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 not observed 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 methylmercury cyanoguanidine (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.
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 Na₂SeO₃ (POCh, Gliwice, Poland), also intragastrically, 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 Balseerska 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.

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.

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

| Tissue | Group | Inorganic Hg | Organic Hg |
|-------|-------|--------------|------------|
| Kidneys | I | 1.75 | 4.21 |
| | Ia | 1.08–2.10 | 3.85–4.89 |
| | II | 1.58 | 2.39 |
| | IIa | 0.78–2.43 | 1.54–3.19 |
| | III | 14.28 | 47.02 |
| | IIIa | 12.19–16.49 | 44.67–51.80 |
| | IV | 9.41 | 47.83 |
| | IVa | 7.20–13.81 | 37.37–61.93 |
| Liver | I | 0.82 | 0.77 |
| | Ia | 0.33–0.53 | 0.76–0.96 |
| | II | 0.51 | 0.70 |
| | IIa | 0.24–0.99 | 0.22–0.97 |
| | III | 0.13 | 13.29 |
| | IIIa | 0.08–1.27 | 12.18–13.34 |
| | IV | 0.55–2.39 | 6.52–8.36 |
| Brain | I | 0.08 | 0.49 |
| | Ia | 0.06–0.10 | 0.47–0.52 |
| | II | 0.08 | 0.68 |
| | IIa | 0.00–0.12 | 0.64–0.76 |
| | III | 0.69 | 3.70 |
| | IIIa | 0.39–1.34 | 3.07–3.99 |
| | IV | 0.90 | 6.37 |
| | IVa | 0.78–1.03 | 6.24–6.50 |
| Blood | I | 0.64 | 6.77 |
| | Ia | 0.10–1.87 | 5.50–7.11 |
| | II | 0.27 | 2.91 |
| | IIa | 0.22–0.34 | 2.84–2.96 |
| | III | 5.82 | 52.86 |
| | IIIa | 2.42–10.50 | 34.60–98.39 |
| | IV | 3.11 | 65.69 |
| | IVa | 1.80–3.90 | 49.80–85.08 |

*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 increase 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, irrespective 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 25% (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 proteins (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 µg Cu/g, and 2.0 µg Cu/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/
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 administered simultaneously with methylmercury (groups Ia and Ila) 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](https://example.com/figures2.png)

**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 LD₅₀.

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 (78), 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 alkylmercurials 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 methoxethylmercuric 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 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-
The neurotoxicity coefficients for various doses of methylmercuric chloride are similar to those 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 HgCl₂. 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.

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

| Treatment | Se | Kidney/brain total Hg ratio | Kidney/brain inorganic Hg (by AAS) |
|-----------|----|-----------------------------|----------------------------------|
| MeHg, 0.25 mg Hg/kg | No | 0.12 | 0.10 | 0.12 |
| MeHg, 2.50 mg Hg/kg | No | 0.09 | 0.06 | 0.08 |
| 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 | 0.20 |
| 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 |

* Ratios were calculated from tissue concentrations measured 24 hr after 2-week exposure to methyl- or ethylmercuric chloride with and without Se.

* Determined as ³⁰₀₂Hg (51,52).

* Determined by AAS according to Balcerska et al. (64).

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

| Treatment | Se | Brain/blood total Hg ratio | Brain/blood inorganic Hg (by AAS) |
|-----------|----|-----------------------------|----------------------------------|
| MeHg, 0.25 mg Hg/kg | No | 0.12 | 0.10 | 0.12 |
| MeHg, 2.50 mg Hg/kg | No | 0.09 | 0.06 | 0.08 |
| 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 | 0.20 |
| 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 |

* Ratios were calculated from tissue concentrations measured 24 hr after 2-week exposure to methyl- or ethylmercuric chloride with and without Se.

* Determined as ³⁰₀₂Hg (51,52).

* Determined by AAS according to Balcerska et al. (64).
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