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Interaction of Alkylmercuric Compounds with Sodium Selenite. III. Biotransformation, Levels of Metallothioneinlike Proteins and Endogenous Copper in Some Tissues of Rats Exposed to Methyl or Ethylmercuric Chloride with and without Sodium Selenite

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The biotransformation efficiency of alkylmercurial compounds was studied in rat liver, kidneys, blood, and brain after 2-week administration of methylmercuric chloride (MeHg) and ethylmercuric chloride (EtHg) at doses of 0.25 or 2.5 mg Hg/kg, alone or in combination with sodium selenite (Se) at a level of 0.5 mg Se/kg. Simultaneously, the level of metallothioneinlike proteins (MTP) and endogenous copper (Cu) was monitored in tissues of control rats and intoxicated rats.

Regardless of the dose, the highest concentrations of inorganic mercury from both the alkylmercurials was found in the rat kidneys. Sodium selenite had a variable effect on the amount of inorganic mercury liberated, depending on the organ and the molar ratio of Hg:Se administered.

A statistically significant increase in the levels of MTP and endogenous Cu, compared with control group, was found only in the kidneys of intoxicated rats. This increase was dependent on the concentration of inorganic mercury liberated by biotransformation of alkylmercurials. The observed changes appeared when the level of inorganic mercury exceeded 10μg Hg/g tissue and reached a plateau at about 40 μg Hg/g tissue. In the presence of selenium the plateau of MTP and Cu levels were no oberved in the kidneys, regardless of the amount of inorganic mercury liberated.

Introduction

Changes in the homeostasis of essential elements, especially zinc and copper, by toxic metals have been studied from the standpoint of understanding of the harmful action of the metals on the organism (*1,2*). Disturbances in the metabolism of endogenous metals may be induced in experimental animals by administration of cadmium (*3–17*), nickel (*3,18,19*), silver (*18,20–22*), bismuth (*23,24*), gold (*25,27*), tin (*28*), molybdenum (*29*), and both inorganic mercury (*22,23,26,30–32*) and methylmercury (*31*).

In general, these changes occur in those tissues of experimental animals in which increase in the level of metallothioneinlike proteins by toxic metals have been noted previously (*2,19,23,24*). Studies of those proteins revealed that they differ in the content of endogenous metals depending on the organ in which the stimulation took place. In the metallothioneinlike protein of the kidneys, copper dominates as endogenous metal (*23,33–36*) while in the liver this role is played by zinc (*25,36–39*). In the rat, inorganic mercury stimulates the formation of metallothionein in the kidneys (*26,40–47*). On the other hand, stimulation of metallothionein by alkyl mercurials, is still a controversial subject (*48–50*).

An increase in the concentration of metallothioneinlike proteins, highly correlated with the level of inorganic mercury, was observed after prolonged exposure of rats to ethylmercuric chloride and methylmercuricyanoguanidine (*48*). It was also demonstrated that Me ^{203}Hg (*49,51*), methoxyethyl ^{203}Hg (*49*) and Et^{203}Hg (*52*) are bound by low molecular weight proteins of the soluble fraction of rat kidneys.

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Liberation *in vivo* of inorganic mercury from organic compounds of this metal has been demonstrated by many authors (*50,53,54*). The efficiency of biotransformation of methylmercurials in the rat as a function of dose and type of exposure is still a matter of controversy. Moreover, only fragmentary data concerning biotransformation of ethylmercury, another alkyl mercuric compound dangerous to living organisms, are available in the literature (*55–58*).

The presence of selenium inhibits the stimulation of biosynthesis of metallothioneinlike proteins by inorganic mercury (*47,48,59–63*). Our previous studies (*51,52*) have dealt with the metabolism of methyl- and ethylmercuric chloride in rats in the presence and absence of selenium. the present study was designed to determine if there is a relationship between the amount of inorganic mercury liberated from these alkylmercurial compounds in the presence and absence of selenium and the concentration of metallothionein proteins and endogenous copper in some rat tissues.

Materials and Methods

The experiments were made on female Wistar rats of body weight 150 to 250 g which were standard LSM granules and allowed to drink tap water *ad libitum*.

Table 1 shows the pattern of experimental groups employed, single doses of mercury and selenium administered and molar ratios of these elements in appropriate groups. Methylmercuric chloride (MeHg) and ethylmercuric chloride (EtHg) (K & K Laboratories Inc., Plainview, NY, USA) in 0.1% sodium carbonate (POCh, Gliwice, Poland) were administered intragastrically (seven times in a two-week period). Selenium (Se) was given as an aqueous solution of Na^2SeO^3 (POCh, Gliwice, Poland), also intragastically, every day over a 14-day period. At 24 hr after administration of the last dose, the animals were sacrificed under ether narcosis by heart puncture, and then the appropriate tissues were isolated.

The content of metallothioneinlike proteins was determined radiochemically according to Piotrowski et al. (*64*) as modified by Zelazowski and Piotrowski (*65*). Metallothionein isolated from horse kidney cortex according to a method similar to that described by Pulido et al. (*66*) was used as a standard (*67*). The standard had three -SH groups per milligram protein. Under the conditions employed, 1 mg of the standard protein bound about 200 μg of mercury. The content of endogenous copper was determined by a spectrophotometric method with zinc dibenzyldithiocarbamate after mineralization of the tissues with a mixture of concentrated sulfuric and perchloric acids (*68*).

The content of inorganic and organic mercury was estimated by cold atomic spectroscopy according to Magos (*69*) in the modification of Balcerska et al. (*70*), by use of equipment with a Handrey Relays mercury detector (mercury vapor concentration meter Type E 3472).

Ceruloplasmin activity in blood serum was determined by the colorimetric method of Lehman et al. (*71*), *o*-dianisidine being used as a substrate.

Table 1. Groups of animals.

Group[a]	Treatment	Mercury, mg/kg	Selenium, mg/kg	Molar ratio Hg:Se
I	MeHg	0,25		
Ia	MeHg + Se	0.25	0.5	1:10
II	MeHg	2.50		
IIa	MeHg + Se	2.50	0.5	1:1
III	EtHg	0.25		
IIIa	EtHg + Se	0.25	0.5	1:10
IV	EtHg	2.50		
IVa	EtHg + Se	2.50	0.5	1:1
V	Control			
Va	Control + Se		0.5	

[a]Six animals in each group.

Results

The concentrations of inorganic and organic mercury in the kidneys, liver, brain and blood for MeHg and EtHg are presented in Tables 2 and 3, respectively. The

Table 2. Levels of inorganic and organic mercury in the kidneys, liver, brain, and blood of rats poisoned for 2 weeks with methylmercury chloride as functions of dose and of presence of sodium selenite.

		μg Hg/g tissue[a]	
Tissue	Group	Inorganic Hg	Organic Hg
Kidneys	I	1.75	4.21
		1.08–2.10	3.85–4.89
	Ia	1.58	2.39
		0.78–2.43	1.54–3.19
	II	14.28	47.02
		12.19–16.49	44.67–51.80
	IIa	9.41	47.83
		7.20–13.81	37.37–61.93
Liver	I	0.52	0.77
		0.33–0.53	0.76–0.96
	Ia	0.51	0.70
		0.24–0.99	0.22–0.97
	II	0.13	13.29
		0.08–1.27	12.18–13.34
	IIa	0.55–2.39	6.52–8.36
Brain	I	0.08	0.49
		0.06–0.10	0.47–0.52
	Ia	0.08	0.68
		0.00–0.12	0.64–0.76
	II	0.69	3.70
		0.39–1.34	3.07–3.99
	IIa	0.90	6.37
		0.78–1.03	6.24–6.50
Blood	I	0.64	6.77
		0.10–1.87	5.50–7.11
	Ia	0.27	2.91
		0.22–0.34	2.84–2.96
	II	5.82	52.86
		2.42–10.50	34.00–98.39
	IIa	3.11	65.69
		1.80–3.90	49.80–85.08

[a]Mean values and ranges from six animals.

Table 3. Levels of inorganic and organic mercury in the kidneys, liver, brain, and blood of rats poisoned for 2 weeks with ethylmercuric chloride as functions of dose and of presence of sodium selenite.

Tissue	Group	μg Hg/g tissue[a]	
		Inorganic Hg	Organic Hg
Kidneys	III	33.05	14.73
		28.81–37.14	10.51–20.28
	IIIa	12.59	5.22
		12.47–12.78	5.03–5.34
	IV	73.48	29.80
		58.11–91.86	11.41–45.17
	IVa	97.76	73.43
		71.81–128.66	42.53–99.38
Liver	III	1.41	0.44
		1.21–1.50	0.34–0.65
	IIIa	2.25	0.87
		2.17–2.33	0.71–1.03
	IV	6.92	20.86
		6.05–8.60	19.18–21.73
	IVa	12.30	17.62
		8.26–20.71	9.21–22.76
Brain			
	III	0.09	0.36
		0.08–0.09	0.34–0.38
	IIIa	0.35	1.02
		0.30–0.41	0.77–1.23
	IV	1.21	3.23
		0.92–1.56	2.71–3.65
	IVa	3.69	9.14
		3.18–4.12	8.72–9.65
Blood	III	3.83	3.06
		3.26–4.36	2.53–3.64
	IIIa	3.21	1.52
		3.07–3.37	1.36–1.72
	IV	36.14	56.04
		28.66–42.93	46.64–66.39
	IVa	24.16	40.02
		20.57–36.60	27.58–43.61

[a]Mean values and ranges from six animals.

percent contribution of the two forms of mercury accumulating in these tissues after a 2 weeks exposure to the alkylmercuric compounds and selenium is shown in Figure 1.

The extent of biotransformation of the alkylmercurials studied in the rat depends on the dose and tissue. In the liver a high dose (2.5 mg Hg/kg) almost completely inhibits the capacity for dealkylation of methylmercuric chloride. After 2-week poisoning of rats with MeHg at doses of 0.25 mg Hg/kg (group I) and 2.5 mg Hg/kg (group II), inorganic mercury liberated constituted about 40% and 1%, respectively, of the total mercury incorporated in this tissue. On the other hand, in the blood the differences in the extent of biotransformation as a function of dose were much smaller than in the liver (Fig. 1). The contribution of inorganic mercury to the total blood mercury after methylmercury intoxication ranged from 8 to 15% for doses of 0.25 and 2.5 mg Hg/kg, respectively. In the kidneys, the extent of biotransformation of this compound was about 29% in group I and about 23% in group II (Fig. 1). In the brain, the content of inorganic mercury after administration of methylmercuric chloride at doses of 0.25 and 2.5 mg Hg/kg was 0.08 and 0.69 μg Hg/g tissue, respectively (Table 2), which amounted to about 15% of the total mercury accumulated in this tissue, irrespective of the dose (Fig. 1).

Administration of selenite simultaneously with the high dose of methylmercury (Table 2) resulted in a decrease of the total mercury content of the liver accompanied by an increased in the extent of biotransformation to inorganic mercury, from about 1% in group II to about 10% in group IIa (Fig. 1).

In the kidneys, selenium brought about a uniform diminution of concentrations of both forms of mercury, irrespectively of the dose of MeHg. On the other hand, in the brain the total mercury concentration was considerably augmented by selenium (Table 2), but efficiency of biotransformation remained unchanged (Fig. 1).

Ethylmercuric chloride was biotransformed more efficiently than methylmercuric chloride in all rat tissues studied. However, as in the case of MeHg (Table 2), dealkylation of EtHg in the liver was dependent on the dose (Table 3). The amount of inorganic mercury liberated after administration of EtHg for 2 weeks at doses of 0.25 mg Hg/kg group (III) and 2.5 mg Hg/kg (group IV) was about 77.1% and 24.0%, respectively, of the total mercury accumulated in the liver (Fig. 1). No analogous dependence was found in the kidneys of rats poisoned with ethylmercury, where inorganic mercury amounted to about 70% of total mercury irrespective of the dose administered (Fig. 1).

In the blood and brain of rats given ethylmercury, the mean concentrations of total and inorganic mercury increased proportionally to the dose (Table 3). The contribution of inorganic mercury to the total concentration of the metal was high for both doses, from 23% (group III) to about 35% (group IV) in the brain, and about 50% in the blood (Fig. 1).

In the presence of sodium selenite the amount of inorganic mercury liberated from ethylmercuric chloride in the liver increased slightly, from about 77% in group III to about 83.0% in group IIIa and from about 25.0% in group IV to about 41.0% in group IVa (Fig. 1), a phenomenon similar to that observed for methylmercuric chloride. No significant effect of selenium on the efficiency of biotransformation of EtHg was found in the kidneys, brain, and blood (Fig. 1).

The appearance of inorganic mercury in some rat tissues due to biotransformation of alkylmercurial compounds (Fig. 1) prompted us to undertake studies of the level of mercury-induced metallothioneinlike protains (MTP) and concentration of endogenous copper. In the kidneys, liver, brain, spleen, and blood of control rats the level of copper was, respectively, 13.4, 4.4, 3.1, 1.9 μgCu/g, and 2.0 μgCu/mL. The level of metallothioneinlike proteins in kidney, liver, and brain of control animals was respectively, 0.24, 0.09, and 0.07 mg MTP/

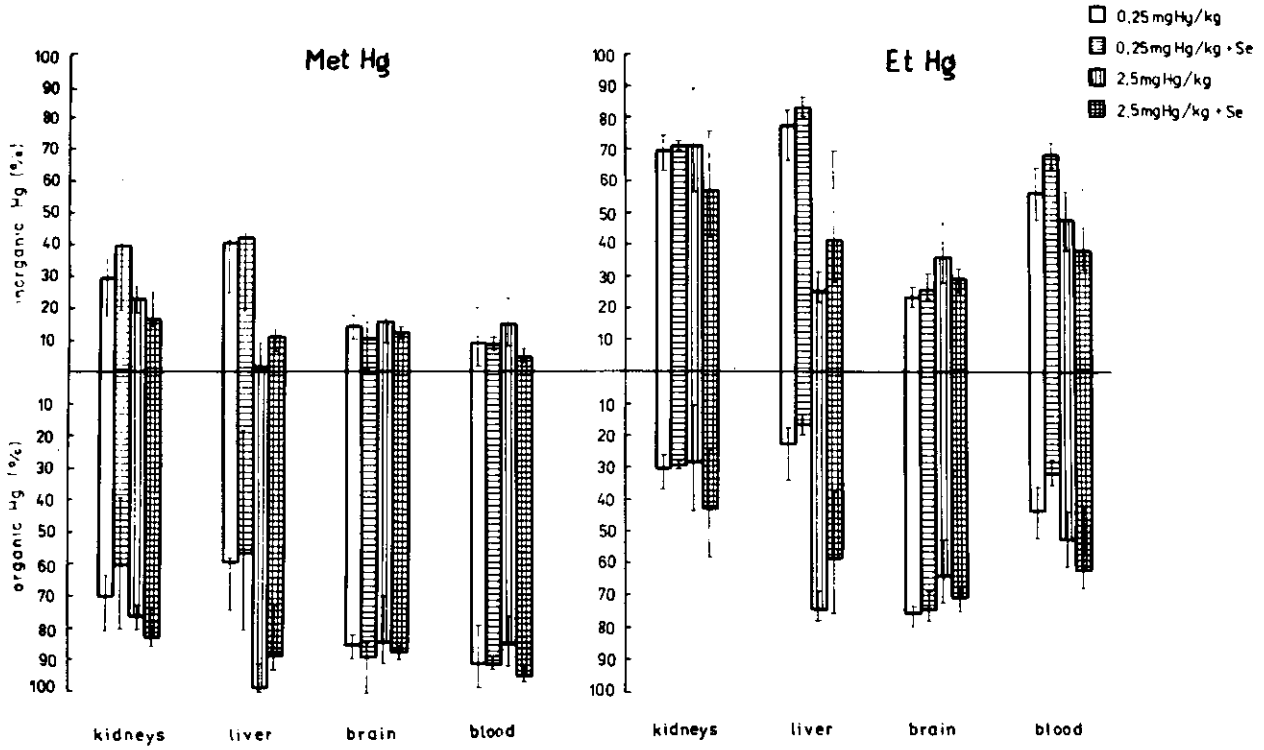


FIGURE 1. Percent contributions of inorganic and organic mercury to the total concentration of this metal in rat kidneys, liver, brain and blood after 2 weeks exposure to methyl-and ethylmercuric chloride with and without Se (mean values and range.from six animals).

Table 4. Levels of metallothioneinlike proteins (mTP), endogenous copper (Cu) and inorganic mercury in the kidneys of control rats with and without Se and of rats poisoned for 2 weeks with alkylmercurials.

Group	Alkylmercurial treatment	Se	MTP, mg/g tissue[a]	Copper, μg/g tissue	Inorganic Hg, μg/g tissue[a]
I	MeHg, 0.25 mgHg/kg	No	0.25 ± 0.05	15.81 ± 4.66	1.75 ± 0.80
Ia	MeHg, 0.25 mgHg/kg	Yes	0.23 ± 0.03	12.50 ± 4.52	1.58 ± 0.40
II	MeHg, 2.50 mgHg/kg	No	0.51 ± 0.17*	20.00 ± 7.21*	14.28 ± 2.28
IIa	MeHg, 2.50 mgHg/kg	Yes	0.27 ± 0.23	14.98 ± 2.13	9.41 ± 2.52
III	EtHg, 0.25 mgHg/kg	No	0.70 ± 0.01†	32.66 ± 1.87†	33.05 ± 3.32
IIIa	EtHg, 0.25 mgHg/kg + Se	Yes	0.33 ± 0.03	20.91 ± 3.42*	12.67 ± 2.23
IV	EtHg, 2.5 mgHg/kg	No	0.68 ± 0.03†	33.73 ± 1.04†	69.97 ± 19.67
IVa	EtHg, 2.5 mgHg/kg + Se	Yes	0.67 ± 0.03†	39.08 ± 6.74†	97.76 ± 19.44
V	(control)	No	0.24 ± 0.09	13.44 ± 4.09	—[b]
Va	None (control)	Yes	0.13 ± 0.01*	33.52 ± 15.52	—[b]

[a]All values means ± SD.
[b]Below the detectability level of the method (50 ng Hg/g tissue).
*Difference statistically significant, $\alpha = 0.05$.
†Difference statistically significant at $\alpha = 0.001$.

g tissue. A significant increase in the level of endogenous Cu and metallothioneinlike proteins in the examined tissues of exposed animals as compared to control ones was shown only in the kidneys (Table 4).

Changes in endogenous copper levels were not connected with ceruloplasmin because no alterations of its activity were noted in the exposed rats as compared with the control.

Sodium selenite adminstered simultaneously with methylmercury (groups Ia and IIa) and the low dose of ethylmercury (groups IIIa) decreased the concentration of this protein in the kidneys as compared with the level typical for animals exposed to alkylmercurials alone (Table 4). Administration of only selenium (group Va) also decreased about the concentration of these proteins in rat kidneys twofold as compared with the control group (Table 4).

The changes in the level of metallothioneinlike proteins in exposed animals were accompanied by an statistically significant increase of the concentration of endogenous copper (Table 4).

The concentration of copper in the kidneys was normalized after combined administration of methylmercury and selenium (Table 4). On the other hand,

administration of ethylmercuric chloride elevated the level of endogenous copper in the kidneys significantly (Table 4), even in the presence of an excess of selenium (groups IIIa).

The dependence of the level of metallothioneinlike proteins and of endogenous copper in rat kidneys on the amount of inorganic mercury accumulated in these organs after a 2-week exposure is presented in Figure 2. It can be seen from these data that the increase of values of the parameters studied was parallel and began when the concentration of inorganic mercury liberated from alkyl compounds by biotransformation exceeded 10 μg Hg/g tissue.

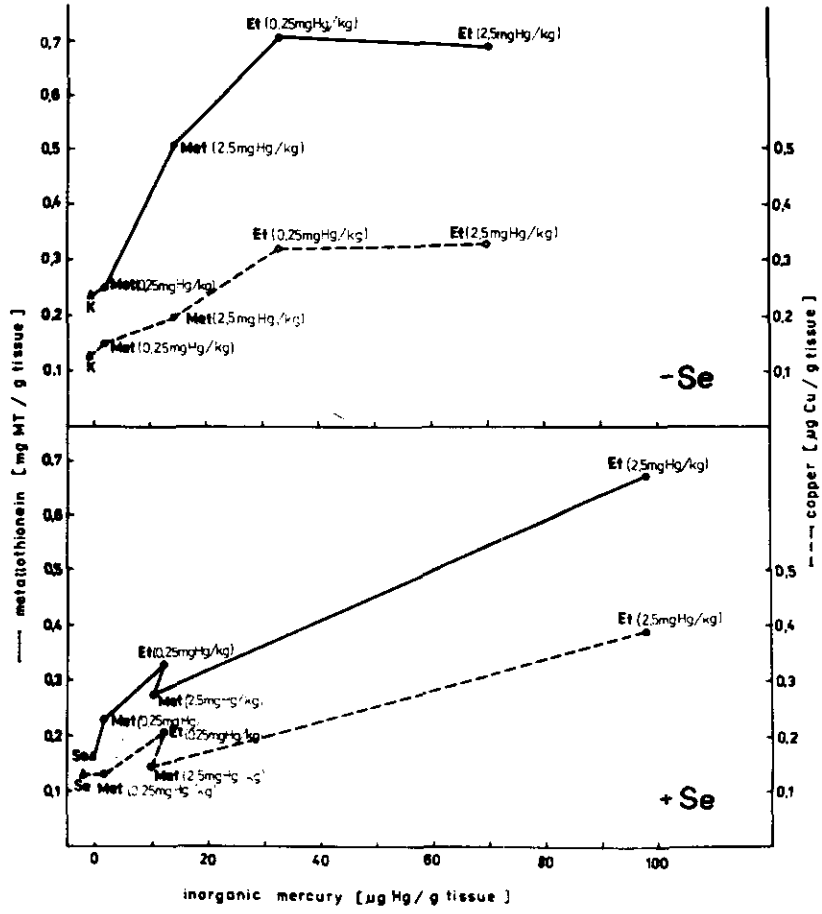
Discussion

Results of this study permit comparison of the efficiency of biotransformation of methyl- and ethylmercuric chloride in rats including dose dependence. We found that irrespective of the dose of methylmercury, about 23 to 29% of inorganic mercury was liberated in rat kidneys (Fig. 1). This process was augmented during prolonged exposure of rats to a low dose of MeHg when liberated inorganic mercury amounted to 40% of total mercury accumulated in this tissue (*58*). A similar efficiency of this process in rat kidneys was reported by Klein et al. (*72*) after single administration of two different doses of methylmercury and by Norseth (*73*) as well as Magos and Butler (*74*) after repeated exposure of rats to this compound. It results even from the experiments of Omata et al. (*50*) employing a high dose of MeHg (25 mg Hg/kg).

After administration of ethylmercury, inorganic mercury amounted to 70% of total mercury in the kidneys of rats (Fig. 1). A similar efficiency of EtHg biotransformation in the rats kidneys was maintained even when the time of exposure was prolonged (*58*).

Biotransformation of EtHg in the brain is also even more efficient than that of methylmercury chloride, and concentrations of both total and inorganic mercury in the brain of animals poisoned with ethylmercury are higher than after poisoning with the same dose of methylmercury (Fig. 1).

In the rat brain, inorganic mercury constituted about



FIGURES 2. Dependence of the levels of—endogenous copper and–metallothioneinlike proteins in rat kidneys on the concentration of inorganic mercury in kidney after 2 weeks exposure to various doses of methyl- and ethylmercuric chloride administered without or with sodium selenite.

15% of total mercury, irrespective of the dose MeHg (Fig. 1). A similar efficiency of methylmercury biotransformation in the brain was found by Mengel and Karlog (*49*) after chronic exposure of rats to this compound and by Omata et al. (*50*) after single administration of methylmercury 925 mg Hg/kg. On the other hand, Magos and Butler (*74*) reported a considerably lower efficiency of biotransformation of this compound in the rat brain, probably due to the use of a very high cumulative dose, close to LD_{50}.

In the liver, biotransformation of methylmercury as well as ethylmercury is closely related to the dose of these compounds (Fig. 1). This process proceeds more intensely at the lower dose when the contribution of inorganic mercury to the total mercury content is about 40% and 77% for MeHg and EtHg, respectively. At higher doses, the efficiency of biotransformation decreases to about 25% for ethylmercury and vanishes almost completely for methylmercury (Fig. 1). A diminution of the extent of mercury biotransformation in the liver with increasing doses was also reported by Norseth (*73*), Mengel and Karlog (*49*), and Magos and Butler (*74*). It seems that, in contrast to the kidneys, the liver is an organ very sensitive to the action of alkylmercurials, and the microsomal fraction of hepatic cells is responsible for the diminution of the efficiency of biotransformation with increasing doses of methylmercury (and probably also ethylmercury). Results of many studies (*75–78*) indicate that the biotransformation of methylmercuric compounds proceeds in the microsomal fraction of the liver by microsomal enzymes, including cytochrome P-450. Depending on the doses of methylmercury employed, some authors observed stimulation (*75,76*) while others found inhibition of these enzymes (*77,78*). It was also demonstrated that methylmercury given at high doses induces destruction of endoplasmic reticulum of liver cells (*79*), which may explain the inhibition of methylmercury biotransformation in the liver observed in this study.

Our results permit determination of the threshold level of action of inorganic mercury liberated from these alkyl compounds on the level of metallothioneinlike proteins and on disturbance of homeostasis of endogenous copper in some tissues. As we showed in our previous studies, Hg binds to low molecular weight (metallothioneinlike) protein fraction of the kidneys to a higher extent after administration of ethylmercuric chloride (*52*) than after methylmercuric chloride (*51*), which is due to the higher efficiency of biotransformation of ethylmercury (*58*).

We observed changes in the levels of metallothionein and endogenous copper (Table 4 and Fig. 2) when the level of inorganic mercury exceeded 10 μg Hg/g tissue. This value can be assumed to be a threshold concentration for the phenomena in question. An increase in the levels of metallothioneinlike proteins and endogenous copper proceeded until the content of inorganic mercury reached 40 μg/g tissue (Fig. 2). Above this value, a plateau was maintained for both MTP and Cu. Analogous dependence was not observed in any other tissue, probably since the amount of inorganic mercury liberated there was much lower than 10 μg Hg/g tissue. Only in the liver was a tendency for increase of the levels of both endogenous copper and metallothioneinlike proteins seen after simultaneous administration of a high dose of EtHg and Se when the level of inorganic mercury liberated was about 12 μg Hg/g tissue (Table 2), but these changes were devoid of statistical significance.

The effect of interaction between the alkylmercurials and sodium selenite (depends on the molar Hg:Se ratio) is found to consist of a change of the distribution and therefore of concentrations of total mercury (*51,52*) and both its forms in individual tissues (Table 2 and 3). However, no significant differences in the percent contribution of inorganic mercury to the total mercury content in the tissues were found between animal groups poisoned with alkymercurials alone and those receiving sodium selenite in addition to mercury (Fig. 1). It can therefore be concluded that selenium does not accelerate the dealkylation of alkylmercurials as it was suggested for methylmercury by Yamane et al. (*80*).

Changes in the distribution of mercury by selenium in rat tissues are apparently due to the binding by selenium of inorganic mercury liberated from alkylmercurials by biotransformation and to alterations in the tissue distribution of mercury-selenium complexes as it probably may be occurs in the case of interaction between inorganic mercury and selenium (*81*). This phenomenon is expressed especially well in the case of ethylmercuric chloride due to the high efficiency of biotransformation of this compound to inorganic mercury *in vivo*.

On the other hand, at low selenium concentrations in the kidneys with respect to high concentrations of inorganic mercury, an increase in the concentration of mercury in this organ was observed (Table 3). A similar phenomenon was noted by Burk et al. (*61*) in the case of a high excess of inorganic mercury and by Mengel and Karlog (*82*) as an effect of interaction between selenium and a methoxyethylmercuric compound. Our studies indicate that an interaction effect between ethylmercury and selenium leading to a decrease in the concentration of mercury in the kidneys occurs only at a high excess of selenium (group IIIa). Administration of a slight excess of this element did not change the mercury content of the kidneys (*58*), while an equimolar dose, sufficient to induce redistribution of mercury in the case of exposure to an inorganic mercury compound (*56*), not only did not decrease but even increased the mercury concentration in this organ (Table 3). In the presence of Se there are also changes in the amount of ^{203}Hg bound by low- molecular weight proteins of this fraction the kidneys (*51,52*) accompanied by decreases in the concentrations of metallothioneinlike proteins and endogenous copper, probably due to the binding by selenium of inorganic mercury liberated and probably also of other native metals. In this way their effects on the homeostasis of copper and synthesis of metallothionein-

Table 5. Ratios of kidney to brain total and inorganic mercury concentrations as an index of neurotoxic potential.[a]

Treatment	Se	Kidney/brain total Hg ratio		Kidney/brain inorganic Hg (by ASS[c])
		As^{203}Hg[b]	By AAS[c]	
MeHg, 0.25 mg Hg/kg	No	9.66	10.28	21.87
MeHg, 2.50 mg Hg/kg	No	9.44	14.03	20.69
EtHg, 0.25 mg Hg/kg	No	145.90	129.13	367.22
EtHg, 2.50 mg Hg/kg	No	15.78	23.26	60.73
MeHg, 0.25 mg Hg/kg	Yes	2.77	6.54	19.75
MeHg, 2.50 mg Hg/kg	Yes	4.34	7.87	10.45
EtHg, 0.25 mg Hg/kg	Yes	7.72	13.00	35.97
EtHg, 2.50 mg Hg/kg	Yes	16.64	13.34	26.49

[a]Ratios were calculated from tissue concentrations measured 24 hr after 2-week exposure to methyl- or ethylmercuric chloride with and without Se.
[b]Determined as ^{203}Hg (*51,52*).
[c]Determined by AAS according to Balcerska et al. (*64*).

like proteins are limited (Fig. 2).

On the other hand, the increase in the concentrations of metallothioneinlike proteins and endogenous copper in the kidneys of rats given ethylmercury and an equimolar dose of selenium should be ascribed to the too low amount of selenium with respect ot the amount of inorganic mercury liberated in the kidneys from this compound.

Alkylmercuric compounds have both neuro- and nephrotoxic actions in the rat (*72,83–85*). The nephrotoxic effect is ascribed to the effect of inorganic mercury liberated in the kidneys of these animals with a considerable efficiency.

Magos (*85*) suggested that the ratio between the concentration of mercury in the kidneys and brain can be used as an indicator of the neurotoxic potential. This ratio is high for inorganic mercury and phenylmercury (respectively about 102 and 83); and low for methylmercury (about 4.5).

Table 5 shows numerical values of this coefficient calculated from our data. These results indicate that values of the neurotoxicity coefficients for various doses of methylmercuric chloride are similar to these obtained by Magos (*85*). Comparison of these values with those for ethylmercuric chloride demonstrates a higher neurotoxicity of methylmercury, in agreement with the common view.

In the case of ethylmercuric chloride, however, numerical values of this coefficient were dependent on the dose (Table 5). For the low dose of EtHg (group II) this value was higher than that of the kidney/brain coefficient given by Magos (*85*) for MeHg, especially when the concentration of inorganic mercury liberated from ethylmercury in the kidneys and brain were taken into account. This suggests that low doses of EtHg maybe more nephrotoxic than $HgCl_2$. The conclusion is in essential disagreement with reports on the toxicity of these compounds.

It seems that a coefficient calculated on the basis of the brain blood mercury concentration might be a better index of neurotoxicity, especially if concentrations of inorganic mercury in both these tissues (Table 3) were considered in the calculations. That value is the higher, the more neurotoxic a given compound is (Table 6).

The presence of selenium, inducing the above described changes in the distribution of both forms of mercury in the rat has a significant influence on values of the discussed indices of neuro- and nephrotoxicity (Tables 5 and 6). These coefficients should be therefore regarded with care and not employed for estimation of the action of a mercuric compound when administered simultaneously with selenium.

Table 6. Ratios of brain to blood total and inorganic mercury concentrations as an index of neurotoxic potential.[a]

Treatment	Se	Brain/blood total Hg ratio		Brain/blood inorganic Hg[c]
		As ^{203}Hg[b]	By AAS[c]	
MeHg, 0.25 mg Hg/kg	No	0.12	0.10	0.12
MeHg, 2.50 mg Hg/kg	No	0.09	0.10	0.12
EtHg, 0.25 mg Hg/kg	No	0.06	0.06	0.02
EtHg, 2.50 mg Hg/kg	No	0.05	0.06	0.03
MeHg, 0.25 mg Hg/kg	Yes	0.29	0.30	0.30
MeHg, 2.50 mg Hg/kg	Yes	0.20	0.20	0.30
EtHg, 0.25 mg Hg/kg	Yes	0.40	0.40	0.11
EtHg, 2.50 mg Hg/kg	Yes	0.20	0.20	0.15

[a]Ratios were calculated from tissue concentrations measured 24 hr after 2-week exposure to methyl- or ethylmercuric chloride with and without Se.
[b]Determined as ^{203}Hg (*51,52*).
[c]Determined by AAS according to Balcerska et al. (*64*).

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REFERENCES

1. Nordberg, G. F. (Ed.). Effect and Dose-Response Relationships of Toxic Metals. Elsevier, Amsterdam, 1976.
2. Kagi, J. H. R., and Nordberg, M. Metallothionein and other low molecular weight metal-binding proteins. In: Metallothionein (J. H. R. Kagi and M. Nordberg, Eds.), Birkheuser Verlag, Basel-Boston-Stuttgart, 1979, pp. 41– 124.
3. Schroeder, H. A., and Nason, A. P. Interactions of trace metals in rat tissues. Cadmium and nickel with zinc, chromium, copper, manganese. J. Nutr. 104: 167–178 (1974).
4. Seth, T. D., Agarwall, L. N., Satija, N. K., and Hasan, M. Z. Effect of lead and cadmium on liver, kidney and brain levels of cadmium, copper, lead, manganese and zinc and on erythrocyte ALA-D activity in mice. Bull. Environ. Contam. Toxicol. 16: 190–196 (1976).
5. Stonard, M. D., and Webb, M. Influence of dietary cadmium on the distribution of the essential metals copper, zinc and iron in tissues of the rat. Chem.-Biol. Interact. 15: 349– 363 (1976).
6. El-Gazzar, R. M., Boyle, J., and Petering, H. G. Effect of cadmium ingestion on cadmium and zinc profile in male and female rat liver cytosol. Annual Report, Center for the Study of the Human Environment, University of Cincinnati, 1977 p. 53.
7. Julshamn, K., Utne, F., and Brackkan, O. R. Interactions of cadmium with copper, zinc and iron in different organs and tissues of the rat. Acta Pharmacol. Toxicol. 41: 515–524 (1977).

8. Elinder, C. G., and Piscator, M. Cadmium and zinc relationships. Environ. Health Perspect. 25: 129–132 (1978).
9. Petering, H. G. Some observations on the interaction of zinc, copper and iron metabolism in lead and cadmium toxicity. Environ. Health Perspect. 25: 141–145 (1978).
10. Suzuki, K. T. Copper content in cadmium exposed animal kidney metallothionein. Arch. Environ. Contam. Toxicol. 8: 255–268 (1979).
11. Bonner, F. W., King, L. J., and Parke, D. V. The tissue disposition and urinary excretion of cadmium, zinc, copper and iron following repeated parenteral administration of cadmium to rats. Chem. -Biol. Interact. 27: 343–351 (1979).
12. Ashby, S. L., King, L. J., and Parke, D. V. Effect of acute administration of cadmium on the disposition of copper, zinc and iron in the rat. Environ. Res. 21: 177–185 (1980).
13. Bonner, F. W., King, L. J., and Parke, D. V. The effect of dietary cadmium on zinc, copper and iron levels in the bone of rats Toxicol. Letters 5: 105–108 (1980).
14. Perry, M. H., Jr., Erlanger, M. W., and Perry, E. F. Inhibition of cadmium-induced hypertension in rats. Sci. Total. Environ. 14: 153–166 (1980).
15. Sato, M., and Nagi, Y. Mode of existence of cadmium in rat liver and kidney after prolonged subcutaneous administration. Toxicol. Appl. Pharmacol. 54: 90–99 (1980).
16. Suzuki, K. T., Yamamura, M., Yamada, Y. K., and Shimizu, F. Decreased copper content in rat kidney metallothionein and its relation to acute cadmium nephropathy. Toxicol. Letters 7: 137–142 (1980).
17. Suzuki, Y. Cadmium, copper and zinc distribution in blood of rats after long-term cadmium administration. J. Toxicol. Environ. Health 7: 251–262 (1981).
18. Suzuki, Y., and Yoshikawa, H. Induction of hepatic zinc binding proteins of rats by various metals. Ind. Health 14: 25–30 (1976).
19. Chmielnicka, J., Szymandka, J. A., and Tyfa, J. Disturbances in the metabolism or endogenous metals Zn and Cu in nickel-exposed rats. Environ. Res. 27: 216–221 (1982).
20. Van Campen, D. R. Effects of zinc, cadmium silver and mercury on the absorption and distribution of copper-64 in rats. J. Nutr. 88: 125–130 (1966).
21. Whanger, P. D., and Weswig, P. H. Effect of some copper antagonists on induction of ceruloplasmin in the rat. J. Nutr. 100: 341–348 (1969).
22. Winge, D. R., Premakumar, R., and Rajagoplan, K. V. Metal-induced formation of metallothionein in rat liver. Arch. Biochem. Biophys. 170: 242–252 (1975).
23. Szymanska, J. A., and Zelazowski, A. J. Effect of cadmium, mercury and bismuth on the copper content in rat tissues. Environ. Res. 19: 121–126 (1979).
24. Szymanska, J. A., Zelazowski, A. J., and Kawiorski, S. Some aspects of bismuth metabolism. Clini. Toxicol. 18: 1291–1298 (1981).
25. Winge, D. R., Premakumar, R., and Rajagopalan , K. W. Studies on the zinc content of Cd-induced thionein. Arch. Biochem. Ciophys. 188: 466–475 (1978).
26. Piotrowski, J. K., Szymańska, J. A., Mogilnicka, E. M., and Zelazowski, A. J. Renal metal binding proteins. In: Metallothionein (J. H. R. Kagi and M. Nordberg, Eds.), Experientia 1979, Suppl. 34, pp. 363–371.
27. Mogilnicka, E. M., and Webb, M. The distribution of gold, copper, and zinc in animals treated with Au (III); species differences in the binding of these metals in the kidney. J. Appl. Toxicol. 1: 42–50 (1981).
28. Chmielnicka, J., Szymańska, J. A., and Snieć, J. Distribution of tin in the rat and disturbances in the metabolism of zinc and copper due to repeated exposure to SnCl. Arch. Toxicol. 47: 263–266 (1981).
29. Nederbragt, H. The influence of molybdenum on the copper medtabolism of the rat at different Cu levels of the diet. Brit. J. Nutr. 43: 329–338 (1980).
30. Martin, M., and Brady, F. O. Interaction of rat liver zinc metallothionein by mercuric chloride. Proc. S. D. Acad. Sci. 56: 72–76 (1977).
31. Bogden, J. D., Kemp, F. W., Troiano, R. A., Jortner, B. S., Timpone, C., and Guiliani, D. Effect of mercuric chloride and methylmercury chloride exposure on tissue concentrations of six essential minerals. Environ. Res. 21: 350–359 (1980).
32. Tandon, S. K., Magos, L., and Cabral, J. R. P. Protection against mercuric chloride by nephrotoxic agents which do not induce thionein. Toxicol. Appl. Pharmacol. 52: 227–236 (1980).
33. Suzuki, T. K., Kubota, K., and Takenaka, S. Copper in cadmium exposed rat kidney metallothionein. Chem. Pharm. Bull. 25: 2794–2796 (1977).
34. Suzuki, K. T., and Yamamura, M. Gel and anion exchange chromatographic properties of copper-containing metallothioneins. Arch. Environ. Contam. Toxicol. 8: 471–485 (1979).
35. Zelazowski, A. J., and Szymańska, J. A. Low molecular height cadmium-and copper-binding proteins from rat kidneys. Biol. Trace Elements Res. 2: 137–148 (1980).
36. Zelazowski, A. J., Szymańska, J. A., and Cierniewski, C. S. Immunological properties of low-molecular weight proteins binding heavy metals in rat kidneys and liver. Chem.-Biol. Interact. 39: 115–125 (1980).
37. Szymańska, J. A., Chmielnicka, J., and Jaskulska, J. The copper and zinc content of low molecular weight hepatic proteins from bismuth-exposed rats. Biol. Trace Elements Res. 3: 265–170 (1981).
38. Suzuki, K. T., and Yamamura, M. Changes of metal contents and isometallothionein levels in rat tissues after cadmium loading. Biochem. Pharmacol. 29: 2407–2412 (1980).
39. Zelazowski, A. J., and Piotrowski, J. K. Mercury-binding, copper- zinc proteins from rat kidney, amino acid composition molecular weight and metal content. Biochem. Biophys. Acta 625: 89–99 (1980).
40. Kagi, J. H. R., and Valle, B. L. Metallothionein: a cadmium and zinc-containing protein from equine renal cortex. J. Biol. Chem. 235: 3460–3465 (1960).
41. Piotrowski, J. K., Trojanowska, B., Wiśniewska-Knypl, J. M., and Bolanowska, W. Further investigations on binding and release of mercury in the rat. In: Mercury, Mercurials and Mercaptans (M. W. Miller and T. W. Clarkson, Eds.), C. C Thomas, Springfield, Ill, 1973, pp. 247–263.
42. Piotrowski, J. K., Trojanowska, B., and Sapota, A. Binding of cadmium and mercury by metallothionein in the kidneys and liver of rats following repeated administration. Arch. Toxicol. 32: 351–360 (1974).
43. Piotrowski, J. K., Trojanowska, B., and Wiśniewska-Knypl, J. M., and Bolanowska, W. Mercury binding in the kidney and liver of rats repeatedly exposed to mercuric chloride: induction of metallothionein by mercury and cadmium. Toxicol. Appl. Pharmacol. 27: 11–19 (1974).
44. Mogilnicka, E. M., Piotrowski, J. K., and Trojanowska, B. Effect of some metals (Cd, Hg, Zn, Cu, Pb, Mn, V) on metallothionein level in the liver and kidneys of rat. Med. Pracy 26: 147–155 (1975).
45. Cherian, M. G., and Clarkson, T. W. Biochemical changes in rat kidney on exposure to elemental mercury vapor. Effect on biosynthesis of metallothionein. Chem.-Biol. Interact. 12: 109–120 (1976).
46. Komsta-Szumska, E., Chmielnicka, J., and Piotrowski, J. K. The influence of selenium on binding of inorganic mercury by metallothionein in the kidney and liver of the rat. Biochem. Pharmacol. 25: 2539–2540 (1976).
47. Komsta-Szumska, E., and Chmielnicka, J. Binding of mercury and selenium in subcellular fractions of rat liver and kidneys following separate and joint administration. Arch. Toxicol. 38: 217–228 (1977).
48. Chmielnicka, J., and Brzeźnicka, E. A. The influence of selenium on the level of mercury and metallothionein in rat kidneys in prolonged exposure to different mercury compounds. Bull. Environ. Contam. Toxicol. 19: 183–190 (1978).
49. Mengel, H., and Karlog, O. Studies on the interaction and distribution of selenite, mercuric metoxyethyl mercuric and methyl mercuric choride in rats. II. Analysis of the soluble proteins and the precipitates of liver and kidney homogenates. Acta Pharmacol. Toxicol. 46: 25–31 (1980).
50. Omata, S., Sato, M., Sakimura, K., and Sugano, H. Time- de-

pendent accumulation of inorganic mercury in subcellular fractions of kidney, liver, and brain of rats exposed to methylmercury. Arch. Toxicol. 44: 231–241 (1980).
51. Brzeźnicka, E. A., and Chmielnicka, J. Interaction of alkylmercuric chloride compounds with sodium selenite. II. Metabolism of methylmercuric chloride administered alone and in combination with sodium selenite in rats. Environ. Health Perspect. 60: 411–421 (1985).
52. Brzeźnicka, E. A., and Chmielnicka, J. Interaction of alkyl mercuric compounds with sodium selenite. I. Metabolism of ethylmercuric choride administered alone and in combination with sodium selenite in rats. Environ. Health Perspect. 39: 131–142 (1981).
53. Gage, J. C. Distribution and excretion of methyl and phenyl mercury salts. Brit. J. Ind. Med. 21: 197–202 (1964).
54. Norseth, T., and Clarkson, T. W. Studies on the biotransformation of ^{203}Hg-labelled methyl mercury choride in rats. Arch. Environ. Health 21: 717–727 (1970).
55. Miller, V. K., Klavano, P. A., Jerstad, A. C., and Csonke, E. Absorption, distribution and excretion of ethylmercuric chloride. Toxicol. Appl. Pharmacol. 3: 459–468 (1961).
56. Takeda, Y., and Ukita, T. Metabolism of ethylmercuric chloride Hg in rats. Toxicol. Appl. Pharmacol. 17: 181– 188 (1970).
57. Suzuki, T., Takemoto, H., Kashiwazaki, H., and Miyama, T. Metabolic fate of ethylmercury salts in man and animal. In: Mercury, Mercurials and Mercaptans (M. W. Miller and T. W. Clarkson, Eds.), C. C Thomas, Springfield, IL 1973, pp. 209– 232.
58. Brzeźnicka, E., Chmielnicka, J., and Wachocka, A. Effect of sodium selenite on mercury level in kidneys, liver, and blood of rats intoxicated with different mercuric compounds. Bromat. Chem. Toksykol. 11: 313–322 (1978).
59. Chen, R. W., Whanger, P. D., and Fang, S. C. Diversion of mercury binding in rat tissues by selenium: a possible mechanism of protection. Pharmacol. Res. Comm. 6: 571–579 (1974).
60. Fang, S. C. Interaction of selenium and mercury in the rat. Chem.-Biol. Interact. 17: 25–40 (1977).
61. Burk, R. F., Jordan, H. F., and Kiker, K. W. Some effects of selenium status on inorganic mercury metabolism in the rat. Toxicol. Appl. Pharmacol. 40: 71–82 (1977).
62. Chmielnicka, J., Komsta-Szumska, E., and Jedrychowski, R. Organ and subcellular distribution of mercury in rats as dependent on the time of exposure to sodium selenite. Environ. Res. 20: 80–86 (1979).
63. Chmielnicka, J., and Komsta-Szumska, E. Variation of the level of mercury and metallothionein in the kidneys and liver of rats with time of exposure to sodium selenite. Biol. Trace Elements Res. 2: 109–120 (1980).
64. Piotrowski, J. K., Bolanowska, W., and Sapota, A. Evaluation of metallothionein content in animal tissues. Acta Biochem. Pol. 20: 207–215 (1973).
65. Zelazowski, A. J., and Piotrowski, J. K. A modified procedure for the determination of metallothionein like proteins in the animal tissues. Acta Biochem. Pol. 24: 97–104 (1977).
66. Pulido, P., Kagi, J. H. R., and Allee, B. L. Isolation and some properties of human metallothionein. Biochemistry 5: 1768–1777 (1966).
67. Zelazowski, A. J., Piotrowski, J. K., Mogilnicka, E. M., Szymańska, J., and Kaszper, W. The preparation of metallothionein from equine renal cortex as a standard protein for analytical purposes. Bromat. Chem. Toksykol. 11: 51–57 (1978).
68. Szymanska, J. A., and Zychowicz, M. Simultaneous spectrophotometric determination of bismuth and copper in biological material by using zinc dibenzyldithiocarbamate. Chem. Anal. 22: 985–988 (1977).
69. Magos, L. Selective atomic-absorption determination of inorganic mercury and methylmercury in undigested biological samples. Analyst 96: 847–853 (1971).
70. Balcerska, I., Brzeźnicka, E., and Chmielnicka, J. Determination of inorganic and organic mercury in nonmineralized samples of biological material by cold vapour atomic absorption. Bromat. Chem. Toksykol. 10: 101–104 (1977).
71. Lehman, H. P., Schosinsky, K. H., and Beeler, M. F. Standardization of serum ceruloplasmin concentrations in international enzyme units with o-dianisidine dihydrochloride as substrate. Clin. Chem. 20: 1564–1567 (1974).
72. Klein, R., Herman, S. P., Bullock, B. C., and Talley, F. A. Methyl mercury intoxication in rat kidneys. Functional and pathological changes. Arch. Pathol. 96: 83–90 (1973).
73. Norseth, T. Biotransformation of methylmercuric salts in the rat with chronic administration of methyl mercuric cysteine. Acta Pharmacol. Toxicol. 31: 138–148 (1972).
74. Magos, L., and Butler, W. H. The kinetics of methylmercury administered repeatedly to rats. Arch. Toxicol. 35: 25–39 (1976).
75. Pekkanen, T. J., and Pekkarinen, K. The effect of methylmercury pretreatment on the duration of hexabarbital hypnosis on rat. Acta. Vet. Scand. 13: 149–150 (1972).
76. Pekkanen, T. J., and Salminen, K. Inductive effects of methyl mercury on the hepatic microsomes of mice. Acta Pharmacol. Toxicol. 32: 289–293 (1973).
77. Lucier, G. W., Matthews, H. B., Brubaker, P. E., Klein, R., and McDaniel, O. S. Effects of methylmercury on microsomal mixed-function oxidase components of rodents. Mol. Pharmacol. 9: 237–246 (1973).
78. Alvares, A. R., Cohn, J., and Kappas, A. Studies on the effects of methylmercury on ethylmorphine-N-demethylase and aniline hydroxylase activities and on the conversion of cytochrome P-450 to cytochrome P-420. Drug. Metab. Dispos. 2: 259–266 (1974).
79. Desnoyers, P. A., and Chang, L. W. Ultrastructural changes in rat hepatocytes following acute methylmercury intoxication. Environ. Res. (224–239) (1975).
80. Yamane, J., Fukino, H., Aida, Y., and Imagawa, M. Studies on the mechanism of protective effects of selenium against the toxicity of methylmercury. Chem. Pharm. Bull. 25: 2831–2837 (1977).
81. Groth, D. H., Stettler, L., and Mackay, G. Interactions of mercury, cadmium, selenium, tellurium, arsenic and beryllium. In: Effect and Dose-Response Relationships of Toxic Metals (G. F. Nordberg, Ed.), Elsevier, Amsterdam, 1976, pp. 527–543.
82. Mengel, H., and Karlog, O. Studies on the interaction and distribution of selenite, mercuric, methoxyethylmercuric and methyl mercuric chloride in rats. I. Analysis of brain liver, kidney and faeces. Acta Pharmacol. Toxicol. 46: 14–24 (1980).
83. Fowler, B. A. The morphologic effect of dieldrin and methyl mercuric chloride on pars recta segments of rat kidney proximal tubules. Am. J. Pathol. 69: 163–178 (1972).
84. Fowler, B. A., and Woods, J. S. Ultrastructural and biochemical changes in renal mitochondria during chronic oral methyl mercury exposure. The relationship to renal function. Exptl. Mol. Pathol. 27: 403–412 (1977).
85. Magos, L. Factors effecting the neurotoxicity of mercury and mercurials. In: Advances in Neurotoxicology (L. Manzo Ed.), Pergamon Press, New York, 1980, pp. 17–25.