaptatory response. Positive correlation observed between nonSe-GPX and GST reflects the fact that nonSe-GPX is an isoenzyme of GST (Mannervik 1988; Sun et al. 1996).

6.3.3. Mutual compensation may exist only under specific circumstances

Tested hypothesis on mutual compensation between antioxidative enzymes having similar physiological role has been supported by results obtained in experiments with rodents. In mice treated with cadmium and selenite a decrease of Se-GPX activity was accompanied by an increase of CAT activity, which was proved by means of Multivariate Analysis of Variance (Tab. 2–5 and 6–9). An opposite relation, an increase of Se-GPX accompanied by a decrease of CAT activity was also observed. At the same time, GST activity rose under both treatments. This pattern was particularly evident for renal CAT activity increased in the kidney of mice following to Cd-treatment, while activity of SOD, Se-GPX and GR was inhibited. Opposite alterations of CAT and Se-GPX activity in comparison to parallel alterations of SOD, Se-GPX and nonSe-GPX activity were also observed in the hepatopancreas and intestine of slugs *Agrolimax* treated with paraquat and carbon tetrachloride. Moreover, in the frogs, renal and intestinal activity of Se-GPX and CAT was also negatively correlated, as mentioned in the previous section.

Unchanged (or slightly enhanced) activity of CAT, accompanied by Cd-induced decrease of Se-GPX in the body wall of earthworms *Dendrobaena* may also be a sign of compensation. The mechanism of postulated compensation was for the first time proposed for rats intoxicated with cadmium. Cd-induced stimulation of SOD and CAT activity in these animals was accompanied by a decrease of Se-GPX activity, while selenium compounds which stimulated Se-GPX had depressed CAT activity (Łaszczyca et al. 1993). Similar signs of mutual compensation of GPXs activity by CAT activity were observed by several investigators (Burk et al. 1978; Olsson 1986; Ji et al. 1992; Olsson et al. 1993; Lin et al. 1993). Catalytic properties of CAT make its role distinct from the role of GPXs. CAT activity is important at higher concentrations of hydrogen peroxide with comparison to those necessary for GPXs (Grisham and McCord 1986; Southorn and Powis 1988; Gaetani et al. 1996). The reaction catalysed by CAT does not need reduced nucleotides, while GPXs reduce hydroperoxides at the expense of NADPH. Moreover, CAT may be considered as a nonregulatory enzyme, which acts at "near to equilibrium conditions". The rate of its reaction is described by the law of mass, that means, its reaction rate under physiologic conditions depends on H_2O_2 concentration, and may immediately adjust to the concentration of this substrate. Thus, CAT activity should not limit the decomposition of H_2O_2 over the wide range of its concentrations (Reich 1976; Newsholme and Crabtree 1986). On the other hand, CAT cannot metabolise organic hydroperoxides and its activity is limited to the hydrophilic compartment of cell (Grisham and McCord 1986) while GPX activity is dependent on selenium availability in the cell.

Another compensatory mechanism may be proposed in the frogs treated with PQ and Cd, where decrease of hepatic Se-GPX was coupled with an increase of GR and GST activity. Possible compensation of decreased Se-GPX by increased GR in the body wall of earthworms *Dendrobaena* was superimposed over biphasic pattern of enzymatic response of both enzymes. Signs of compensation were also revealed in the intestine of cockroaches *Gromphadorhina* treated with increasing doses of PQ. In these insects, increased activity of Se-GPX and nonSe-GPX after the treatment with lower doses of PQ followed by a decrease after higher doses was accompanied by opposite alterations of GR.

A compensation of decreased activity of a particular antioxidative enzyme by another one having similar substrate specificity was described in several papers. For example, enhanced activity of CAT in Se-GPX depressed house fly was reported by Allen et al. (1985), in rats by Burk et al. (1978), while in cellular cultures by Lin et al. (1993) and Martins et al. (1991). In the brain of developing rats CAT and Se-GPX activities undergo opposite changes (Maestro and McDonald 1987), which may be regarded as a mutual, compensatory mechanism. The hypothesised inverse relations among activity of SOD, CAT and GR with respect to GPX activity were also observed in the brain tissue of rat foetuses intoxicated with Cd in utero (Gupta et al. 1996). Time course of carbon tetrachloride induced alterations of rat hepatic

Se-GPX, G6PDH, GST and GR activity, reported by Nishida et al. (1996), also seems to obey the discussed principle. Interesting results were obtained by Lopez-Torez et al. (1993) who demonstrated a huge compensatory increase of GR activity accompanied by minor change of GPX activity in the liver and kidney of rats chronically treated with a catalase inhibitor. Despite a direct induction of antioxidative enzymes by xenobiotics or by an increased level of free-radical species, another possible triggering mechanism of compensation might be proposed on the basis of results obtained by Baker et al. (1996) who found that GR activity in the rat brain is susceptible and directly proportional to the cellular content of glutathione. Depressed activity of GPXs which consume GSH may influence this mechanism.

The results discussed above demonstrate that postulated compensatory mechanisms are factor specific and dose related. The processes involved in these adaptatory responses seem to be also organ and species specific. Thus, hypothesised pattern of enzymatic compensation may be disclosed only under some characteristic circumstances, which – nevertheless – does not exclude its regular but transient existence as an early stage in the course of adaptative response. Interactions among cadmium and selenium seem to be the best models to evoke this effect.

On the basis of discussed results, the hypothesis on mutual compensatory mechanism may be drawn and summarised as follows. In animals in which toxic agent inhibits Se-GPX dependent pathway of free radical scavenger system a compensatory stimulation of SOD and CAT activity may develop to replace inhibited process. Moreover, partially depressed activity of GPXs may be compensated by increasing a "drive" and actual reaction rate of these enzymes, due to an improved flux and availability of reduced glutathione, if only activity of GR rises sufficiently and the pool of reduced nicotinamide dinucleotide phosphate (NADPH) is maintained. This mechanism may also act in the opposite direction compensating decreased activity of CAT by GPX or GST activity. Moreover, selenium compounds may stimulate Se-GPX, but at the same time exert antagonistic action upon CAT activity, which undergoes a reduction, as it was observed in this study. The process of compensation is mediated rather by a cellular content of free-radical intermediates, than by common genetical regulatory mechanism. The specific species which induces compensatory response is not oxygen free-radical since oxygen radical generating compounds (PQ) do not produce this effect. Opposing results of experiments with transfectionally manipulated cell lines (Amstad et al. 1994; Michiels et al. 1994) do not settle the doubts on proposed regulatory mechanism, as transfected genes need not be incorporated into the exact regulatory unit of the host genome.

Concomitant cadmium and selenium administration might accomplish this mechanism because of possible Se-Cd chemical complexation (Mochizuki et al. 1982; Naganuma et al. 1982; Nath et al. 1984) and a competition for cysteine which reduces the content of GSH available for GPX isozymes (Kawate and Suzuki 1983). Various chemical forms of supplementary selenium may exert slightly different

effects on these processes because of their diverse metabolism and action on the isozymes of GPX (Lane et al. 1991; Ip et al. 1991; Beilstein and Whanger 1986 a, b; Mason and Weaver 1986).

Some interesting questions arising from presented hypothesis consist in estimations of substrate and product fluxes across the GPX – GR system, which can be done only by sophisticated analyses of kinetic properties of these enzymes and then modelled mathematically or in the *in vitro* reconstituted system. Another question lies in the compartmentation pattern and substrate affinity of CAT and various isoforms of GPXs, which cause that the substrate available for one enzyme need not be available for the other (Michiels et al. 1994; Agar et al. 1986; Clemens and Waller 1987).

The results summarised and reviewed above allow to speculate that the relations among enzyme activity have to be more complicated and dynamically adjustable to momentary conditions than in the discussed hypothesis. Moreover, they do not seem to be limited to the main links of antiperoxidative system, especially to the simple relations among two particular enzymes, but they would rather constitute a wide multidirectional network of relations. Signs of this complexity were represented in the reviewed material, for example, as the correlations among CAT and GR or among SOD and GST. If such a system acts in each animal cell as well as in whole organs, the discovery of any particular element of this network may be done only under very specific conditions. Simpler models based on cell cultures may be more suitable for the search of these relations.

Nevertheless, the final statement answering the questions posed in the aims of the present study is that the causal relationships among examined indices of antioxidative processes do exist and are universal in the sense of taxonomic variability. Moreover, these relationships may be described as a kind of a mutual compensation among the activities of particular enzymatic components of antioxidative system.

6.4. Summary of supplemental observations

Despite the major conclusions of the study (presented in the next chapter) several miscellaneous observations and conclusions may be drawn on the basis of obtained results and are worth stressing here:

- The protection exerted by vitamin E against cadmium or paraquat enhanced lipid peroxidation and against paraquat induced alterations of enzyme activity (SOD, GPX, CAT – Tab. 6–9) suggests that products of peroxidation (but not

cadmium or paraquat themselves) participate in the process of enzyme inhibition and act as enzyme inductors.

- The results of hydrocortisone administration to mice demonstrate that reactions to prooxidative factors do not consist in stress response, especially in the processes regulated by steroid hormones of adrenals.
- Despite the similarity of effects of oral and parenteral acute treatment with Cd in respect to the tissue content of cadmium, physiological results vary with regard to the direction and magnitude of alterations of Se-GPX, GR, CAT activity and FeAsc-LP in the organs of mice. These differences may be attributed to a faster turnover of injected metal and to the direct toxic effects of metals which were not attenuated by defensive mechanisms in the digestive tract and liver.
- The brain of mice and rats shows signs of better protection against peroxidative damage than the liver, which was evidenced by the effects of cadmium poisoning on lipid peroxidation as well as on enzyme activity.
- Low activity of glutathione peroxidases in organs of invertebrate species may reflect diversity of defensive mechanisms characterised by relatively high activity of catalase and a presence of ascorbate peroxidase which replaces activity of glutathione peroxidase.
- Surprisingly high activity of glutathione reductase in relation to the activity of glutathione peroxidase was discovered in the examined slugs, but not in other species used in the experiments. This pattern of enzyme activity may reflect an unknown aspect of diversity of defensive mechanisms in various species.

7. Conclusions

1. A biphasic pattern of enzymatic response to various doses of prooxidant factors observed in the present experiments is an illustration of the hormetic effects of tested agents. Biphasic dose – response of enzyme activity reflects a variability of the induction threshold of particular enzymes and the complexity of balance among enzyme induction as well as all the deletorious effects on their activity. Biphasic effects may also reflect kinetics of turnover, accumulation and "effective" concentration of free active agents in the organs.

2. A complex "mosaic" response of several parameters in the organs of animals treated with prooxidants and antioxidants (i.e. opposite alterations of CAT activity in various organs of acutely poisoned mice) may be a result of a diverse distribution pattern of applied factors within organism as well as of specific susceptibility of particular organs and enzymes. Additionally, these observations of "mosaic" response pattern may reflect another aspect of biphasic dose – response relationships within the organs.

3. In the organs of animals, in which toxic agent inhibits Se-dependent glutathione peroxidase related pathway of free-radical scavenger system, a compensatory stimulation of catalase and superoxide peroxidase activity may develop to replace the inhibited process. This mechanism possibly acts also in the opposite direction, thus compensating for the decreased CAT by GPX or GST activity, as it was observed under selenite treatment in this study.

4. Partially depressed or apparently unchanged activity of glutathione peroxidases (GPXs) in the animals poisoned with tested prooxidants may be compensated by increasing the reaction rate of these enzymes due to the improved flux and availability of reduced glutathione, on the condition that activity of glutathione reductase (GR) will rise sufficiently and that the pool of reduced nicotinamide dinucleotide phosphate (NADPH) is maintained. This may be proved by the opposite alterations of GPX and GR in mice and frogs under paraquat treatment. This model of enzyme interactions should be reexamined in order to consider other relations among activities of GST, CAT and GR.

5. Postulated relationships among enzymes are mediated by cellular content of metabolites of toxic species rather, than by common genetical regulatory

mechanism, since observed alterations of particular enzymes are not concordant, unidirectional but vary, reflecting the diversity of the inducing stimuli.

6. The main frame of mutual compensatory mechanism within the antioxidative enzymatic system seems to be universal with regard to taxonomic differences, at least in the tested vertebrate species, insects and earthworms. However, snails demonstrated indirect signs of higher resistance against tested prooxidative factors, which was reflected by similar alterations of examined enzymes, with respect to direction and magnitude, as well as insignificant or marginal symptoms of biphasic response to noxious agents.

8. References

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9. Index of figures and tables

ABBREVIATIONS USED IN THE TABLES:

- Presented as follows: number of individuals (or the count of the group), mean value and ±standard deviation (±SD).
- The same letters – "superscripts" located after the mean value – (a, b, c, d) denote homogeneity of the marked groups for $P < 0.05$.
- The activity of superoxide dismutase (SOD) is expressed in units of Misra (the amount of enzyme causing half inhibition of spontaneous indicative reaction in standard conditions) per mg of protein content [U_{SOD}/mg $_{Prt}$].

- Activity of selenium dependent and selenium independent glutathione peroxidase (Se-GPX, nonSe-GPX), glutathione reductase (GR), glutathione S-transferase (GST) and activity of catalase (CAT) are expressed in micromoles of converted substrate per second and mg of protein [μmol/s/mg].
- The intensity of iron-ascorbate-stimulated lipid peroxidation (LP) is expressed as a yield of thiobarbituric acid reactive substances (TBARS) per 1 min and gram of tissue [nmol TBARS/g/min] for 30 minutes incubation.
- The concentration of TBARS is expressed in nanomoles of thiobarbituric acid reactive substances MDA per milligram of protein content in the examined fraction [nmol/mg protein].

Note: The amount of TBARS is usually considered as nearly equal to the amount of malondialdehyde (MDA).

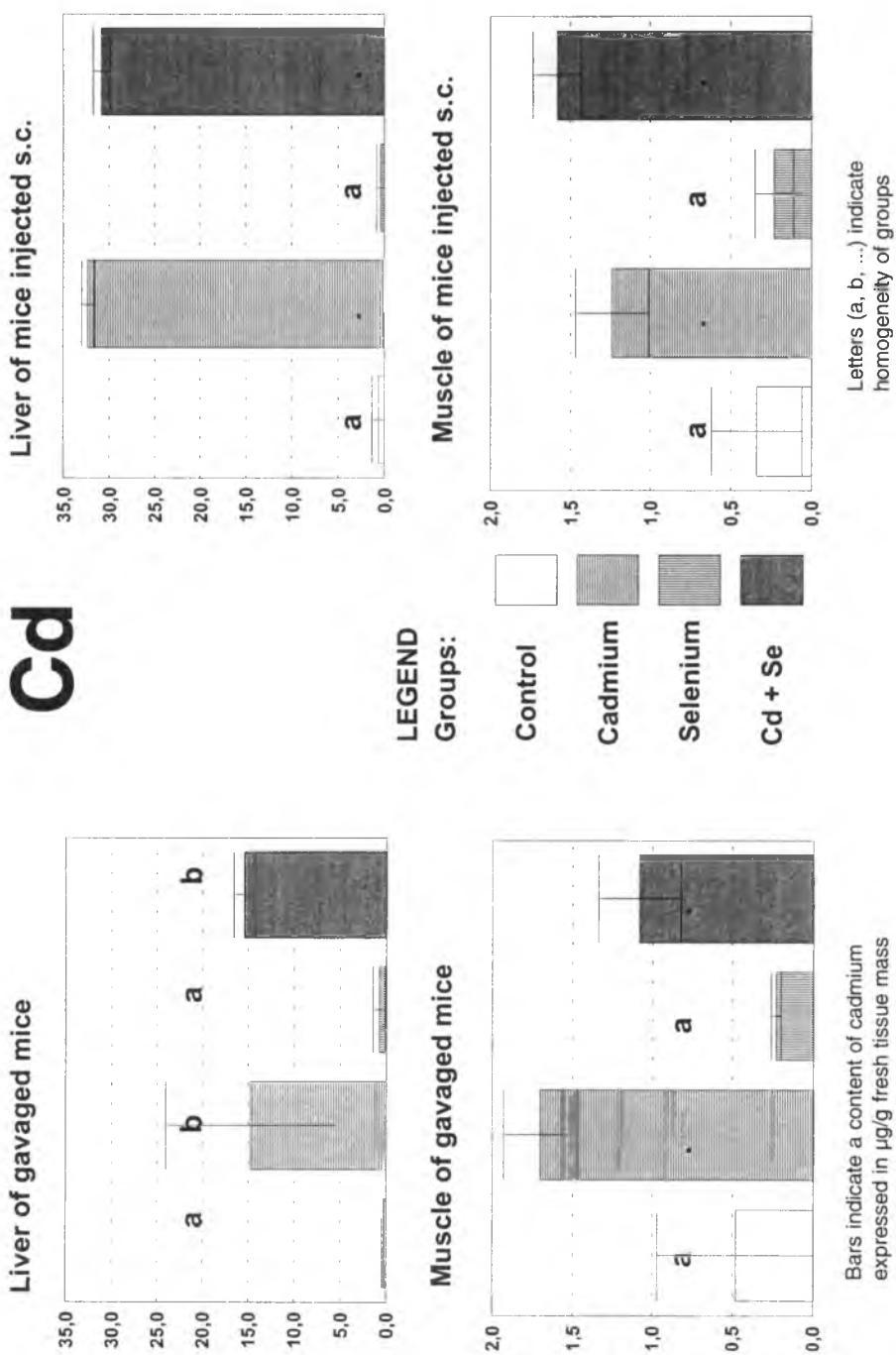


Fig. 3. Content of cadmium in organs mice treated with Cd and Se

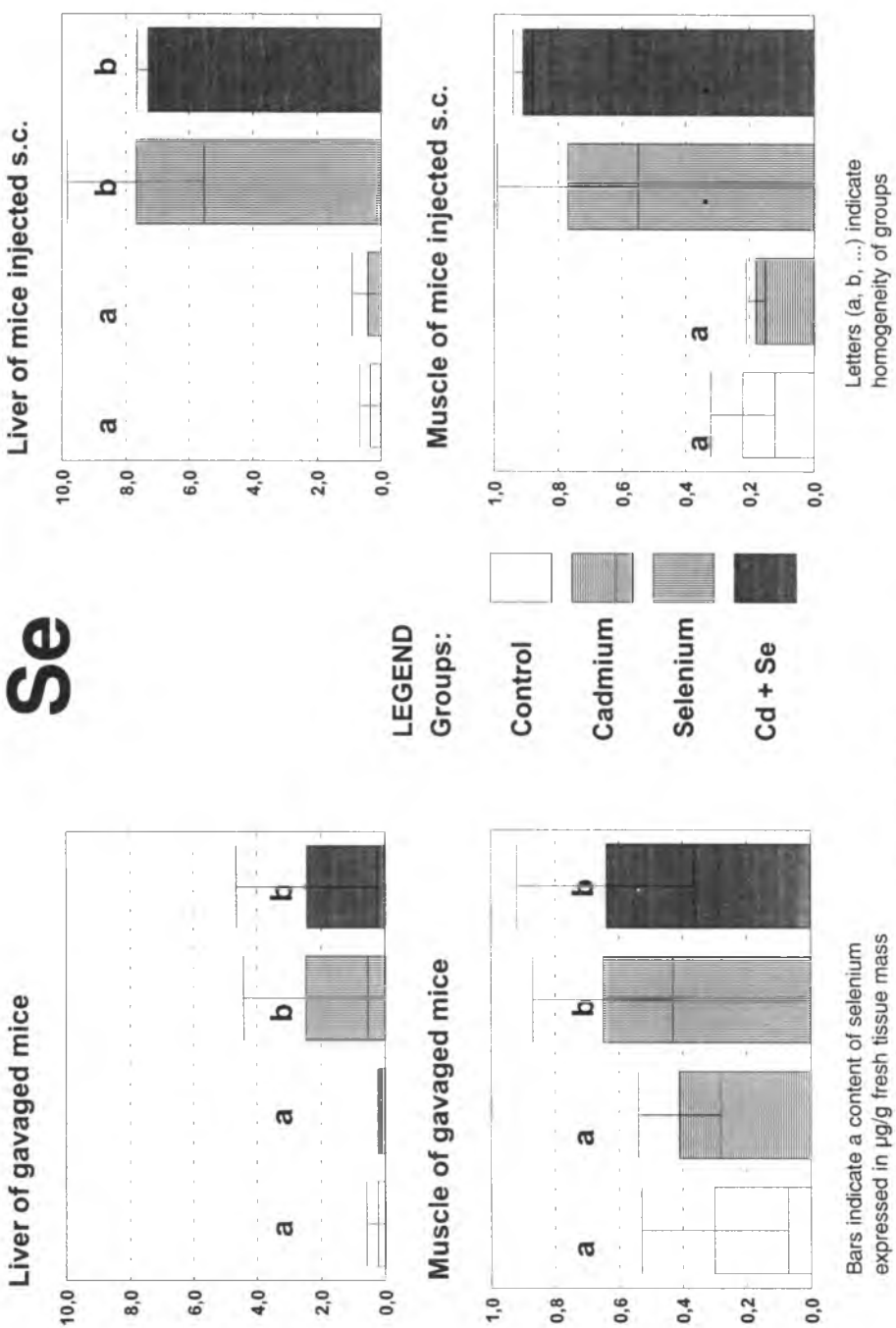
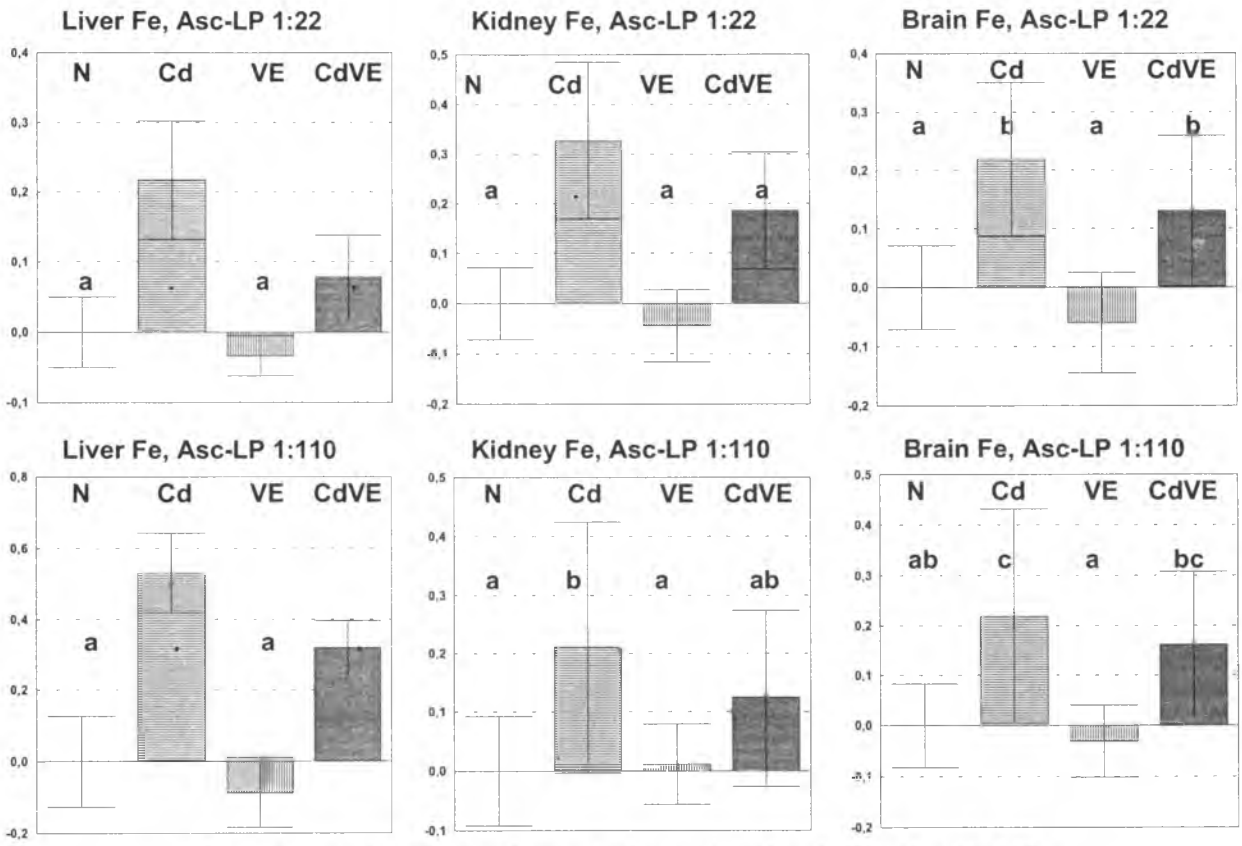


Fig. 4. Content of selenium in organs mice treated with Cd and Se



Bars represent relative change of parameter from control value which is reduced to 0.00.
Letters (a, b, ...) indicate homogeneity of groups

Fig. 5. Effects of Cd and vitamin E on lipid peroxidation in the rat organs

Table 2

The activity of superoxide dismutase (SOD), catalase (CAT), selenium-dependent glutathione peroxidase (Se-GPX), glutathione reductase (GR), glutathione S-transferase (GST) and stimulated lipid peroxidation (LP) in the liver of laboratory B6 mice from the control group (N) and from groups gavaged with cadmium acetate (C), selenite (S) or cadmium with selenite (SC). Presented: group count, mean value, ±SD

Liver	Control (N)	Cadmium (C)	Selenite (S)	Cadmium & Selenite (SC)
SOD	7 3.63 [a] ±0.28	8 2.82 [b] ±0.31	8 2.89 [b] ±0.26	7 3.48 [a] ±0.37
Se-GPX	9 2.85 [a] ±0.43	6 2.12 ±0.55	6 2.90 [a] ±0.37	5 2.84 [a] ±0.51
GR	10 1.14 [a] ±0.15	6 0.94 [b] ±0.13	6 1.03 [ba] ±0.13	6 1.06 [ba] ±0.11
GST	6 15.63 [ab] ±1.50	6 16.76 [b] ±2.10	6 19.81 ±2.34	6 14.06 [a] ±1.86
CAT	6 6.31 [a] ±0.53	5 6.82 [a] ±0.54	6 4.48 ±0.50	6 5.28 ±0.72
$LP_{1:21}$	10 17.65 [a] ±1.54	6 21.34 ±1.95	6 12.75 ±1.88	6 17.98 [a] ±1.74

Abbreviations – see Index of figures and tables on page 85

Table 3

The activity of superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (Se-GPX), glutathione reductase (GR), glutathione S-transferase (GST) and lipid peroxidation (LP) in the kidney of laboratory B6 mice from the control group (N) and groups gavaged with cadmium (C) and/or selenite (S; SC)

Kidney	Control (N)	Cadmium (C)	Selenite (S)	Cadmium & Selenite (SC)
SOD	10 2.49 [a] ±0.47	6 1.99 [b] ±0.35	6 2.29 [ba] ±0.34	6 2.75 [a] ±0.41
Se-GPX	10 2.39 [a] ±0.18	6 2.07 [b] ±0.24	6 2.34 [a] ±0.15	6 2.24 [ba] ±0.21
GR	9 2.56 ±0.12	6 2.24 [a] ±0.16	6 2.27 [a] ±0.14	6 2.13 [a] ±0.24
GST	6 4.21 [a] ±0.52	6 4.62 [a] ±0.42	6 4.60 [a] ±0.72	6 5.61 ±0.39
CAT	6 5.19 [a] ±0.56	6 4.06 ±0.64	6 5.59 [a] ±0.32	5 5.72 [a] ±0.67
$LP_{1:21}$	10 12.88 [ba] ±1.41	6 13.69 [a] ±0.80	6 11.94 [b] ±1.07	6 12.41 [ba] ±1.27

Abbreviations – see Index of figures and tables on page 85

Table 4

The activity of superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (Se-GPX), glutathione reductase (GR), glutathione S-transferase (GST) and lipid peroxidation (LP) in the heart of laboratory B6 mice from the control group (N) and groups gavaged with cadmium (C) and/or selenite (S; SC)

Heart	Control (N)	Cadmium (C)	Selenite (S)	Cadmium & Selenite (SC)
SOD	9 1.32 [a] ±0.20	6 1.31 [a] ±0.17	6 1.10 ±0.17	6 1.47 [a] ±0.15
Se-GPX	10 0.269 [a] ±0.033	6 0.195 [b] ±0.043	5 0.261 [a] ±0.039	6 0.235 [ba] ±0.044
GR	10 0.312 [a] ±0.057	6 0.382 ±0.045	6 0.299 [a] ±0.039	6 0.298 [a] ±0.049
GST	6 1.60 [a] ±0.15	6 1.51 [ba] ±0.09	6 1.44 [b] ±0.15	6 1.41 [b] ±0.07
CAT	6 0.84 [ba] ±0.14	6 0.87 [a] ±0.17	6 0.69 [b] ±0.12	6 0.67 [b] ±0.11
$LP_{1:21}$	10 9.39 [a] ±1.81	6 12.41 [b] ±1.88	6 10.07 [ab] ±1.81	6 11.47 [ab] ±2.75

Abbreviations – see Index of figures and tables on page 85

Table 5

The activity of superoxide dismutase (SOD), catalase (CAT), selenium dependent glutathione peroxidase (Se-GPX), glutathione reductase (GR), glutathione S-transferase (GST) and stimulated lipid peroxidation (LP) in the brain of laboratory B6 mice from control group (N) and groups gavaged with cadmium (C) and/or selenite (S; SC)

Brain	Control (N)	Cadmium (C)	Selenite (S)	Cadmium & Selenite (SC)
SOD	10 1.78 [ab] ±0.35	6 1.91 [ab] ±0.42	6 1.57 [a] ±0.25	6 2.05 [b] ±0.47
Se-GPX	10 0.374 [ab] ±0.084	6 0.319 [a] ±0.100	6 0.461 [b] ±0.160	6 0.447 [ab] ±0.133
GR	10 0.408 [a] ±0.067	6 0.281 [b] ±0.052	5 0.261 [b] ±0.077	6 0.340 [ba] ±0.092
GST	6 1.84 [ba] ±0.32	6 1.89 [a] ±0.19	6 1.80 [ba] ±0.341	6 1.55 [b] ±0.21
CAT	5 0.373 [a] ±0.053	6 0.400 [a] ±0.088	6 0.392 [a] ±0.066	6 0.332 [a] ±0.087
$LP_{1:21}$	10 33.1 [a] ±2.2	6 36.5 [b] ±2.1	6 34.2 [ab] ±2.3	6 34.6 [ab] ±2.3

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Table 6

The activity superoxide dismutase (SOD), selenium-dependent glutathione peroxidase (GPX), glutathione reductase (GR), glutathione S-transferase (GST), catalase (CAT) and iron-ascorbate stimulated lipid peroxidation (LP) in the liver of mice from the control group (N) and from groups injected subcutaneously with cadmium acetate (C), selenite (S), paraquat (P), vitamin E (tocopherol = T) and hydrocortisone (HC). Presented as follows: number of individuals, mean value, ±standard deviation (±SD)

Liver	Control (N)	Cadmium (C)	Selenite (S)	Cadmium & Selenite (SC)	Paraquat (P)	Toco-pherol (T)	Paraquat & Toco-pherol (PT)	Hydro-cortisone (HC)
SOD	7 3.55 ^{ab} ±0.25	6 3.33 ^{a} ±0.23	6 3.60 ^{b} ±0.21	6 3.67 ^{bc} ±0.21	6 4.03 ^{d} ±0.19	6 3.51 ^{ab} ±0.17	6 3.91 ^{cd} ±0.20	6 3.35 ^{a} ±0.18
Se-GPX	12 2.76 ^{a} ±0.32	6 2.15 ±0.24	6 3.23 ^{b} ±0.41	6 3.16 ^{b} ±0.26	6 3.15 ^{b} ±0.34	6 2.97 ^{ab} ±0.27	6 3.03 ^{ab} ±0.40	6 2.99 ^{ab} ±0.20
GR	12 1.18 ^{a} ±0.10	6 1.25 ^{ab} ±0.15	6 1.43 ^{c} ±0.11	6 1.32 ^{bc} ±0.09	6 1.28 ^{ab} ±0.10	6 1.16 ^{a} ±0.13	6 1.27 ^{abc} ±0.10	6 1.37 ^{bc} ±0.14
GST	12 15.7 ^{a} ±1.4	6 18.0 ^{b} ±1.4	6 20.1 ^{c} ±1.3	6 20.3 ^{c} ±1.5	6 16.3 ^{ab} ±1.9	6 15.7 ^{a} ±0.9	6 16.5 ^{ab} ±1.4	6 19.9 ^{b} ±2.2
CAT	12 5.61 ^{ab} ±0.62	6 7.26 ^{c} ±0.87	6 5.97 ^{bc} ±0.50	6 5.65 ^{ab} ±0.61	6 6.39 ^{c} ±0.49	6 5.02 ^{a} ±0.52	6 5.87 ^{bc} ±0.54	6 5.75 ^{bc} ±0.52
$LP_{1:21}$	12 17.98 ^{bc} ±1.94	6 21.14 ^{cd} ±2.41	6 14.16 ^{a} ±3.82	6 15.70 ^{a} ±1.54	6 21.87 ^{b} ±1.94	6 14.36 ^{a} ±2.08	6 16.37 ^{ab} ±2.21	6 19.06 ^{cd} ±1.68

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Table 7

The activity superoxide dismutase (SOD), selenium-dependent glutathione peroxidase (GPX), glutathione reductase (GR), glutathione S-transferase (GST), catalase (CAT) and iron-ascorbate stimulated lipid peroxidation (LP) in the kidney of mice from the control group (N) and from groups injected with cadmium acetate (C), selenite (S), paraquat (P), vitamin E (tocopherol = T) and hydrocortisone (HC)

Kidney	Control (N)	Cadmium (C)	Selenite (S)	Cadmium & Selenite (SC)	Paraquat (P)	Toco-pherol (T)	Paraquat & Toco-pherol (PT)	Hydro-cortisone (HC)
SOD	7 2.57^{ab} ±0.17	6 2.32^{a} ±0.23	6 2.63^{bc} ±0.18	6 2.67^{bc} ±0.29	6 2.83^{c} ±0.29	6 2.52^{ab} ±0.20	6 2.73^{bc} ±0.23	6 2.49^{ab} ±0.25
Se-GPX	12 2.37^{a} ±0.09	6 1.91 ±0.16	6 2.80^{b} ±0.28	6 2.66^{bc} ±0.28	6 2.46^{ac} ±0.35	6 2.39^{a} ±0.26	6 2.20^{a} ±0.26	6 2.47^{ac} ±0.21
GR	11 2.58^{a} ±0.16	6 2.70^{ab} ±0.20	6 2.58^{a} ±0.19	6 2.67^{ab} ±0.11	6 2.64^{ab} ±0.20	6 2.64^{ab} ±0.23	6 2.80^{b} ±0.24	6 2.84^{b} ±0.13
GST	12 4.37^{a} ±0.60	6 4.50^{a} ±0.57	6 4.28^{a} ±0.43	6 4.37^{a} ±0.77	6 5.26^{b} ±0.70	6 4.59^{ab} ±0.63	6 4.62^{ab} ±0.74	6 4.98^{ab} ±0.29
CAT	12 5.20^{abc} ±0.47	6 6.17^{c} ±0.38	6 5.58^{cd} ±0.51	6 5.84^{dc} ±0.63	6 5.61^{cde} ±0.42	6 4.96^{ab} ±0.43	6 5.52^{acd} ±0.48	6 4.82^{b} ±0.57
$LP_{1\text{-}21}$	12 12.88^{ab} ±1.48	6 15.50^{c} ±1.27	6 11.34^{d} ±1.01	6 12.35^{ad} ±1.34	6 15.70^{c} ±1.94	6 13.02^{ab} ±1.48	6 14.22^{abc} ±0.94	6 13.69^{ab} ±1.21

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Table 8

The activity of superoxide dismutase (SOD), selenium-dependent glutathione peroxidase (GPX), glutathione reductase (GR), glutathione S-transferase (GST), catalase (CAT) and iron-ascorbate stimulated lipid peroxidation (LP) in the heart of mice from the control group (N) and from groups injected with cadmium acetate (C), selenite (S), paraquat (P), vitamin E (tocopherol = T) and hydrocortisone (HC)

Heart	Control (N)	Cadmium (C)	Selenite (S)	Cadmium & Selenite (SC)	Paraquat (P)	Toco-pherol (T)	Paraquat & Toco-pherol (PT)	Hydro-cortisone (HC)
SOD	7 1.30 ^{ab} ±0.11	6 1.02 ±0.08	6 1.29 ^{ab} ±0.06	6 1.24 ^{a} ±0.09	6 1.47 ^{c} ±0.08	6 1.23 ^{a} ±0.10	6 1.35 ^{b} ±0.12	6 1.26 ^{ab} ±0.06
Se-GPX	12 0.257 ^{a} ±0.036	6 0.242 ^{a} ±0.030	6 0.265 ^{ab} ±0.026	6 0.266 ^{ab} ±0.032	6 0.304 ^{b} ±0.031	6 0.247 ^{a} ±0.034	6 0.251 ^{a} ±0.041	6 0.299 ^{b} ±0.038
GR	12 0.302 ^{ab} ±0.030	6 0.280 ^{a} ±0.025	6 0.281 ^{a} ±0.030	6 0.282 ^{ab} ±0.027	6 0.349 ^{c} ±0.027	6 0.315 ^{b} ±0.026	6 0.295 ^{ab} ±0.033	6 0.310 ^{ab} ±0.030
GST	12 1.54 ^{ab} ±0.09	6 1.65 ^{b} ±0.09	6 1.72 ^{b} ±0.10	6 1.57 ^{b} ±0.13	6 1.41 ^{c} ±0.09	6 1.43 ^{ac} ±0.16	6 1.50 ^{abc} ±0.12	6 1.69 ^{b} ±0.14
CAT	12 0.772 ^{a} ±0.068	6 0.763 ^{ab} ±0.062	6 0.648 ^{dc} ±0.065	6 0.580 ^{e} ±0.061	6 0.873 ^{c} ±0.057	6 0.665 ^{dc} ±0.102	6 0.847 ^{ac} ±0.116	6 0.692 ^{bd} ±0.060
$LP_{1:21}$	12 10.60 ^{bc} ±1.01	6 10.93 ^{bcd} ±0.80	6 9.93 ^{ab} ±0.74	6 9.53 ^{a} ±0.94	6 11.47 ^{cd} ±1.21	6 9.33 ^{a} ±1.48	6 9.80 ^{a} ±1.61	6 11.74 ^{d} ±0.74

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Table 9

The activity of total superoxide dismutase (SOD), selenium-dependent glutathione peroxidase (GPX), glutathione reductase (GR), glutathione S-transferase (GST), catalase (CAT) and iron-ascorbate stimulated lipid peroxidation (LP) in the brain of mice from the control group (N) and from groups injected with cadmium acetate (C), selenite (S), paraquat (P), vitamin E (tocopherol = T) and hydrocortisone (HC)

Brain	Control (N)	Cadmium (C)	Selenite (S)	Cadmium & Selenite (SC)	Paraquat (P)	Toco-pherol (T)	Paraquat & Toco-pherol (PT)	Hydro-cortisone (HC)
SOD	7 2.01 ^{bc} ±0.13	6 1.13 ^{a} ±0.12	6 1.89 ^{b} ±0.18	6 2.13 ^{cd} ±0.13	6 2.17 ^{d} ±0.15	6 1.89 ^{b} ±0.15	6 2.07 ^{cd} ±0.13	6 2.12 ^{cd} ±0.11
Se-GPX	12 0.345 ^{a} ±0.057	6 0.321 ^{a} ±0.081	6 0.382 ^{a} ±0.058	6 0.349 ^{a} ±0.041	6 0.359 ^{a} ±0.068	6 0.356 ^{a} ±0.063	6 0.377 ^{a} ±0.062	6 0.331 ^{a} ±0.039
GR	12 0.440 ^{b} ±0.032	6 0.397 ^{a} ±0.024	6 0.443 ^{b} ±0.025	6 0.399 ^{a} ±0.023	6 0.454 ^{b} ±0.023	6 0.465 ^{b} ±0.039	6 0.438 ^{b} ±0.021	6 0.398 ^{a} ±0.024
GST	12 2.19 ^{a} ±0.28	6 2.29 ^{a} ±0.26	6 2.31 ^{a} ±0.21	6 2.05 ^{a} ±0.19	6 2.31 ^{a} ±0.19	6 2.33 ^{a} ±0.20	6 2.10 ^{a} ±0.32	6 2.23 ^{a} ±0.25
CAT	12 0.373 ^{b} ±8.089	6 0.352 ^{ab} ±0.071	6 0.278 ^{a} ±0.065	6 0.443 ^{bc} ±0.104	6 0.411 ^{bc} ±0.066	5 0.412 ^{bc} ±0.101	6 0.461 ^{c} ±0.054	6 0.417 ^{bc} ±0.056
$LP_{1:21}$	7 32.54 ^{b} ±2.41	6 35.23 ^{c} ±3.22	6 31.87 ^{b} ±1.74	6 30.33 ^{b} ±2.41	6 35.70 ^{c} ±2.48	6 31.74 ^{b} ±3.82	6 31.87 ^{b} ±2.15	6 33.01 ^{bc} ±1.34

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Table 10

The activity of selenium-dependent-glutathione peroxidase (Se-GPX), selenium-independent-glutathione peroxidase (nonSe-GPX), glutathione reductase (GR), glutathione S-transferase (GST) and catalase (CAT) in the liver of laboratory strain B6 mice from the control (N) and from paraquat (P-02, P-05, P-12) poisoned groups. Presented as follows: number of individuals, mean value, ±standard deviation

Liver	Control (N)	Paraquat 02 (P-02)	Paraquat 05 (P-05)	Paraquat 12 (P-12)
Se-GPX	6 3.22 [a] ±0.41	6 3.15 [ab] ±0.61	6 2.76 [ab] ±0.77	7 2.12 [b] ±0.43
nonSe-GPX	6 17.31 [ab] ±1.04	6 16.72 [ab] ±1.84	6 17.82 [a] ±1.24	7 16.00 [b] ±1.95
GR	6 1.32 [a] ±0.14	6 1.16 [a] ±0.16	6 1.17 [a] ±0.15	7 1.14 [a] ±0.20
GST	6 20.67 [a] ±2.81	6 21.47 [a] ±4.39	6 20.87 [a] ±3.68	7 18.87 [a] ±3.77
CAT	6 5.32 ±0.77	6 4.37 [a] ±0.33	6 4.40 [a] ±0.51	7 3.33 ±0.74

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Table 11

The activity of selenium-dependent-glutathione peroxidase (Se-GPX), selenium-independent-glutathione peroxidase (nonSe-GPX), glutathione reductase (GR), glutathione S-transferase (GST) and catalase (CAT) in the kidney of laboratory strain B6 mice from the control (N) and from paraquat (P-02, P-05, P-12) poisoned groups

Kidney	Control (N)	Paraquat 02 (P-02)	Paraquat 05 (P-05)	Paraquat 12 (P-12)
Se-GPX	6 2.09 [ab] ±0.34	6 2.36 [a] ±0.38	6 2.21 [ab] ±0.23	7 1.87 [b] ±0.32
nonSe-GPX	6 2.92 [ab] ±0.23	6 3.03 [b] ±0.30	6 3.03 [b] ±0.16	7 2.70 [a] ±0.37
GR	6 2.08 [a] ±0.44	6 2.13 [a] ±0.35	6 2.13 [a] ±0.51	7 2.03 [a] ±0.23
GST	6 4.04 [a] ±0.75	6 3.86 [a] ±1.02	6 4.21 [a] ±0.95	7 3.97 [a] ±0.70
CAT	6 4.81 [a] ±0.71	6 4.64 [a] ±0.74	6 4.87 [a] ±0.59	7 4.13 [a] ±0.65

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Table 12

The activity of selenium-dependent-glutathione peroxidase (Se-GPX), selenium-independent-glutathione peroxidase (nonSe-GPX), glutathione reductase (GR), glutathione S-transferase (GST) and catalase (CAT) in the intestine of laboratory strain B6 mice from the control (N) and from paraquat (P-02, P-05, P-12) poisoned groups

Intestine	Control (N)	Paraquat 02 (P-02)	Paraquat 05 (P-05)	Paraquat 12 (P-12)
Se-GPX	6 0.272 [a] ±0.038	6 0.327 [ab] ±0.073	6 0.275 [a] ±0.055	7 0.372 [ab] ±0.089
nonSe-GPX	6 0.361 [a] ±0.117	6 0.502 [b] ±0.127	6 0.341 [a] ±0.072	7 0.396 [ab] ±0.093
GR	6 2.89 [a] ±0.30	6 3.44 ±0.27	6 2.88 [a] ±0.36	7 2.35 ±0.51
GST	6 1.31 [a] ±0.27	6 1.78 [ab] ±0.62	6 1.41 [ab] ±0.30	7 1.62 [ab] ±0.28
CAT	6 0.239 [a] ±0.047	6 0.284 [a] ±0.054	6 0.264 [a] ±0.063	7 0.237 [a] ±0.104

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Table 13

The activity of selenium-dependent-glutathione peroxidase (Se-GPX), selenium-independent-glutathione peroxidase (nonSe-GPX), glutathione reductase (GR), glutathione S-transferase (GST) and catalase (CAT) in the brain of laboratory strain B6 mice from the control (N) and from paraquat (P-02, P-05, P-12) poisoned groups

Brain	Control (N)	Paraquat 02 (P-02)	Paraquat 05 (P-05)	Paraquat 12 (P-12)
Se-GPX	6 0.241 [a] ±0.093	6 0.255 [a] ±0.076	6 0.244 [a] ±0.052	7 0.231 [a] ±0.058
nonSe-GPX	6 0.231 [a] ±0.103	6 0.237 [a] ±0.093	6 0.220 [a] ±0.065	7 0.248 [a] ±0.052
GR	6 0.750 [ab] ±0.047	6 0.788 [a] ±0.068	6 0.706 [ab] ±0.115	7 0.662 [b] ±0.091
GST	6 1.90 [a] ±0.23	6 1.86 [a] ±0.12	6 1.80 [a] ±0.23	7 1.86 [a] ±0.21
CAT	6 0.336 [a] ±0.063	6 0.285 [a] ±0.164	6 0.428 [a] ±0.146	7 0.285 [a] ±0.134

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Table 14A

The activity of superoxide dismutase (SOD), glutathione peroxidase (GPX), glutathione reductase (GR), glutathione S-transferase (GST) and catalase (CAT) in the liver of frogs (*Rana esculenta L.*) from the control (N) and paraquat (PQ01, PQ04, PQ19) poisoned groups. Presented: count of groups, mean value, ±SD

Liver	Control (N)	Paraquat 01 (PQ01)	Paraquat 04 (PQ04)	Paraquat 19 (PQ19)
SOD	17 1.44 [a] ±0.27	10 1.56 [a] ±0.29	9 1.66 [a] ±0.23	10 1.59 [a] ±0.35
Se-GPX	17 0.439 [a] ±0.089	10 0.491 [ab] ±0.147	9 0.555 [b] ±0.184	10 0.410 [a] ±0.113
GR	17 0.502 [a] ±0.121	10 0.592 [ab] ±0.235	9 0.579 [ab] ±0.133	10 0.680 [b] ±0.159
GST	17 2.72 ±0.48	10 3.84 [a] ±0.50	9 4.18 [a] ±0.63	10 4.23 [a] ±0.78
CAT	16 25.53 [a] ±6.31	8 26.71 [a] ±8.06	7 35.15 ±9.87	9 22.55 [a] ±4.91

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Table 14B

The activity of superoxide dismutase (SOD), glutathione peroxidase (GPX), glutathione reductase (GR), glutathione S-transferase (GST) and catalase (CAT) in the liver of frogs (*Rana esculenta L.*) from the control (N) and cadmium (Cd04, Cd16, Cd64) poisoned groups

Liver	Control (N)	Cadmium 04 (Cd04)	Cadmium 16 (Cd16)	Cadmium 64 (Cd64)
SOD	17 1.43 [a] ±0.26	10 1.47 [a] ±0.30	10 1.56 [a] ±0.34	9 1.53 [a] ±0.28
Se-GPX	17 0.439 [a] ±0.089	10 0.527 [bc] ±0.092	10 0.590 [c] ±0.128	9 0.445 [ab] ±0.088
GR	17 0.502 [a] ±0.121	10 0.627 [ab] ±0.165	10 0.660 [b] ±0.204	9 0.734 [b] ±0.253
GST	17 2.72 ±0.48	10 3.72 [a] ±0.98	10 3.85 [a] ±0.96	9 3.59 [a] ±0.72
CAT	16 25.23 [a] ±6.31	10 28.44 [a] ±5.12	10 28.22 [a] ±5.11	9 24.90 [a] ±5.75

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Table 15A

The activity of superoxide dismutase (SOD), glutathione peroxidase (GPX), glutathione reductase (GR), glutathione S-transferase (GST) and catalase (CAT) in the kidney of frogs (*Rana esculenta L.*) from the control (N) and paraquat (PQ01, PQ04, PQ19) poisoned groups. Presented: count of groups, mean value, ±SD

Kidney	Control (N)	Paraquat 01 (PQ01)	Paraquat 04 (PQ04)	Paraquat 19 (PQ19)
SOD	17 1.39 [a] ±0.29	10 1.43 [a] ±0.17	9 1.43 [a] ±0.18	10 1.35 [a] ±0.21
Se-GPX	17 1.17 [b] ±0.22	10 0.93 [a] ±0.18	9 1.12 [ab] ±0.11	10 1.12 [ab] ±0.33
GR	17 2.42 [a] ±0.50	10 2.64 [a] ±0.75	9 2.83 [ab] ±0.83	10 3.48 [b] ±0.94
GST	17 4.28 [a] ±0.97	10 5.06 [ab] ±0.80	9 5.53 [b] ±1.52	10 5.195 [b] ±1.07
CAT	16 9.56 [b] ±2.82	8 9.61 [b] ±2.58	7 8.28 [ab] ±2.20	9 6.29 [a] ±1.18

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Table 15B

The activity of superoxide dismutase (SOD), glutathione peroxidase (GPX), glutathione reductase (GR), glutathione S-transferase (GST) and catalase (CAT) in the kidney of frogs (*Rana esculenta L.*) from the control (N) and cadmium (Cd04, Cd16, Cd64) poisoned groups

Kidney	Control (N)	Cadmium 04 (Cd04)	Cadmium 16 (Cd16)	Cadmium 64 (Cd64)
SOD	17 1.39 [b] ±0.29	10 1.22 [ab] ±0.31	10 1.01 [a] ±0.37	9 0.87 [a] ±0.11
Se-GPX	17 1.17 [b] ±0.22	10 1.05 [ab] ±0.25	10 0.94 [a] ±0.26	9 0.85 [a] ±0.21
GR	17 2.42 [a] ±0.50	10 2.70 [a] ±0.70	10 2.31 [a] ±0.93	9 2.31 [a] ±0.72
GST	17 4.28 [a] ±0.97	10 4.21 [a] ±0.93	10 3.90 [a] ±1.08	9 3.56 [a] ±0.98
CAT	16 9.56 ±2.82	10 7.25 [a] ±2.25	10 6.94 [a] ±2.51	9 5.79 [a] ±1.35

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Table 16A

The activity of superoxide dismutase (SOD), glutathione peroxidase (GPX), glutathione reductase (GR), glutathione S-transferase (GST) and catalase (CAT) in the intestine of frogs (*Rana esculenta L.*) from the control (N) and paraquat (PQ01, PQ04, PQ19) poisoned groups. Presented: count of groups, mean value, ±SD

Intestine	Control (N)	Paraquat 01 (PQ01)	Paraquat 04 (PQ04)	Paraquat 19 (PQ19)
SOD	16 0.896 [b] ±0.153	10 0.804 [ab] ±0.083	9 0.775 [a] ±0.093	10 0.826 [ab] ±0.082
Se-GPX	17 1.51 [a] ±0.47	10 1.86 [ab] ±0.52	9 2.12 [b] ±0.59	10 2.06 [b] ±0.70
GR	17 2.27 [a] ±0.42	10 2.15 [a] ±0.52	9 1.91 [a] ±0.49	10 2.26 [a] ±0.40
GST	17 2.93 [b] ±0.68	10 2.32 [a] ±0.85	9 2.43 [ab] ±0.68	10 2.54 [ab] ±0.59
CAT	15 1.11 [a] ±0.38	8 1.34 [a] ±0.80	7 1.48 [a] ±0.97	9 1.27 [a] ±0.64

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Table 16B

The activity of superoxide dismutase (SOD), glutathione peroxidase (GPX), glutathione reductase (GR), glutathione S-transferase (GST) and catalase (CAT) in the intestine of frogs (*Rana esculenta L.*) from the control (N) and cadmium (Cd04, Cd16, Cd64) poisoned groups

Intestine	Control (N)	Cadmium 04 (Cd04)	Cadmium 16 (Cd16)	Cadmium 64 (Cd64)
SOD	16 0.896 [b] ±0.153	10 0.801 [ab] ±0.236	10 0.779 [ab] ±0.207	9 0.648 [a] ±0.126
Se-GPX	17 1.51 [a] ±0.47	10 1.73 [ab] ±0.38	10 1.89 [b] ±0.31	9 1.64 [ab] ±0.42
GR	17 2.27 [a] ±0.42	10 2.54 [a] ±0.53	10 2.48 [a] ±0.62	9 2.19 [a] ±0.87
GST	17 2.93 [a] ±0.68	10 2.97 [a] ±0.65	10 3.08 [a] ±1.00	9 2.67 [a] ±0.57
CAT	15 1.11 [a] ±0.38	8 1.12 [a] ±0.33	9 1.06 [a] ±0.35	9 0.86 [a] ±0.23

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Table 17

The activity of superoxide dismutase (SOD), selenium-dependent and selenium-independent glutathione peroxidase (Se-GPX, nonSe-GPX), glutathione S-transferase (GST), catalase (CAT) and content of thiobarbituric acid reactive substances (TBARS) in the hepatopancreas of slugs *Agrolimax* from the control (N), paraquat or carbon tetrachloride poisoned (PQ-02, PQ-05, PQ-10, TC-01, TC-05) groups. Presented: group count, mean, ±SD

Slugs Hepatopancreas	Control (N)	Paraquat 02 (PQ-02)	Paraquat 05 (PQ-05)	Paraquat 10 (PQ-10)	CCl_4 01 (TC-01)	CCl_4 05 (TC-05)
SOD	19 0.99 [a] ±0.31	9 1.39 [b] ±0.29	7 1.22 [ab] ±0.38	6 1.04 [ab] ±0.40	5 1.24 [ab] ±0.35	10 1.06 [ab] ±0.49
Se-GPX	24 0.240 [a] ±0.072	8 0.312 [ab] ±0.134	7 0.350 [b] ±0.041	7 0.233 [a] ±0.109	4 0.295 [ab] ±0.093	10 0.268 [ab] ±0.126
nonSe-GPX	24 0.216 [a] ±0.054	9 0.253 [ab] ±0.097	8 0.283 [b] ±0.081	7 0.201 [a] ±0.074	5 0.256 [ab] ±0.064	10 0.219 [ab] ±0.082
GST	21 1.50 [ab] ±0.44	9 1.63 [ab] ±0.64	8 1.51 [ab] ±0.38	7 1.30 [a] ±0.43	5 1.50 [ab] ±0.29	11 1.81 [b] ±0.64
CAT	24 11.04 [bc] ±2.43	9 9.15 [a] ±2.55	8 9.21 [ab] ±0.72	7 10.53 [abc] ±2.07	5 12.42 [c] ±3.27	11 11.64 [c] ±2.61
TBARS	23 0.0203 [b] ±0.0055	9 0.0185 [b] ±0.0055	8 0.0157 [b] ±0.0039	7 0.0180 [b] ±0.0063	5 0.0156 [ab] ±0.0019	11 0.0107 [a] ±0.0039

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Table 18

The activity of superoxide dismutase (SOD), selenium-dependent and selenium-independent glutathione peroxidase (Se-GPX, nonSe-GPX), glutathione S-transferase (GST), catalase (CAT) and content of thiobarbituric acid reactive substances (TBARS) in the intestine of slugs *Agrolimax* from the control (N), paraquat or carbon tetrachloride poisoned (PQ-02, PQ-05, PQ-10, TC-01, TC-05) groups. Presented: group count, mean, ±SD

Slugs Intestine	Control (N)	Paraquat 02 (PQ-02)	Paraquat 05 (PQ-05)	Paraquat 10 (PQ-10)	CCl_4 01 (TC-01)	CCl_4 05 (TC-05)
SOD	20 1.62 [a] ±0.40	7 1.93 [a] ±0.59	7 1.66 [a] ±0.57	5 1.43 [a] ±0.32	5 2.76 ±1.25	10 1.91 [a] ±0.60
Se-GPX	17 0.262 [a] ±0.093	6 0.337 [a] ±0.168	7 0.308 [a] ±0.150	5 0.250 [a] ±0.082	4 0.280 [a] ±0.138	7 0.292 [a] ±0.155
nonSe-GPX	20 0.268 [a] ±0.072	7 0.376 [b] ±0.105	7 0.326 [ab] ±0.146	5 0.296 [ab] ±0.141	3 0.312 [ab] ±0.037	10 0.323 [ab] ±0.134
GST	21 3.37 [a] ±0.74	7 4.44 [b] ±1.54	7 4.44 [b] ±0.74	5 3.54 [ab] ±1.32	5 3.92 [ab] ±0.82	11 3.86 [ab] ±0.60
CAT	20 29.7 [a] ±7.1	7 31.6 [a] ±15.7	7 32.5 [a] ±14.4	5 30.8 [a] ±8.8	4 31.9 [a] ±8.0	10 33.1 [a] ±15.4
TBARS	17 0.0232 [ab] ±0.0110	7 0.0177 [a] ±0.0042	7 0.0184 [a] ±0.0072	7 0.0180 [a] ±0.0063	6 0.0284 [bc] ±0.0037	6 0.0308 [c] ±0.0041

Abbreviations – see Index of figures and tables on page 85

Table 19

The activity of superoxide dismutase (SOD), selenium-dependent and selenium-independent glutathione peroxidase (Se-GPX, nonSe-GPX), glutathione S-transferase (GST), catalase (CAT) and content of thiobarbituric acid reactive substances (TBARS) in the foot muscle of slugs *Agrolimax* from the control (N) and paraquat or carbon tetrachloride (PQ-02, PQ-05, PQ-10, TC-01, TC-05) poisoned groups. Presented: group count, mean, ±SD

Slugs Foot Muscle	Control (N)	Paraquat 02 (PQ-02)	Paraquat 05 (PQ-05)	Paraquat 10 (PQ-10)	CCl_4 01 (TC-01)	CCl_4 05 (TC-05)
SOD	20 1.69 [a] ±0.56	9 2.28 [b] ±1.11	8 2.44 [b] ±0.79	7 2.31 [b] ±0.64	5 2.89 ±0.49	10 1.88 [ab] ±0.44
Se-GPX	8 0.065 [a] ±0.009	2 0.076 [a] ±0.041	1 0.059 [a] ±0.000	2 0.092 [a] ±0.016	nd	nd
nonSe-GPX	9 0.067 [a] ±0.028	2 0.073 [ab] ±0.001	1 0.112 [ab] ±0.000	2 0.118 [b] ±0.004	nd	nd
GST	13 1.22 [ab] ±0.26	4 0.95 [a] ±0.63	1 0.99 [ab] ±0.00	3 1.06 [ab] ±0.29	5 1.45 [b] ±0.30	6 1.30 [ab] ±0.22
CAT	24 1.56 [a] ±0.54	9 1.44 [a] ±0.60	8 1.32 [a] ±0.54	7 1.50 [a] ±0.84	5 1.44 [a] ±0.72	11 1.26 [a] ±0.36
TBARS	21 0.0058 [b] ±0.0027	9 0.0049 [ab] ±0.0021	8 0.0057 [ab] ±0.0031	7 0.0032 [a] ±0.0009	5 0.0066 [b] ±0.0050	6 0.0038 [ab] ±0.0026

Abbreviations – see Index of figures and tables on page 85

Table 20

The activity of superoxide dismutase (SOD), selenium-dependent glutathione peroxidase (Se-GPX), glutathione reductase (GR) and catalase (CAT) in the hepatopancreas of slugs *Arion* from the control (N), cadmium (Cd04, Cd16, Cd64), selenium (Se) or cadmium and selenium (Cd04Se, Cd16Se, Cd64Se) treated groups. Presented: group count, mean, ±SD

Hepato-pancreas	Control (N)	Cadmium 04 (Cd04)	Cadmium 16 (Cd16)	Cadmium 64 (Cd64)	Selenium (Se)	Cadmium 04 & Selenium (Cd04Se)	Cadmium 16 & Selenium (Cd16Se)	Cadmium 64 & Selenium (Cd64Se)
SOD	9 1.30 ^{ab} ±0.18	9 1.44 ^{b} ±0.32	9 1.49 ^{b} ±0.24	9 1.26 ^{ab} ±0.25	10 1.19 ^{a} ±0.24	10 1.41 ^{ab} ±0.27	10 1.24 ^{ab} ±0.33	10 1.24 ^{ab} ±0.29
Se-GPX	10 0.272 ^{a} ±0.014	9 0.263 ^{a} ±0.013	10 0.311 ±0.018	9 0.251 ^{a} ±0.034	9 0.244 ^{a} ±0.031	9 0.257 ^{a} ±0.060	8 0.247 ^{a} ±0.062	9 0.220 ^{a} ±0.056
GR	9 2.33 ^{b} ±0.41	9 2.20 ^{ab} ±0.74	9 2.34 ^{b} ±0.47	9 2.18 ^{ab} ±0.54	10 2.31 ^{b} ±0.58	10 1.80 ^{a} ±0.41	10 1.97 ^{ab} ±0.53	10 2.01 ^{ab} ±0.58
CAT	10 18.55 ^{a} ±2.99	10 16.82 ^{a} ±3.86	10 15.75 ^{a} ±2.96	10 16.82 ^{a} ±3.90	10 15.65 ^{a} ±4.13	10 15.68 ^{a} ±3.50	10 15.45 ^{a} ±4.58	10 15.54 ^{a} ±2.65

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Table 21

The activity of superoxide dismutase (SOD), selenium-dependent glutathione peroxidase (Se-GPX), glutathione reductase (GR) and catalase (CAT) in the intestine of slugs *Arion* from the control (N), cadmium (Cd04, Cd16, Cd64), selenium (Se) or cadmium and selenium (Cd04Se, Cd16Se, Cd64Se) treated groups

Intes-tine	Control (N)	Cadmium 04 (Cd04)	Cadmium 16 (Cd16)	Cadmium 64 (Cd64)	Selenium (Se)	Cadmium 04 & Selenium (Cd04Se)	Cadmium 16 & Selenium (Cd16Se)	Cadmium 64 & Selenium (Cd64Se)
SOD	10 1.53 ^{bc} ±0.31	10 1.63 ^{bc} ±0.35	10 1.73 ^{c} ±0.29	10 1.60 ^{bc} ±0.41	10 1.61 ^{bc} ±0.28	10 1.53 ^{bc} ±0.46	10 1.20 ^{a} ±0.39	10 1.36 ^{ab} ±0.32
Se-GPX	10 0.263 ^{a} ±0.036	10 0.266 ^{a} ±0.032	10 0.240 ^{a} ±0.045	10 0.234 ^{a} ±0.054	10 0.253 ^{a} ±0.064	9 0.251 ^{a} ±0.056	9 0.233 ^{a} ±0.070	9 0.223 ^{a} ±0.069
GR	10 3.41 ^{a} ±0.90	10 3.94 ^{a} ±0.89	10 3.91 ^{a} ±0.84	10 3.82 ^{a} ±0.82	10 4.00 ^{a} ±0.76	10 3.77 ^{a} ±1.00	10 3.50 ^{a} ±0.69	10 3.50 ^{a} ±0.91
CAT	10 27.19 ^{a} ±5.43	10 23.68 ^{a} ±4.70	10 24.73 ^{a} ±5.18	10 24.59 ^{a} ±10.61	10 25.16 ^{a} ±4.67	10 23.69 ^{a} ±5.51	10 20.36 ^{a} ±5.02	10 21.43 ^{a} ±7.10

Abbreviations – see Index of figures and tables on page 85

Table 22

The activity of superoxide dismutase (SOD), selenium-dependent and independent glutathione peroxidase (Se-GPX, nonSe-GPX), glutathione S-transferase (GST), catalase (CAT) and content of thiobarbituric acid reactive substances (TBARS) in the intestine of earthworms *Lumbricus* from the control (N), paraquat or carbon tetrachloride poisoned (PQ-02, PQ-05, PQ-10, TC-01, TC-05) groups. Presented: group count, mean, ±SD

Earthworm Intestine	Control (N)	Paraquat 02 (PQ-02)	Paraquat 05 (PQ-05)	Paraquat 10 (PQ-10)	CCl_4 01 (TC-01)	CCl_4 05 (TC-05)
SOD	17 1.66 [a] ±0.40	8 2.06 [a] ±0.49	8 2.05 [a] ±0.92	8 2.10 [a] ±0.69	6 2.18 [a] ±0.40	7 2.91 ±0.71
Se-GPX	14 0.115 [ab] ±0.055	7 0.123 [ab] ±0.067	8 0.083 [a] ±0.027	6 0.143 [b] ±0.037	6 0.107 [ab] ±0.010	7 0.119 [ab] ±0.039
nonSe-GPX	17 0.108 [a] ±0.038	8 0.125 [a] ±0.051	8 0.130 [a] ±0.044	7 0.136 [a] ±0.036	6 0.094 [a] ±0.024	7 0.094 [a] ±0.036
GST	17 6.78 [ab] ±2.25	8 8.76 [c] ±1.32	7 8.43 [bc] ±1.24	8 8.23 [bc] ±2.03	6 6.73 [ab] ±1.43	7 5.86 [a] ±2.36
CAT	17 13.74 [ab] ±3.57	8 16.83 [bc] ±3.63	8 14.91 [b] ±4.83	8 20.49 [c] ±5.34	6 9.99 [a] ±2.61	7 12.3 [a] ±3.40
TBARS	17 0.0081 [b] ±0.0045	8 0.0040 [a] ±0.0030	7 0.0046 [a] ±0.0031	8 0.0047 [a] ±0.0030	6 0.0076 [ab] ±0.0013	7 0.0084 [b] ±0.0018

Abbreviations – see Index of figures and tables on page 85

Table 23

The activity of superoxide dismutase (SOD), selenium-dependent and independent glutathione peroxidase (Se-GPX, nonSe-GPX), glutathione S-transferase (GST), catalase (CAT) and content of thiobarbituric acid reactive substances (TBARS) in the body wall of earthworms *Lumbricus* from the control (N), paraquat or carbon tetrachloride poisoned (PQ-02, PQ-05, PQ-10, TC-01, TC-05) groups. Presented: group count, mean, ±SD

Earthworm Body Wall	Control (N)	Paraquat 02 (PQ-02)	Paraquat 05 (PQ-05)	Paraquat 10 (PQ-10)	CCl_4 01 (TC-01)	CCl_4 05 (TC-05)
SOD	19 0.94 [a] ±0.25	8 1.02 [ab] ±0.29	8 0.98 [ab] ±0.16	8 1.26 [b] ±0.34	8 1.07 [ab] ±0.31	8 0.98 [ab] ±0.34
Se-GPX	7 0.043 [a] ±0.015	4 0.039 [a] ±0.019	2 0.033 [a] ±0.020	3 0.036 [a] ±0.024	5 0.047 [a] ±0.029	7 0.039 [a] ±0.009
nonSe-GPX	10 0.058 [a] ±0.022	2 0.038 [a] ±0.024	2 0.040 [a] ±0.030	3 0.046 [a] ±0.036	5 0.039 [a] ±0.017	7 0.052 [a] ±0.026
GST	19 2.16 [a] ±0.58	8 2.21 [ab] ±0.60	8 2.10 [a] ±0.65	8 2.32 [ab] ±0.58	7 2.72 [b] ±0.65	8 2.05 [a] ±0.47
CAT	19 2.85 [ab] ±1.02	8 2.43 [a] ±0.72	8 2.67 [ab] ±0.84	8 2.79 [ab] ±1.02	8 3.63 [b] ±0.72	8 3.39 [ab] ±1.83
TBARS	17 0.0044 [a] ±0.0020	8 0.0038 [a] ±0.0018	8 0.0030 [a] ±0.0026	8 0.0045 [a] ±0.0031	8 0.0046 [a] ±0.0016	8 0.0031 [a] ±0.0011

Abbreviations – see Index of figures and tables on page 85

Table 24

The activity of superoxide dismutase (SOD), selenium-dependent glutathione peroxidase (GPX), glutathione reductase (GR) and catalase (CAT) in the body wall of earthworms *Dendrobaena* from the control (N), cadmium (Cd04, Cd16, Cd64), selenium (Se) or cadmium and selenium (Cd04Se, Cd16Se, Cd64Se) treated groups. Presented: group count, mean, ±SD

Body Wall	Control (N)	Cadmium 04 (Cd04)	Cadmium 16 (Cd16)	Cadmium 64 (Cd64)	Selenium (Se)	Cadmium 04 & Selenium (Cd04Se)	Cadmium 16 & Selenium (Cd16Se)	Cadmium 64 & Selenium (Cd64Se)
SOD	9 1.27 ^{a} ±0.18	9 1.17 ^{a} ±0.19	10 1.26 ^{a} ±0.20	10 1.30 ^{a} ±0.30	10 1.30 ^{a} ±0.30	11 1.32 ^{a} ±0.21	8 1.30 ^{a} ±0.34	10 1.34 ^{a} ±0.38
Se-GPX	9 0.0516 ^{bc} ±0.0162	9 0.0449 ^{bc} ±0.0123	8 0.0312 ^{a} ±0.0062	10 0.0416 ^{ab} ±0.0145	10 0.0537 ^{c} ±0.0107	11 0.0464 ^{bc} ±0.0097	7 0.0499 ^{bc} ±0.0101	10 0.0474 ^{bc} ±0.0111
GR	9 0.113 ^{a} ±0.022	9 0.126 ^{a} ±0.018	9 0.117 ^{a} ±0.018	10 0.124 ^{a} ±0.026	10 0.127 ^{a} ±0.017	11 0.136 ^{b} ±0.019	8 0.130 ^{ab} ±0.029	10 0.152 ^{b} ±0.039
CAT	9 4.46 ^{a} ±0.92	9 4.64 ^{a} ±0.95	10 4.91 ^{a} ±1.00	10 5.30 ^{a} ±1.51	10 4.95 ^{a} ±0.99	11 5.10 ^{a} ±0.82	8 5.05 ^{a} ±0.93	10 5.44 ^{a} ±0.91

Abbreviations – see Index of figures and tables on page 85

Table 25

The activity of superoxide dismutase (SOD), selenium-dependent glutathione peroxidase (Se-GPX), glutathione reductase (GR) and catalase (CAT) in the intestine of earthworms *Dendrobaena* from the control (N), cadmium (Cd04, Cd16, Cd64), selenium (Se) or cadmium and selenium (Cd04Se, Cd16Se, Cd64Se) treated groups

Intestine	Control (N)	Cadmium 04 (Cd04)	Cadmium 16 (Cd16)	Cadmium 64 (Cd64)	Selenium (Se)	Cadmium 04 & Selenium (Cd04Se)	Cadmium 16 & Selenium (Cd16Se)	Cadmium 64 & Selenium (Cd64Se)
SOD	8 1.77 ^{b} ±0.35	9 1.58 ^{ab} ±0.36	10 1.63 ^{ab} ±0.37	10 1.80 ^{b} ±0.44	10 1.70 ^{b} ±0.36	11 1.36 ^{a} ±0.35	8 1.33 ^{a} ±0.30	10 1.34 ^{a} ±0.30
Se-GPX	8 0.168 ^{ab} ±0.052	8 0.152 ^{a} ±0.049	10 0.214 ^{b} ±0.053	9 0.178 ^{ab} ±0.065	10 0.184 ^{ab} ±0.050	10 0.155 ^{a} ±0.061	7 0.142 ^{a} ±0.024	9 0.154 ^{a} ±0.38
GR	8 0.244 ^{ab} ±0.101	9 0.185 ^{a} ±0.078	10 0.185 ^{a} ±0.062	10 0.270 ^{b} ±0.108	9 0.211 ^{ab} ±0.051	11 0.174 ^{a} ±0.087	8 0.235 ^{ab} ±0.057	10 0.182 ^{a} ±0.036
CAT	8 14.80 ^{b} ±3.90	9 12.22 ^{ab} ±2.89	10 13.51 ^{ab} ±2.86	10 13.05 ^{ab} ±2.04	10 12.27 ^{ab} ±2.54	11 12.46 ^{ab} ±4.83	8 12.30 ^{ab} ±2.29	9 11.79 ^{a} ±1.76

Abbreviations – see Index of figures and tables on page 85

Table 26

The activity of selenium-dependent and (Se-GPX), selenium-independent-glutathione peroxidase (nonSe-GPX), glutathione reductase (GR), S-glutathione transferase (GST) and catalase (CAT) in the intestine of Madagascar cockroach *Gromphadorhina portentosa* treated with various doses of paraquat (P-12, P-24, P-47, P-95, P-190). Presented as follows: number of individuals, mean value, ±standard deviation

Gromphadorhina portentosa Intestine	Control (N)	Paraquat 12 (P-12)	Paraquat 24 (P-24)	Paraquat 47 (P-47)	Paraquat 95 (P-95)	Paraquat 190 (P-190)
Se-GPX	14 0.121 [a] ±0.051	10 0.252 [c] ±0.078	11 0.276 [c] ±0.153	9 0.206 [bc] ±0.106	10 0.146 [ab] ±0.041	9 0.171 [ab] ±0.036
nonSe-GPX	15 0.256 [a] ±0.059	10 0.379 [b] ±0.140	12 0.376 [b] ±0.013	11 0.333 [ab] ±0.123	9 0.267 [a] ±0.041	7 0.338 [ab] ±0.072
GR	18 0.110 [a] ±0.042	10 0.144 [ab] ±0.071	10 0.140 [ab] ±0.091	11 0.134 [ab] ±0.048	9 0.164 [b] ±0.084	8 0.175 [b] ±0.058
GST	18 9.07 [a] ±2.31	10 9.91 [a] ±2.07	12 9.89 [a] ±1.70	11 9.97 [a] ±2.81	10 9.02 [a] ±2.19	9 9.20 [a] ±2.83
CAT	16 9.62 [a] ±2.55	10 8.92 [a] ±2.78	12 9.50 [a] ±2.78	11 6.90 [b] ±1.79	10 6.72 [b] ±1.35	8 6.48 [b] ±1.91

Abbreviations – see Index of figures and tables on page 85

Table 27

The activity of superoxide dismutase (SOD), S-glutathione transferase (GST) and catalase (CAT) in the intestine Madagascar cockroach *Gromphadorhina portentosa* treated with various doses of paraquat (P-12, P-47, P-190)

Gromphadorhina portentosa Intestine	Control (N)	Paraquat 12 (P-12)	Paraquat 47 (P-47)	Paraquat 190 (P-190)
SOD	7 2.24 [ab] ±0.35	6 2.70 [b] ±0.66	7 2.12 [ab] ±0.75	10 1.78 [a] ±0.37
GST	6 8.98 [a] ±1.47	8 9.96 [a] ±2.85	8 9.38 [a] ±0.92	10 9.20 [a] ±1.90
CAT	8 9.71 [a] ±1.28	8 9.65 [ab] ±1.99	7 8.22 [ab] ±1.69	8 7.97 [b] ±1.70

Abbreviations – see Index of figures and tables on page 85

Table 28

Index of significant (P<0.05) correlations among enzyme activities in organs of examined species

Correlated enzymes		Mice gavaged	Mice injected	Mice injected	Frogs	Agro-limax	Arion	Lumb-ricus	Dendro-baena	Cock-roach
		Cd+Se	Cd+Se; PQ+VE	PQ	Cd; PQ	PQ; TC	Cd+Se	PQ; TC	Cd+Se	PQ
SOD	Se-GPX	+L, K	+L, H, B	†	+I	+G, I			+W, I	
	nonSe-GPX			†	†		†		†	
	GST			†	+L, K	+G	†		†	+I
	GR		+K, H	†	+L, K, I		+I		+W, I	
	CAT			†	+I		+G		+W	
	LPO TBARS	+H, K		†	†		†		†	†
Se-GPX	nonSe-GPX			+L, K, I	†	+G, I	†	+I	†	+I
	GST			+L	+I	+G, I	†		†	
	GR						+G			+I
	CAT			+L	+I		+G	+W, I	+W, I	
	LPO TBARS		-L, K	†	†		†		†	†
nonSe-GPX	GST			+L, I	†	+G, I	†		†	
	GR				†		†		†	+I
	CAT				†		†		†	
	LPO TBARS			†	†	+I	†		†	†
GST	GR		+L		+L, K, I		†		†	
	CAT					+G	†	+W	†	
	LPO TBARS			†	†		†		†	†
GR	CAT	+H -K	+H, K	+L, K		+I	+G, I		+W, I	
	LPO TBARS			†						†
CAT	LPO TBARS	+L	+L	†		+F	†		†	†

Abbreviations: shadowed area – negative correlation; empty area – nonsignificant; † – not determined; organs: L – liver, K – kidney, H – heart, B – brain, I – intestine, G – hepatopancreas, F – foot, W – body wall, "+" and "–" – the sign of correlation coefficient indicated for given parameters in particular organ. For more details see Tab. 29

Table 29

Values of correlation coefficient for pairs of enzymes in various species – significant r's indicated only

Species	Organ	Group	Enzyme 1	Enzyme 2	Count	r	P
Mice gavage (Tab. 2–5)	Liver	general	SOD	Se-GPX	24	-0,375	=0,05
			SOD	GST	24	-0,47	<0,02
			Se-GPX	LP	26	-0,46	<0,02
			GST	CAT	23	-0,375	=0,05
			GST	LP	24	-0,450	<0,02
			CAT	LP	23	0,723	<0,001
	Kidney	general	SOD	LP	28	-0,394	<0,05
			SOD	Se-GPX	28	0,339	NS
			Se-GPX	GR	27	-0,28	NS
			Se-GPX	LP	26	-0,460	<0,02
			GST	GR	24	0,370	=0,05
			GST	LPO	24	-0,450	<0,02
			GR	CAT	23	-0,3756	NS
	Heart	general	SOD	LP	27	0,337	NS
			Se-GPX	nonSe-GPX	27	-0,375	=0,05
			GR	CAT	24	0,53	<0,005
	Brain	general	GST	CAT	23	0,23	NS
			GST	GR	24	-0,437	<0,05
			GR	LP	23	0,24	NS
Mice injected (Tab. 6–9)	Liver	general	SOD	Se-GPX	49	0,204	NS
			Se-GPX	LP	54	-0,307	<0,05
			GST	GR	54	0,588	<0,01
			CAT	LP	54	0,401	<0,005
	Kidney	general	SOD	GR	49	0,295	<0,05
			Se-GPX	LP	54	-0,340	<0,02
			GST	LP	54	0,273	<0,05
			GR	CAT	54	0,219	NS
	Heart	general	SOD	Se-GPX	49	0,275	NS
			SOD	GR	49	0,344	<0,01
			Se-GPX	GST	54	-0,195	NS
			Se-GPX	GR	54	0,402	<0,005
			GST	GR	54	-0,283	<0,05
			GR	CAT	54	0,305	<0,05
			GR	LP	54	0,402	<0,001
Mice injected (Tab. 10–13)	Liver	general	Se-GPX	nonSe-GPX	25	0,540	<0,01
			Se-GPX	GST	25	0,33	<0,02
			Se-GPX	CAT	25	0,48	<0,01
			nonSe-GPX	GST	25	0,62	<0,001
			GR	CAT	25	0,48	<0,015
	Kidney	general	Se-GPX	nonSe-GPX	25	0,710	<0,0001
			GR	CAT	54	0,340	NS
	Intestine	general	Se-GPX	nonSe-GPX	25	0,390	NS
			nonSe-GPX	GST	25	0,670	<0,0001

Table 29. Cont.

Species	Organ	Group	Enzyme 1	Enzyme 2	Count	r	P
Frog injected (Tab. 14A,B–16A,B)	Liver	N	GPX	CAT	16	-0,52	<0,037
		PQ01	GST	CAT	8	0,78	<0,021
		Cd04	SOD	GST	10	0,52	<0,05
		Cd64	SOD	GST	9	0,84	<0,001
		Cd64	SOD	GR	9	0,64	<0,02
		general	SOD	GR	75	0,29	<0,01
		general	SOD	GST	75	0,42	<0,001
		general	GST	GR	75	0,27	<0,05
	Kidney	N	SOD	Se-GPX	17	0,53	<0,05
		N	SOD	GR	17	0,53	<0,05
		N	SOD	GST	17	0,53	<0,05
		PQ01	SOD	GR	10	0,840	<0,001
		PQ04	GST	GR	9	0,66	<0,05
		PQ19	Se-GPX	GR	10	-0,570	<0,05
		Cd04	Se-GPX	CAT	10	0,63	<0,05
		Cd16	Se-GPX	GR	10	0,71	<0,01
		Cd16	Se-GPX	GST	10	0,72	<0,01
		Cd64	GR	CAT	9	-0,520	=0,05
		general	SOD	Se-GPX	75	-0,40	<0,001
		general	SOD	GST	75	0,33	<0,01
		general	SOD	GR	75	0,38	<0,001
		general	SOD	CAT	69	0,37	<0,001
		general	Se-GPX	GST	75	0,27	<0,02
		general	GST	GR	75	0,54	<0,001
	Intestine	N	GPX	CAT	16	-0,52	<0,037
		PQ01	GST	CAT	8	0,78	<0,021
		Cd04	SOD	GST	10	0,52	<0,05
		Cd64	SOD	GR	9	0,840	<0,001
		Cd64	SOD	GST	9	0,64	<0,02
		general	SOD	GST	75	0,42	<0,001
		general	SOD	GR	75	0,29	<0,01
		general	GST	GR	75	0,27	<0,05
Arion injected (Tab. 20–21)	Hepato-pancreas	Cd64	Se-GPX	CAT	9	0,76	<0,01
		Cd04Se	SOD	GR	10	-0,71	<0,01
		Cd16Se	SOD	Se-GPX	8	0,83	<0,005
		Cd16Se	SOD	CAT	10	0,82	<0,005
		Cd16Se	Se-GPX	CAT	8	0,85	<0,005
		general	SOD	CAT	76	0,27	<0,02
		general	Se-GPX	GR	73	0,46	<0,001
		general	Se-GPX	CAT	73	0,23	<0,05
		general	GR	CAT	76	0,31	<0,01
	Intestine	Cd64	SOD	GR	10	0,64	<0,05
		Cd04Se	SOD	GR	10	0,701	<0,01
		Cd16Se	GR	CAT	10	0,68	<0,002
		Cd16Se	GR	CAT	10	0,70	=0,01
		general	SOD	GR	80	0,46	<0,001

Table 29. Cont.

Species	Organ	Group	Enzyme 1	Enzyme 2	Count	r	P
Dendrobaena injected (Tab. 24–25)	Body Wall	N	Se-GPX	CAT	9	0,66	<0,05
		Cd04Se	Se-GPX	GR	9	0,76	<0,005
		Cd64	SOD	Se-GPX	10	0,79	<0,005
		Cd64	SOD	CAT	10	0,82	<0,001
		Cd04Se	Se-GPX	GR	11	0,75	<0,005
		Cd64Se	SOD	GR	11	0,84	<0,001
		Cd64Se	SOD	CAT	11	0,77	<0,005
		Cd64Se	GR	CAT	11	0,362	<0,01
		general	SOD	Se-GPX	74	0,24	<0,05
		general	SOD	GR	74	0,52	<0,001
		general	SOD	CAT	74	0,54	<0,001
		general	GR	CAT	74	0,50	<0,001
	Intestine	Cd04e	Se-GPX	CAT	8	0,68	<0,05
		Cd64e	Se-GPX	CAT	9	0,72	<0,02
		Cd04Se	Se-GPX	CAT	10	0,67	<0,02
		Cd04Se	GR	CAT	11	0,64	<0,02
		general	SOD	Se-GPX	69	0,37	<0,02
		general	SOD	GR	73	0,35	<0,001
		general	Se-GPX	CAT	69	0,29	<0,02
		general	GR	CAT	69	0,31	<0,02
Agrolimax injected (Tab. 17–19)	Hepatopancreas	general	SOD	GST	61	0,31	<0,027
		general	SOD	Se-GPX	56	0,29	<0,04
		general	Se-GPX	nonSe-GPX	61	0,31	<0,047
		general	Se-GPX	GST	61	0,33	<0,02
		general	GST	CAT	61	0,37	<0,009
	Intestine	general	SOD	Se-GPX	46	0,40	<0,016
		general	Se-GPX	nonSe-GPX	46	0,65	<0,001
		general	Se-GPX	GST	46	0,33	<0,048
		general	nonSe-GPX	GST	52	0,33	<0,047
		general	nonSe-GPX	TBARS	52	0,35	<0,043
	Foot	general	SOD	CAT	59	-0,74	<0,037
		general	SOD	TBARS	56	-0,74	<0,036
		general	nonSe-GPX	CAT	14	-0,77	<0,025
		general	nonSe-GPX	TBARS	14	-0,80	<0,017
		general	CAT	TBARS	56	0,87	<0,005
Lumbricus injected (Tab. 22–23)	Intestine	general	Se-GPX	nonSe-GPX	48	0,38	<0,008
		general	nonSe-GPX	CAT	48	0,36	<0,014
		general	nonSe-GPX	TBARS	18	-0,32	<0,029
		general	CAT	TBARS	54	-0,31	<0,036
	Body Wall	general	Se-GPX	CAT	28	0,19	<0,037
		general	GST	CAT	58	0,46	<0,055
Cockroach fed (Tab. 26)	Intestine	general	SOD	GST	30	0,51	<0,011
		general	Se-GPX	nonSe-GPX	63	0,60	<0,001
		general	Se-GPX	GR	63	0,280	NS
		general	nonSe-GPX	GR	64	0,310	<0,037

Abbreviations: r – coefficient of correlation, P – probability of correlation, NS – nonsignificant; abbreviations of enzyme names as in the text – see Preface; Tab. 2–5 – number of table containing corresponding mean values and standard deviations; N, Cd04Se, PQ01 etc. – group codes as in corresponding tables; general – correlation estimated for all the groups

Piotr Łaszczyca

Współzależności między wskaźnikami aktywności antyoksydacyjnej u zwierząt traktowanych wybranymi czynnikami prooksydacyjnymi i antyoksydacyjnymi

Streszczenie

Celem pracy jest sprawdzenie, czy u zwierząt poddanych stresowi środowiskowemu zachodzą współzależności między aktywnościami enzymów uznawanych za wskaźniki procesów antyoksydacyjnych. Zwierzęta reprezentujące różne grupy systematyczne: myszy laboratoryjne, żaby, ślimaki z rodzaju *Agrolimax* i *Arion*, dżdżownice *Dendrobaena* i *Lumbricus* oraz karaluch madagaskarski *Gromphadorhina* traktowano wybranymi czynnikami prooksydacyjnymi lub antyoksydacyjnymi: związkami kadmu, parakwatem, czterochlorkiem węgla, seleninem i witaminą E. Mierzono aktywność dysmutazy ponadtlenkowej, peroksydazy glutationowej, reduktazy glutationowej, S-transferazy glutationowej i katalazy oraz nasilenie peroksydacji lipidów stymulowanej przez jony żelaza i askorbinian lub zawartość substancji reagujących z kwasem tiobarbiturowym. Zastosowano zróżnicowane dawki i schematy czasowe podawania wymienionych czynników w celu wywołania zmian wskaźników procesów antyoksydacyjnych i sprawdzenia, czy pomiędzy wskaźnikami tymi zachodzą istotne współzależności o charakterze kompensacyjnym.

Dawki parakwatu i kadmu zastosowane wobec myszy, żab i wybranych zwierząt bezkręgowych powodowały dwufazowe zmiany aktywności badanych enzymów, scharakteryzowane przez wzrost aktywności po zastosowaniu niskich dawek i obniżenie aktywności po dawkach wysokich. W niektórych przypadkach obserwowano wzorzec odwrotny: obniżenie aktywności po niskich i wzrost po wysokich dawkach. Obserwowano też złożony, „mozaikowy" charakter reakcji, cechujący się przeciwnymi zmianami analizowanych wskaźników w poszczególnych narządach. Efekt ten można tłumaczyć jako skutek zróżnicowanej dystrybucji zastosowanego związku w narządach, jak również niejednakowej wrażliwości poszczególnych narządów i wskaźnikowych enzymów. „Mozaikowy" wzorzec odpowiedzi różnych narządów może także odzwierciedlać dwufazowy charakter ich odpowiedzi na rosnące dawki.

Przeciwne kierunki zmian aktywności enzymów szlaku peroksydazy względem zmian aktywności dysmutazy ponadtlenkowej lub katalazy pozwalają sformułować hipotezę o ist-

nieniu wzajemnej kompensacji w tym systemie. Zgodnie z hipotezą wywołane przez czynniki prooksydacyjne obniżenie aktywności w układzie enzymów selenozależnej peroksydazy glutationowej może wywoływać wzrost aktywności katalazy lub dysmutazy ponadtlenkowej, które kompensują zaburzenie. Prawdopodobnie mechanizm ten działa także w przeciwnym kierunku, kompensując obniżoną aktywność katalazy przez wzrost aktywności peroksydazy glutationowej lub S-transferazy glutationowej. Wyniki wskazują też na kompensację obniżonej aktywności peroksydazy glutationowej przez aktywność enzymów, które zwiększają przepływ i dostępności zredukowanego glutationu. Warunkiem jest wzrost aktywności reduktazy glutationowej i niezmieniona pula zredukowanego fosforanu dwunukleotydu nikotyno-adeninowego w komórce.

Piotr Łaszczyca

Les relations entre les indices de l'activité des antioxydants chez les animaux traités avec les prooxydants et les antioxydants sélectionnés

Résumé

L'objectif du présent travail est de vérifier si chez les animaux soumis au stress environnemental il existe des interdépendances entre l'activité des enzymes reconnus comme indice des processus antioxydants. Les animaux représentant les différents groupes systématiques tels: les souris de laboratoire, les grenouilles, les escargots *Agrolimax* et *Arion*, les lombrics *Dendrobaena* et *Lumbricus* ainsi que la blatte de Madagaskar *Gromphadorhina* ont subi le traitemant par les agents peroxydants et antioxydants sélectionnés: les composés de cadmium, le paraquat, le tétrachlorue de carbone, le sélénite, et la vitamine E. On mesurait l'activité de la dismutase superoxygénée, la glutathion-peroxydase, la glutathion-réductase, la S-glutathion-transferase et la catalase ainsi que l'intensité de la peroxydation des lipides stimulée par les ions de fer et l'ascorbate ou bien la teneur des substances réagissant avec l'acide thiobarbiturique. On a appliqué de différents doses et schémas temporels de traitement par les agents cités ci-dessus afin de provoquer les variations des indices des processus antioxydants dans le but de vérifier si entre ces agents il existe des interdépendances essentielles à caractère compensatif.

Les doses du paraquat et du cadmium utilisées sur les souris, les grenouilles et différents intervertèbres sélectionnés ont entraîné les variations diphasées de l'activité des enzymes étudiés se caractérisant par l'accroissement de l'activité après l'application des doses basses et la baisse de l'activité après l'application des doses importantes. Dans certains cas, on a également observé le phénomène contraire: la baisse de l'activité après l'application des doses faibles et l'accroissement de celle-ci après l'application des doses fortes. On s'est également aperçu du caractère complexe des réactions dont le trait caractéristique étaient les variations divergantes des indices dans les organes respectifs. Ce phénomène peut être perçu comme l'effet de la distribution inégale des composés dans les organes mais il peut être aussi dû au degré différent de la susceptibilité des organes respectifs et des indices d'enzymes. La forme «de mosaïque» de la réponse des organes différents peut également refléter le caractère diphasé des réponses de ceux-ci aux doses augmentées.

Les directions divergentes de l'activité des enzymes par la voie de la glutathion-peroxydase par rapport aux variations de l'activité de la dismutase suroxygénée ou la catalase

permettent de formuler l'hypothèse sur l'existence de la compensation réciproque dans ce système. Conformément à celle-ci, la baisse de l'activité provoquée par les agents prooxydants dans le système des enzymes séléno-dépendants de la glutathion-peroxydase peut entraîner l'augmentation de l'activité de la catalase ou bien celle de la dismutase suroxygénée compensant les perturbations. Ce mécanisme, probablement, a son effet contraire et compense la basse activité de la catalase par la croissance de l'activité de la glutathion--peroxydase ou de la S-glutathion-réductase. Les résultats démontrent aussi la compensation de la baisse de l'activité de la glutathion-peroxydase à travers l'activité des enzymes augmentant le débit du glutathion sous forme réduite et l'accessibilité à celui-ci. Les conditions à remplir c'est l'augmentation de l'activité de la glutathion-réductase ainsi que de la teneur constante du nicotinamide adénine dinucléotide phosphate (NADPH) à l'état réduit.

Piotr Łaszczyca

Relationships among indices of antioxidative activity in animals treated with selected prooxidants and antioxidants

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