In Vitro Effects of Selenite and Mercuric Chloride on Liver Thiobarbituric Acid–Reactive Substances and Non-Protein Thiols From Rats: Influences of Dietary Cholesterol and Polyunsaturated and Saturated Fatty Acids

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OBJECTIVE: We measured the in vitro effects of mercuric chloride (Hg^{2+}) and selenite (Se^{4+}) on hepatic 2-thiobarbituric acid–reactive substances (TBARS) and non-protein sulfhydryl (NPSH) levels of rats fed diets enriched with polyunsaturated or saturated fatty acids with and without cholesterol.

METHODS: Male Wistar rats (21 d old) were assigned to one of four groups and fed diets containing 20% soybean oil, 20% soybean oil plus 1% cholesterol, 20% coconut oil, or coconut oil plus 1% cholesterol. After the feeding period (6 wk), body weight gain was equal in all groups. TBARS levels and NPSH content were measured after in vitro exposure to mercuric chloride (100 μM) and sodium selenite (25 μM) for 1 h.

RESULTS: The lipid peroxidation, measured as TBARS levels in the control group, were statistically higher in hepatic homogenates of rats fed diets containing soybean oil than in groups fed coconut oil ($P = 0.009$). However, cholesterol supplementation did not change TBARS levels. Selenite alone did not modify TBARS production, whereas mercury alone significantly increased TBARS levels. Moreover, Se^{4+} protected against mercury-induced lipid peroxidation only in rats fed diets containing coconut oil. In the control group, dietary fat acids did not change NPSH levels. Selenite produced higher oxidative effects toward NPSH content, whereas Hg^{2+} decreased NPSH levels only in liver from rats fed diets containing soybean oil. NPSH levels were higher after concomitant exposure to Se^{4+} and Hg^{2+} chloride that after exposure to Se^{4+} alone, suggesting an interaction between Hg^{2+} and Se^{4+}. Catalase activity was higher in animals fed diets containing soybean oil. Dietary cholesterol decreased glutathione peroxidase activity.

CONCLUSION: Together these results indicated that the protective effect of Se^{4+} against mercury-induced lipid peroxidation depends on dietary fat saturation. *Nutrition* 2003;19:531–535. ©Elsevier Inc. 2003

KEY WORDS: non-protein thiols, lipid peroxidation, selenite, mercuric chloride, coconut oil, soybean oil, cholesterol, liver

INTRODUCTION

The protective effect of sodium selenite against mercuric chloride toxicity in mammals has been known for three decades.[1] This effect has been demonstrated in many species such as rat,[1,2] mouse,[3,4] pig,[5] and rabbit.[6] Although the mechanism underlying the protective effects of selenite against mercury toxicity is not known, there is evidence that selenide (produced in vitro from selenite in the presence of glutathione, Se/S) forms a metal-Se/S complex that binds to selenoprotein P (Sel-P) to form a ternary complex, metal-Se/S-Sel-P.[7]

Lipid peroxidation, a free radical–mediated mechanism, leads to oxidative destruction of polyunsaturated fatty acids constitutive of cellular membranes.[8,9] In particular, mercuric chloride[10–12] and selenite[13,14] can stimulate lipid peroxidation. Mercuric chloride (Hg^{2+}) can stimulate lipid peroxidation by enhancing H_2O_2 formation in mitochondria,[15] whereas selenite-induced lipid peroxidation seems to be related to generation of superoxide.[16] However, selenite can act as a radical scavenger.[17]

The activity of reactive oxygen species such as hydrogen peroxide and superoxide anion can be countered by a system of antioxidant defenses, of which tissues and erythrocyte enzymes contribute to avoid cell injury. These enzymes include superoxide

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dismutase, which catalyzes dismutation of superoxide anions into hydrogen peroxide, and catalase and glutathione peroxidase (GSH-Px), which detoxify H_2O_2 and convert lipid hydroperoxides into non-toxic alcohol.[18,19]

It has been demonstrated that the types and levels of dietary fat affect the susceptibility of lipid peroxidation and oxidative damage to cells.[20] Polyunsaturated fatty acids are susceptible to oxidation, and the resulting products may be toxic to the cell.[21] Saturated fatty acids are less susceptible than unsaturated fatty acids to oxidation.[22] Working with human umbilical vein endothelial cells, Vossen and collaborators[23] showed that the extent of phospholipid peroxidation increases with increasing content of polyunsaturated fatty acids added in a culture medium and that the sensitivity of individual polyunsaturated fatty acids to peroxidation is directly proportional to the number of double bonds present. In addition, Varghese and Oommen[24] showed that liver lipid peroxidation is lower in teleosts of animals fed coconut oil than those in animals fed cod liver oil. Some studies showed that cholesterol feeding also modifies antioxidant status, apparently by altering GSH-Px and catalase activities.[19,25–27]

Fatty acid composition of diets can affect membrane composition of cells[28] and, consequently, change the susceptibility of such cells to pro-oxidant agents. However, a possible interaction between the fatty acid composition of diets and the response of liver to agents such as mercuric chloride and selenite is lacking in the literature. We investigated the in vitro effects of mercuric chloride and selenite on liver lipid peroxidation (hepatic 2-thiobarbituric acid–reactive substances [TBARS]) and non-protein sulfhydryl (NPSH) levels in rats fed diets enriched with polyunsaturated or saturated (soybean oil or coconut oil) fatty acids and with or without cholesterol. Further, we analyzed the effects of diet on hepatic catalase and GSH-Px activities to identify some factors that might change the response of liver membranes to the pro-oxidant effects of mercury or selenite.

MATERIALS AND METHODS

Compounds

Reduced glutathione, 5,5′-dithio-bis(2-nitrobenzoic acid), 2-thiobarbituric acid, and acetic acid were obtained from Sigma Chemical Company (St. Louis, MO, USA). All other chemicals used were of analytical reagent grade and purchased from Merck (Darmstadt, Germany).

Animals and Diets

Male Wistar rats (21 d old) were from the local breeding colony (ICBS-UFRGS). They were maintained on a 12-h light, 12-h dark cycle in a ventilated room at 21°C with free access to food and water. All animal procedures were in accordance with the National Institutes of Health Guidelines for the Care and Use of Laboratory Animals and were approved by the local authorities.

Twenty animals were assigned to one of four groups with five animals per group: 20% soybean oil (SO), 20% soybean oil plus 1% cholesterol (SO + CHOL), 20% coconut oil (CO), or coconut oil plus 1% cholesterol (CO + CHOL; Table I). Fatty acid composition of the dietary fats (SO and CO) is summarized in Table II. Food consumption was measured every 3 d, and body weight gain was measured every week. After 6 wk of dietary treatment, rats were killed by decapitation, and livers were rapidly removed, weighed, rinsed, and homogenized in 10 vol of saline solution containing 100 mM of phosphate buffer, pH 7.4. The fresh tissue homogenate was used for the measurement of NPSH and TBARS.

In Vitro Exposure and Biochemical Analysis

Incubations of fresh liver homogenates were done for 1 h at 37°C in a medium containing buffered saline solution (100 mM of phosphate buffer, pH 7.4) with or without mercuric chloride (100 μM) or sodium selenite (25 μM). Each tube (total volume of 2.5 mL) contained 10 to 12 mg of protein. The compounds were dissolved in physiologic saline solution. Mercury dose was based on the study of Fujimoto et al.[29] Because Se^{4+} has a catalytic oxidative effect on NPSH,[16] the selenite dose was four-fold less than the mercury dose.

TABLE I.

COMPOSITION OF THE EXPERIMENTAL DIETS

Component (g/100 g)	SO	SO + CHOL	CO	CO + CHOL
Soybean oil	20	20	—	—
Coconut oil	—	—	20	20
Cholesterol	—	1	—	1
Soybean protein*	25	25	25	25
Sucrose	24	24	24	24
Cornstarch	24	24	24	24
Nonnutritive fiber	1	1	1	1
Salt mix†	4	4	4	4
Vitamin mix‡	1.5	1.5	1.5	1.5
L-methionine§	0.15	0.15	0.15	0.15

* Soybean protein from Samprosoy (Bunge Alimentos, Porto Alegre, Brazil).

† Mineral mixture (Roche, São Paulo, Brazil); (mg/100 g of ration): NaCl, 557; KCl, 3.2; KH_2PO_4, 1556; $MgSO_4$, 229, $CaCO_3$, 1526; $FeSO_4 \cdot 7H_2O$, 108; $MnSO_4 \cdot H_2O$, 16; $ZnSO_4 \cdot 7H_2O$, 2.2; $CuSO_4 \cdot 5H_2O$, 1.9; and $CoCl_2 \cdot 6H_2O$, 0.09.

‡ Vitamin mixture (Roche, São Paulo, Brazil; mg/100 g of ration): vitamin A, 4; vitamin D, 0.5; vitamin E, 10; menadione, 0.5; choline, 200; p-aminobenzoic acid (PABA), 10; inositol 10mg; niacin, 4; panthothenic acid, 4; riboflavin, 0.8; thiamin, 0.5; pyridoxine, 0.5; folic acid, 0.2; biotin, 0.04; and vitamin B12, 0.003.

§ L-methionine (Merck, Rio de Janeiro, Brazil)

CO, 20% coconut oil; CO + CHOL, coconut oil plus 1% cholesterol: SO, 20% soybean oil; SO + CHOL, 20% soybean oil plus 1% cholesterol

TABLE II.

FATTY ACID COMPOSITION OF THE DIETARY FAT

Fatty acid	Soybean oil (%)	Coconut oil (%)
C8:0	0.0	2.4
C10:0	0.0	2.7
C12:0	0.0	44.4
C14:0	0.1	16.6
C16:0	11.6	9.6
C16:1(ω-7)	0.3	0.0
C18:0	4.1	2.8
C18:1(ω-9)	26.1	17.8
C18:2 (ω-6)	48.9	3.1
C18:3 (ω-3)	3.8	0.0
C20:0	0.4	0.1
C20:1	1.2	0.1
C22:0	0.5	0.0
C24:0	0.2	0.0
Other	0.3	0.1
Saturated fatty acids	17.3	78.7
Monounsaturated fatty acids	27.6	18.0
Polyunsaturated fatty acids	52.6	3.1

TABLE III.

EFFECT OF MERCURIC CHLORIDE OR SODIUM SELENITE ON TBARS LEVELS FROM RAT LIVER HOMOGENATES*

Diets	Control	Hg	Se	Hg + Se
Soybean oil	$2.29 \pm 0.19^{a,1}$	$5.75 \pm 0.59^{a,2}$	$2.97 \pm 0.15^{a,1}$	$5.14 \pm 0.76^{a,2}$
Soybean oil + Cholesterol	$2.10 \pm 0.13^{a,b,1}$	$6.44 \pm 0.22^{a,2}$	$2.50 \pm 0.30^{a,1}$	$6.38 \pm 1.04^{a,2}$
Coconut oil	$1.82 \pm 0.13^{b,c,1}$	$3.36 \pm 0.16^{b,2}$	$1.72 \pm 0.15^{b,1}$	$1.32 \pm 0.04^{b,3}$
Coconut oil + Cholesterol	$1.58 \pm 0.08^{c,1}$	$3.15 \pm 0.30^{b,2}$	$1.67 \pm 0.06^{b,1}$	$1.27 \pm 0.05^{b,1}$

* Presented as nanomoles of malondialdehyde per milligram of protein. The incubation (1 h at 37°C) was initiated by adding hepatic homogenates (10 to 12 mg of protein) to reaction mixtures containing phosphate buffered saline solution (pH 7.4) with or without mercuric chloride (100 μM) or sodium selenite (25 μM). The results are presented as mean ± standard error of the mean. Means not sharing the same superscript letters within the same column (diet effect) were different at $P < 0.05$ (Duncan's multiple range test). Means not sharing the same superscript number within the same row (compound effect) were different at $P < 0.05$ (Duncan's multiple range test).
TBARS, thiobarbitoric acid–reactive substances

To determine NPSH, 500 μL of 20% trichloroacetic acid was added to 500 μL of fresh tissue homogenate. After centrifugation (4000*g* at 4°C for 10 min), the protein pellet was discarded and free SH− groups were determined in the clear supernatant (which was neutralized with 0.1 M NaOH) by the method of Ellman.[30] TBARS levels were determined in tissue homogenates by the method of Ohkawa et al.,[31] in which malondialdehyde, an end product of fatty acid peroxidation, reacts with thiobarbituric acid to form a colored complex. Malondialdehyde values were determined with the absorbance coefficient of the malondialdehyde–thiobarbituric acid complex at 532 nm (1.56×10^5/cm/mM).

For enzyme assays, fresh liver homogenates not exposed to mercury or selenite were centrifuged for 10 min at 15 800*g* in an Eppendorf Model 5417 R centrifuge at 4°C. The supernatant fraction was used to determine GSH-Px and catalase activities.

GSH-Px activity was measured by the method of Paglia and Valentine.[32] Liver homogenate supernatant (200 to 400 μg of protein) was added to the assay mixture, and the reaction was started by the addition of 0.1 mL of 4 mM H_2O_2 for a final concentration of 0.4 mM. Conversion of reduced nicotinamide adenine dinucleotide phosphate to oxidized nicotinamide adenine dinucleotide phosphate was monitored continuously at 340 nm for 5 min. GSH-Px activity was expressed as nanomoles of reduced to oxidized nicotinamide adenine dinucleotide phosphate per minute per milligram of protein of the liver homogenate supernatant by using an extinction coefficient of 6.22×10^6 for nicotinamide adenine dinucleotide phosphate. Catalase activity was measured by the method of Aebi.[33] Supernatant of the liver homogenate (200 to 500 μg of protein) was added to a cuvette, and the reaction was started by the addition of 1.0 mL of freshly prepared 30 mM H_2O_2 in phosphate buffer (50 mM, pH 7.0). The rate of decomposition of H_2O_2 was measured spectrophotometrically from changes in absorbance at 240 nm during the first 30 s. The activity of catalase was expressed as the rate constant of a first-order reaction (k) per second per milligram of protein for the liver homogenate. Protein was measured by the method of Lowry et al.[34] by using bovine serum albumin as the standard.

Statistical Analysis

The individual effects of compounds and diets were analyzed by one-way analysis of variance (ANOVA), followed by Duncan's multiple range test, when appropriate. Three-way ANOVA, 4 (in vitro treatments: control, Se, Hg, or Se + Hg) × 2 (types of fatty acids: SO or CO) × 2 (with or without cholesterol addition), also was performed to detect possible interactions between cholesterol, fatty acid type, and in vitro treatment. Differences between groups were considered significant at $P \leq 0.05$. Pearson's correlation (TBARS × NPSH) also was performed in all conditions of in vitro exposure.

RESULTS

The effect of diets on food consumption and body weight gain did not differ between groups (data not shown).

Table III summarizes TBARS levels of fresh liver homogenates of rats fed diets containing CO or SO and with or without cholesterol after additional in vitro exposure to mercuric chloride and sodium selenite. Under basal conditions (without mercuric ion and selenite), TBARS levels were statistically higher in hepatic supernatants of rats fed a diet containing SO than in rats fed CO, however, cholesterol supplementation did not modify TBARS levels. Mercuric chloride alone increased TBARS levels independently of diet type, and the highest values were found in animals fed diets containing SO. Selenite alone did not change TBARS levels. Moreover, selenite protected against mercury-induced lipid peroxidation in CO and CO + CHOL groups. In all conditions of in vitro exposure, two-way ANOVA showed no significant interaction between cholesterol and fatty acid type for TBARS levels. In contrast, a significant interaction between mercury and selenite was observed in the CO ($F_{1,9} = 38.43$, $P < 0.001$) and CO + CHOL ($F_{1,9} = 24.54$, $P < 0.001$) groups. The three-way interaction showed a significant interaction between mercury, selenite, and fatty acid type ($F_{1,9} = 14.78$, $P < 0.001$).

In the absence of compounds, the effects of dietary fat saturation were non-significant with regard to NPSH levels (Table IV). Interestingly, mercuric chloride alone decreased liver NPSH levels only in animals fed diets containing SO. Selenite alone produced a greater oxidative effect than mercuric chloride on NPSH content, and this effect was lower in animals fed diets containing CO. After concomitant in vitro exposure to selenite and mercuric chloride, hepatic NPSH levels were higher that NPSH levels after exposure to selenite alone. In all conditions of in vitro exposure, two-way ANOVA showed no significant interaction between cholesterol and fatty acid type with regard to NPSH levels. In contrast, two-way ANOVA showed a significant interaction between mercury and selenite for all diet types with regard to NPSH.

A negative correlation between NPSH and TBARS was significant at 0.05 (two-tailed Pearson's correlation) only for incubations containing mercuric chloride alone ($r = -0.533$, $P = 0.015$) and those containing mercuric chloride and selenite ($r = -0.487$, $P = 0.029$).

Table V summarizes the effects of diet on catalase and GSH-Px activities. Two-way ANOVA showed a significant effect of dietary fatty acid type ($F_{1,9} = 15.75$, $P = 0.001$) on catalase activity. In fact, catalase activity was higher in animals fed diets containing

TABLE IV.

EFFECT OF MERCURIC CHLORIDE OR SODIUM SELENITE ON NON-PROTEIN SULFHYDRYL LEVELS FROM RAT LIVER HOMOGENATES*

Diets	Control	Hg	Se	Hg + Se
Soybean oil	$4.94 \pm 0.39^{a,b,1}$	$3.99 \pm 0.42^{a,2}$	$0.98 \pm 0.02^{a,3}$	$2.10 \pm 0.18^{a,4}$
Soybean oil + Cholesterol	$4.72 \pm 0.35^{a,b,1}$	$3.69 \pm 0.39^{a,2}$	$0.98 \pm 0.07^{a,3}$	$2.18 \pm 0.19^{a,4}$
Coconut oil	$5.65 \pm 0.22^{a,1}$	$5.22 \pm 0.22^{b,1}$	$1.18 \pm 0.1^{b,2}$	$2.83 \pm 0.11^{b,3}$
Coconut oil + Cholesterol	$4.46 \pm 0.29^{b,1}$	$4.00 \pm 0.18^{a,1}$	$1.23 \pm 0.02^{b,2}$	$2.22 \pm 0.18^{a,3}$

* Presented as micromoles per gram of tissue. The incubation (1 h at 37°C) was initiated by adding hepatic homogenates (10 to 12 mg of protein) to reaction mixtures containing phosphate buffered saline solution (pH 7.4) with or without mercuric chloride (100 μM) or sodium selenite (25 μM). The results are presented as mean ± standard error of the mean. Means not sharing the same superscript letters within the same column (diet effect) were different at $P < 0.05$ (Duncan's multiple range test). Means not sharing the same superscript number within the same row (compound effect) were different at $P < 0.05$ (Duncan's multiple range test).

SO, independent of the presence of cholesterol. GSH-Px activity was lower in animals fed diets enriched with CHOL ($F_{1,9} = 17.05$, $P = 0.001$), independent of the type of dietary fatty acid. The interaction between CHOL and dietary fatty acid was non-significant for GSH-Px and catalase activities.

DISCUSSION

About three decades ago, Parizek and Ostadalova[1] reported that small amounts of selenite can protect against signs of mercury intoxication in rats. After the evidence of an interaction between selenium and mercury in the body, many efforts have been made to clarify the mechanism related to the protective action of selenite against mercury-induced toxicity. Naganuma and Imura[6] showed that this detoxification process seems to occur predominantly in blood. Further, Sasakura and Suzuki[7] showed that selenide (produced from selenite in the presence of glutathione) forms a complex (metal-Se/S) that binds to Sel-P to form a ternary complex, metal-Se/S-Sel-P. Our results demonstrated that the in vitro oxidative effect of mercuric chloride and the protective effect of selenite against mercuric chloride–induced lipid peroxidation in rat liver homogenates depend on the dietary fat saturation.

The peroxidative effect of mercury on membrane lipids is well known[10–12] and seems to be related to thiol depletion and the formation of H_2O_2 in mitochondria.[15] Our results showed that the peroxidative effect of mercuric chloride was 1.7-fold higher in SO animals than in CO animals and 2.0-fold higher in SO + CHOL animals than in CO + CHOL animals. These results demonstrated that dietary polyunsaturated fatty acids influence the susceptibility to mercury-induced lipid peroxidation in rat liver. Because dietary fatty acids modify the fatty acid composition of phospholipids,[28] it is reasonable to suppose that the greater peroxidative effect of mercuric chloride on liver homogenates of animals fed diets containing polyunsaturated fatty acid (SO) is related to the qualitative composition of membrane phospholipids in liver. This possibility is reasonable because the amount of phospholipids and triacylglycerols did not change in response to different compositions of dietary fat (data not shown).

TABLE V.

EFFECT OF DIETS ENRICHED WITH COCONUT OR SOYBEAN OIL AND WITH OR WITHOUT CHOLESTEROL ON THE ACTIVITIES OF HEPATIC CATALASE AND GLUTATHIONE PEROXIDASE*

Diets	GSH-Px	Catalase
Soybean oil	10.50 ± 0.34^{a}	35.15 ± 2.12^{c}
Soybean oil + Cholesterol	8.21 ± 0.57^{b}	$28.58 \pm 3.27^{b,c}$
Coconut oil	11.02 ± 0.36^{a}	$21.45 \pm 3.30^{a,b}$
Coconut oil + Cholesterol	8.45 ± 0.23^{b}	18.89 ± 2.76^{a}

* Catalase activity is expressed as the rate constant of a first-order reaction per second per milligram of protein for the liver homogenate. Glutathione peroxidase is expressed as nanomoles of nicotinamide adenine dinucleotide phosphate oxidation per milligram of protein per minute. For details, see MATERIALS AND METHODS. The results are presented as mean ± standard error of the mean. Means not sharing the same superscript letters within the same column were different at $P < 0.05$ (Duncan's multiple range test).

Data reported in the literature indicating a pro-oxidant effect of selenite are rare. There are few reports showing that in vivo exposure to selenite can induce lipid peroxidation.[2,35] In vitro studies on the effect of selenite on lipid peroxidation are even more scarce, and selenite apparently does not stimulate lipid peroxidation of unsaturated fatty acids.[36] In line with this idea, in the present study, 25 μM of selenite did not increase lipid peroxidation in liver homogenates.

Although dietary fatty acids did not affect the basal NPSH levels, two-way ANOVA showed a significant effect of dietary fatty acid type on mercury-induced depletion of NPSH. After in vitro exposure to mercuric chloride, NPSH levels were lower in liver homogenates of SO (polyunsaturated fat) rats than in CO (saturated fat) rats. This observation suggested that depletion of NPSH after exposure to mercuric chloride may be a consequence and not the cause of lipid peroxidation because in vitro exposure to mercuric chloride did not change NPSH levels of fresh liver homogenates of CO rats. The presence of mercury-induced NPSH depletion only in liver homogenates of SO rats could be related to the detoxification of lipid hydroperoxides of polyunsaturated fatty acids via GSH-Px reaction. In these animals, NPSH levels probably decreased by detoxifying lipid hydroperoxides to non-toxic alcohol.

Although mercuric chloride concentration was similar to the NPSH concentration (around 100 μM) in the reaction medium, Hg^{2+} oxidized only 8% to 19% of total NPSH, depending on the diet composition. Even though the high affinity of Hg^{2+} by glutathione is well established,[37] it is impossible to assume the stoichiometric interaction between mercuric ions and NPSH in a medium containing liver homogenate because Hg^{2+} can bind in sites distinct from NPSH groups, including the cysteinyl residues of thiol-containing proteins.[4] Moreover, the Hg^{2+}-induced lipid peroxidation also may be causally linked to depletion of glutathione, which can detoxify lipid hydroperoxides.

The interaction between selenium and mercury seems to depend on the reduction of selenite to selenide, and this reduction is mediated by glutathione.[7] Interestingly, the present data demonstrated a high rate of NPSH depletion after selenite exposure, and the NPSH levels were higher after concomitant exposure to selenite and mercuric chloride than after exposure to selenite alone. Two-way ANOVA showed a significant interaction between mercury and selenite for all diet types with regard to NPSH. This finding could be due to a direct chemical interaction between selenium (as selenide) and mercury. A previous study demonstrated that the interaction of mercuric ion with selenite inhibits selenite-induced thiol oxidation.[38] Our results demonstrated a similar inhibition of selenite-induced thiol (NPSH) oxidation, but in a biological system. This phenomenon could be due the fact that selenide is much more reactive than thiols toward mercuric ions.[39]

Although the interaction between mercury and selenite with regard to NPSH was significant for all diet types, the protective effect of selenite against mercury-induced lipid peroxidation was observed only for CO animals. Thus, this protective effect cannot be explained as a consequence of the chemical interaction of the selenide ion with the mercuric ion.

Cholesterol levels represent a major determinant affecting membrane fluidity. Moreover, some studies have shown that cholesterol feeding also modifies antioxidant status.[19,26,27] Our results showed that dietary cholesterol did not affect susceptibility to lipid peroxidation under basal conditions and after additional exposure to mercuric chloride. However, cholesterol feeding decreased activities of liver GSH-Px. This data are in agreement with previous results[25,27] showing decreased GSH-Px activity in CHOL rats.

The greater effect of catalase activity on SO animals may be related to the effect of polyunsaturated fatty acids on peroxisomes. Accordingly, De Craemer et al.[40] reported that hepatic catalase activity increases in rats fed a diet enriched with polyunsaturated fatty acids, which induce the proliferation of liver peroxisomes.

In conclusion, fatty acids in the diet affected mercury-induced lipid peroxidation in rat liver homogenates, and the dietary fat saturation seemed to be a major factor determining the lipid peroxide content of tissues. Moreover, the protective effect of selenide against mercuric chloride–induced lipid peroxidation depended on the dietary fat saturation. This protective effect cannot to be explained as a consequence of a chemical interaction of the selenide ion with the mercuric ion.

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