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Mercury distribution and renal metallothionein induction after subchronic oral exposure in rats

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The effects of long-term daily intake of low and high levels of mercury on its organ distribution and binding to renal metallothionein (MT) in male rats were studied. The animals were exposed to mercuric chloride labelled with ^{203}Hg via drinking water for 8 weeks (5, 50 and 500 μM Hg). The greatest concentration of mercury was found in the kidneys. Similar levels of radioactivity in the buccal cavity and oesophagus were also observed by whole-body autoradiography. In the kidneys, the mercury was accumulated in the outer stripe of the outer zone of the medulla and, to a minor degree, in the renal cortex. Almost 50% the total renal mercury was associated to MT. The binding capacity of the renal MT for mercury tends to saturate with increasing doses, thus this means that the capacity of the kidneys to accumulate mercury is limited.

Keywords: mercury, kidney, metallothionein, oral exposure

Introduction

Environmental and occupational exposure to the various forms of mercury remain a serious problem throughout the world. It is known that mercury has a high affinity for ectodermal and endodermal epithelial cells and glands (WHO 1991). The distribution of mercury within the body and specific organs changes with dose and time after exposure (Berlin 1986). Furthermore, the route of administration affects the organ distribution of absorbed mercury (Nielsen & Andersen 1990). The relative organ distribution of retained mercury 14 days after a single oral administration of $HgCl_2$ depends on the dose, and is characterized by an increase of relative deposition in the liver, stomach, intestines, testes, spleen and carcass, and by a decrease of relative deposition in the kidneys with increasing dose (Nielsen & Andersen 1989). The kidneys are the main depositories of mercury after the administration of mercury vapour or inorganic mercury compounds (Nordlind 1990, WHO 1991). Renal accumulation of mercury occurs mainly in the cortex and outer stripe of the outer medulla (Zalups & Diamond 1987, Zalups *et al.* 1987), specifically in the pars recta of the proximal tubule (Zalups 1991). Tissue fractionation studies have shown that mercury is enriched in the renal soluble fraction, where it induces the synthesis and binds to metallothionein (MT), a low molecular weight, cysteine-rich, heavy metal binding protein (Jakubowski *et al.* 1970, Nordberg *et al.* 1974, Piotrowski *et al.* 1974, Webb & Magos 1980, Nolan & Shaikh 1987, Morcillo & Santamaria 1993). The fact that MTs are metal-binding proteins induced by the metals that bind to them suggests a role for MT in the basic cellular metabolism of metals (Bremner 1991). Although the protective effects of MT against exposure to toxic heavy metals have been studied extensively in animal experiments and cell culture systems, the detoxification is not universally accepted as a primary function of MT (Karin 1985). Such detoxification of heavy metals represents an important role for MT, although it could be an adventitious consequence of the ability of these metals to induce and bind to a protein that is primarily concerned with the metabolism of zinc and copper (Bremner 1991). MT is strongly involved in cadmium metabolism. Many reports have documented the synthesis of MT protein in the liver and kidney following cadmium exposure (Lehmann-McKeeman & Klaassen 1987). Furthermore, in studies conducted *in vitro*, the induction of MT gene expression in a variety of cell types in response to cadmium treatment has been shown (Jahroudi *et al.* 1990). On the other hand, whereas the induction of MT allows most cells to sequester intracellular cadmium in a non-toxic form, at least temporarily, extracellular Cd–MT is a potent nephrotoxin that affects proximal tubule function. This has

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been demonstrated by i.v. injection of Cd–MT into rats (Cherian *et al.* 1976, Squibb & Fowler 1984) and in primary cultures of renal epithelial cells (Maitani *et al.* 1988). With regard to mercury, it has been argued that the impact of MT induction on mercury nephrotoxicity is probably small (Webb 1987) and only a small part of the renal burden of Hg(II) is bound to MT (Templeton & Cherian 1991). On the contrary, it has been suggested that renal MT could play an important role in the binding of mercury when it is present at low levels (Whanger & Deagen 1983, Nolan & Shaikh 1987, Morcillo *et al.* 1992).

Most of the toxicokinetic studies of mercury compounds have used either single-dose administration or short-term exposures; however, humans are either chronically or subchronically exposed to mercury. Moreover, human exposure to inorganic mercury compounds occurs via oral or pulmonary routes. Therefore, research of the correlation between the toxicokinetics of mercury compounds after a single administration and after chronic or subchronic exposure via the oral route in experimental animals would be relevant for risk evaluation with regard to human mercury exposure (Nielsen & Andersen 1989, Nielsen 1992). The effects of long-term daily intake of mercury on its urinary and fecal excretion, whole-body retention and blood concentration in male rats were previously studied (Morcillo & Santamaria 1995). The present study has been carried out to explain the effects of long-term daily intake of mercury on its quantitative tissue and organ distribution in rats exposed to low and high levels of mercuric chloride. At the same time, it will provide us with detailed knowledge of the relation between tissue and organ concentrations of mercury (mainly the kidneys) and its toxic symptoms in experimental animals subchronically exposed to mercury via the oral route. In order to determine the importance of the association of mercury with MT on mercury toxicity, the binding of mercury to MT in the liver and the kidneys of exposed rats was studied.

Materials and methods

Animals

Twenty-four male Sprague-Dawley rats weighing about 130 g were used. After a conditioning period of 4 days in a temperature/light controlled environment, they were randomly assigned to three experimental groups of six animals each and one as a control group. Animals were marked, weighed and housed in metabolic cages. At the beginning of the exposure period, the rats were fed with 12 g day^{-1} of standard laboratory rodent diet (Rodent Toxicology Diet, B&K Universal, Barcelona, Spain) and this amount was gradually increased to 20 g day^{-1} with the purpose to avoid excessive weight of the animals at the end of the exposure period. Separate groups of six rats received oral doses of mercuric chloride labeled with $^{203}HgCl_2$ (Amersham, Amersham, UK) via drinking water *ad libitum* for 8 weeks (about 3.7×10^3 Bq ml^{-1}). Mercury levels were 5, 50 and 500 μM. Rats exposed to 500 μM Hg neither drank nor ate because of an excessive salty taste of the water; therefore, to avoid this problem, drinking water containing 500 μM Hg was supplemented with 5% sugar. Mean daily doses of mercury were 0.1, 1 and 7 mg kg^{-1} for the rats exposed to 5, 50 and 500 μM Hg, respectively.

Radioactivity determination

The amount of radioactivity in each sample was measured in all cases by solid scintillation counting using an automatic gamma spectrometer (LKB Model 1275). As the isotope decay must be taken in account throughout the exposure period, 1 ml ^{203}Hg standard with a known initial amount of radioactivity was counted at the beginning of each counting session. The gamma counter background was below 700 c.p.m. Main ingested doses of ^{203}Hg were calculated by means of an aliquot of water taken directly from the drinking bottle of each rat at the end of each day of the study, because of the problem of absorption of mercury to the glass plastic containers. As the different organs and tissues varied in size, we evaluated this effect on the counting efficiency for different sample volumes. Increasing amounts of water were added to a vial starting with an initial amount of ^{203}Hg, and a curvilinear relationship between counting efficiency and sample volume was found. The counting efficiency was around 45%, which was considered satisfactory. Thus, the c.p.m. were converted into d.p.m. after correction for background and sample volume.

Organ distribution

At the end of the experimental period, five rats of each group were sacrificed by total exanguination (by cardiac puncture under deep ether anaesthesia) and immediately after sacrifice the following tissues/organs were removed from each carcass: kidneys, liver, spleen, perirenal fat, testes, muscle, bone marrow (from a femur), lungs, heart, salivary glands and brain. Subsequently, the samples were weighed and radioactivity measured as described above. The kidneys and liver were frozen at $-70°C$ for subsequent analysis by subcellular fractionation.

Whole-body autoradiography

One animal of each group (except the control group) was sacrificed at the end of the experimental period. After sacrifice, each rat was pinned out on a board and rapidly frozen by total immersion in a bath of solid CO_2/hexane at about $-70°C$ for about 30 min. After trimming off the legs and tail with a power saw, the frozen carcass was set in a block of an aqueous solution of carboxymethylcellulose (1.5% w/v) at about $-80°C$ and mounted onto the stage of a microtome in a cryostast maintained at $-20°C$. Sagittal sections (100 μm) were then obtained. The sections were mounted on a transparent adhesive tape and freeze-dried at $-40°C$ and 0.05 torr in a VirTis-Consol 4.5 freeze drier before placing them in contact with Hyperfilm-MP film (Amersham) at $-60°C$. Once exposure time had been

completed (1 month), the autoradiographs on MP film were developed by standard photographic procedures. Some of the autoradiographs were also used to furnish enlargements of areas of interest.

Distribution of mercury in cytosolic proteins of kidney and liver

Frozen liver and kidneys were minced and homogenized in two volumes of 0.01 M Tris–HCl buffer (pH 8.6)/0.25 M Sucrose/0.005 M 2-mercaptoethanol (bubbled with nitrogen before use) using a Potter homogenizer (B. Braun Melsungen AG, Melsungen, Germany) and the homogenate was centrifuged at 105 000 *g* for 90 min in a Centrikon T-1055 ultracentrifuge with a TFT 65.13 rotor (Kontron Instruments, Milan, Italy) at 4°C. The homogenates and cytosols obtained were placed in tubes, and their radioactivity measured by solid scintillation counting using a LKB MiniGamma automatic analyzer. The liver and renal supernatant were applied to a Sephadex G-75 column (70 × 26 cm; Pharmacia, Uppsala, Sweden) which had been pre-equilibrated with 0.02 M ammonium formiate buffer (pH 7.65, adjusted with ammonia solution) and the column was eluted with the same buffer (bubbled with nitrogen before use). Although MT is usually eluted from Sephadex G-75 with Tris–HCl buffer, ammonium formiate was used to prevent the binding of mercury to the Sephadex gel (Wilcox & Lisowski 1960). Fractions (4.2 ml) were collected and radioactivity measured by solid scintillation counting techniques.

MT determination

MT was indirectly quantitated by measuring its SH content. The sensitive Ellman's reagent DTNB [5,5'-dithiobis(2-nitrobenzoic acid)] was used for sulphydryl determination (Ellman 1959). The sulphydryl reagents react with the sulphydryl groups of MT leading to the displacement of the bound metals. The kinetic behaviour of Ellman's reagent depends on the metal composition of MT due to the different affinities for metals to the protein. To determine the kinetic of the reaction of DTNB with the mercury-thionein, immediately after adding DTNB to a sample of ^{203}Hg-thionein the cuvette was placed into a spectrophotometer set to measure the change in absorbance of 412 nm as a function of time against a blank sample. The measured absorbance reached its maximum at approximately 3 h. The following procedure for sulphydryl determination was used: 2 ml of each fraction of rat MT peak isolated onto the Sephadex G-75 column were dried in a Speed Vac Concentrator and redissolved in 2 ml of a solution containing 0.1 M phosphate buffer (pH 8.0). Immediately, a volume of 100 µl DTNB (39.6 mg DTNB in 10 ml 0.1 M phosphate buffer, pH 8.0) was added to each fraction and the resulting absorbance, at 412 nm after 3 h, was used to calculate the amount of sulphydryl groups in each fraction of the MT peak ($\varepsilon_{412} = 13\,600\ \text{M}^{-1}\ \text{cm}^{-1}$). Although it has been described that SH-containing compounds other than MT can alter MT quantification by this method (Summer & Kelin 1991), we have previously observed, by HPLC analysis, that renal MT from rats exposed to inorganic mercury and isolated on a Sephadex G-75 column was absent of non-MT proteins that might interfere with the quantification of MT via its SH content (Morcillo & Santamaria 1993). Therefore, this method seems to be appropriate for the determination of rat renal Hg–MT.

Statistical analysis

Statistical differences between means of exposed and control groups were examined by Student's two-tailed *t*-test using the statistical package of CSS:STATISTICA from Statsoft. All data are expressed as mean ± SEM.

Results

Organ and tissues distribution

Mean organ weights are presented in Table 1. The results show that the weight of the animals decreased gradually with the increasing doses of mercury and the weight of all organs also decreased, except the kidneys. The mean weight

Table 1. Organ weight changes of rats exposed to mercuric chloride.

Organ	Exposure level (µM Hg)			
	Control	5	50	500
Body weight	318 ± 6	300 ± 5	297 ± 5[a]	257 ± 9[c]
Kidneys	1.81 ± 0.03	1.79 ± 0.03	2.02 ± 0.03[b]	2.39 ± 0.08[c]
Liver	11.56 ± 0.22	8.33 ± 0.14[c]	9.75 ± 0.16[c]	7.95 ± 0.28[c]
Spleen	0.50 ± 0.01	0.51 ± 0.01	0.42 ± 0.01[c]	0.42 ± 0.01[c]
Testes	3.48 ± 0.07	3.41 ± 0.06	3.03 ± 0.05[c]	3.41 ± 0.12
Lungs	1.12 ± 0.02	1.09 ± 0.02	1.08 ± 0.02	1.07 ± 0.04
Heart	0.87 ± 0.02	0.74 ± 0.01[c]	0.69 ± 0.01[c]	0.84 ± 0.03
Salivary glands	0.63 ± 0.01	0.62 ± 0.01	0.55 ± 0.01[c]	0.55 ± 0.02[b]
Brain	1.88 ± 0.04	1.84 ± 0.03	1.74 ± 0.03[a]	1.69 ± 0.06[a]

[a]Statistically significant differences with control group ($P < 0.05$).
[b]Statistically significant differences with control group ($P < 0.01$).
[c]Statistically significant differences with control group ($P < 0.001$).

of the kidneys in rats exposed to 50 and 500 μM was 1.1 and 1.3 times significantly higher than in the control group, respectively.

The mean mercury concentration increased with the exposure level in all examined organs (Table 2). The greatest concentration of mercury was found in the kidneys in all exposed animals; however, the mean mercury concentration in the kidneys of the animals exposed to 50 and 500 μM Hg was similar (about 87 and 94 μg Hg g^{-1} tissue, respectively). This fact suggests that the kidneys have a limited capacity to accumulate mercury. Apart of the kidneys, the greatest concentration of mercury was found in the liver, lungs and spleen in all exposed animals, although in rats exposed to 500 μM, the mean mercury concentration in the brain and bone was similar to that in the spleen (around 1 μg Hg g^{-1} tissue). Mercury concentrations in all organs were 8–13 times higher in rats exposed to 50 μM Hg than in rats exposed to 5 μM. The most significant increase was observed in the brain where the mercury concentration was 16 times higher. Concentrations of mercury in selected organ and tissues were 3–6 times higher in rats exposed to 500 μM Hg than in rats exposed to 50 μM, with the exception of the muscle and brain. Since the error of the mean concentration of mercury in the muscle of rats exposed to 500 μM was high, not much emphasis can be placed on this value. With regard to the concentration of mercury in the brain, this was 14 times higher in the rats exposed to 500 μM than in rats exposed to 50 μM.

Table 2. Mercury concentration in organs and tissues of rats exposed to mercuric chloride.

Organ	Concentration (ng Hg g^{-1} tissue)		
	5 μM	50 μM	500 μM
Kidneys	8625 ± 486	86 788 ± 5891	94 030 ± 4417
Liver	47.9 ± 4.1	560 ± 60	3350 ± 377
Spleen	27.6 ± 2.5	356 ± 89	973 ± 87
Fat	15.6 ± 3.9	106 ± 22	262 ± 13
Testes	9.0 ± 0.8	118 ± 13	533 ± 25
Muscle	2.4 ± 0.2	20.2 ± 1.5	571 ± 344
Bone	15.5 ± 1.7	176 ± 14	851 ± 48
Lungs	27.4 ± 1.6	358 ± 123	2192 ± 189
Heart	8.3 ± 0.3	83.1 ± 8.1	468 ± 45
Salivary glands	12.4 ± 0.3	116 ± 8	357 ± 32
Brain	5.4 ± 0.2	87.7 ± 8.6	1238 ± 108

Autoradiography

The results obtained by whole-body autoradiography after oral exposure to 500 μM Hg (as $^{203}HgCl_2$) for 8 weeks are shown in Figure 1. Whole-body autoradiograms of the medial sagittal sections of rats exposed to 5 and 50 μM (not shown) were similar to those exposed to 500 μM. The greatest concentration of radioactivity, which was similar to the concentration found in the kidneys, was present in the buccal cavity and oesophagus; this mercury deposition is not surprising since exposure to mercury was by the oral route. Lower concentrations of radioactivity were present in the stomach, intestine and rectum. In sagittal lateral sections, the radioactivity was preferentially located in the kidneys (Figure 2). In this organ, the mercury was mainly concentrated in the outer stripe of the outer zone of the medulla (medullar rays) and in a lower concentration in the renal cortex of both groups exposed to 50 and 500 μM. The difference observed between the level of radioactivity in the outer stripe of the medulla and in the renal cortex was less for rats exposed to 5 μM. As it can be observed, the level of radioactivity in the kidneys of rats exposed to 500 μM was less than the level found in rats exposed to 5 and 50 μM. This was expected since the concentration of mercury was similar in the kidneys of rats exposed to 50 and 500 μM, while the specific activity of ^{203}Hg in the drinking water was 10 times lower for rats exposed to 500 μM than for rats exposed to 50 μM.

Liver and kidney distribution

No changes in the percentage of the total liver ^{203}Hg distributed in the cytosol with increasing exposure level were observed (around 56% for all exposed groups). Although

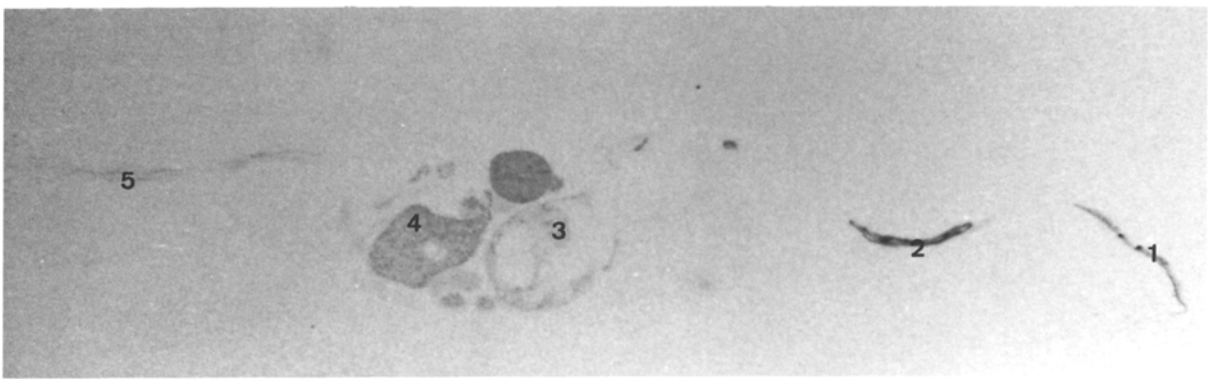

Figure 1. Autoradiograph corresponding to sagittal medial section of a rat exposed to 500 μM Hg (as $^{203}HgCl_2$) for 8 weeks. (1) Buccal cavity, (2) oesophagus, (3) stomach, (4) intestine and (5) rectum.

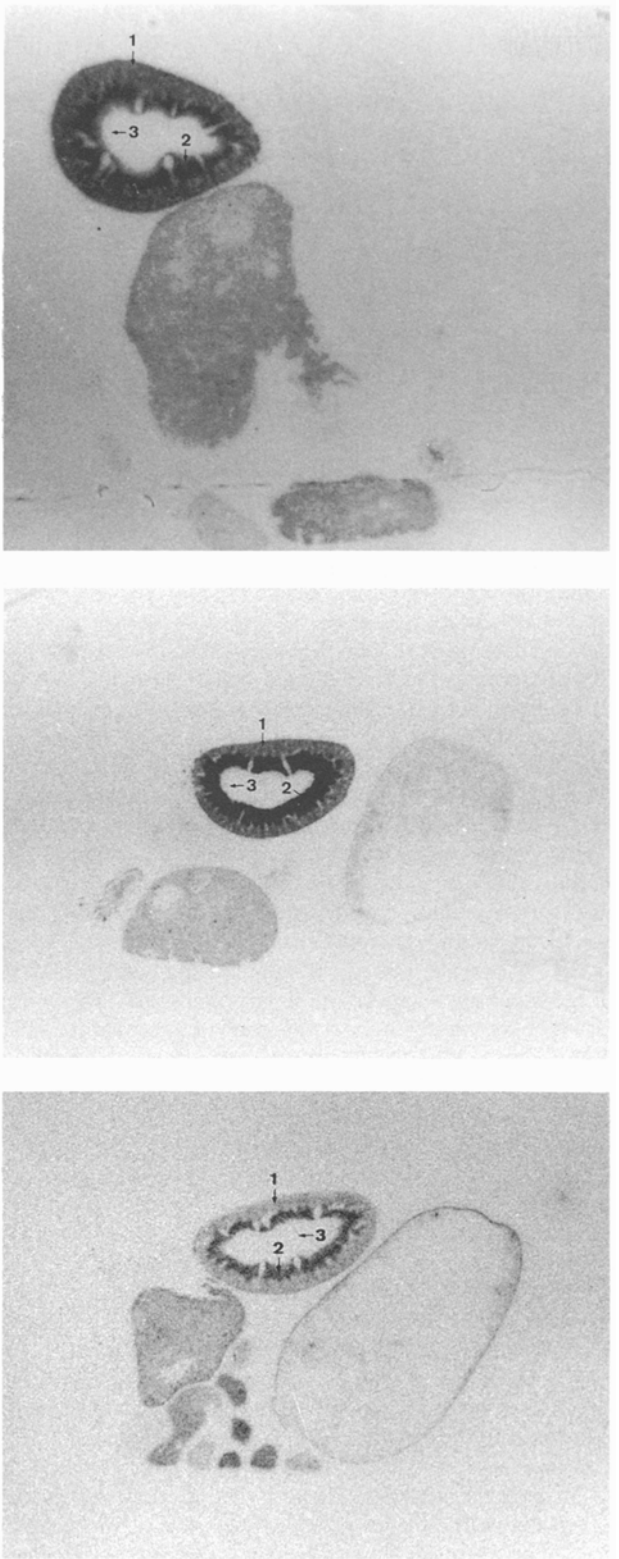

Figure 2. Autoradiographs corresponding to sagittal lateral sections of rats exposed to 5 (top), 50 (middle) and 500 (bottom) μM Hg (as $^{203}HgCl_2$) for 8 weeks. In these sections only the zone around kidney is shown. (1) Renal cortex, (2) outer stripe of the outer zone of medulla and (3) inner zone of medulla.

there was an apparent decrease in the proportion of the total renal ^{203}Hg in the cytosol with increasing exposure level (68, 62 and 60% in rats exposed to 5, 50 and 500 μM Hg, respectively), no statistically significant change in the relative distribution of ^{203}Hg with increasing mercury doses was observed. Liver cytosol fractions analysis revealed that the chromatographic profiles from gel filtration on Sephadex G-75 were similar for each mercury dose studied (Figure 3). Most of the eluted cytosolic radioactivity was associated with the void volume proteins (high weight molecular proteins) and only a small quantity of ^{203}Hg in association with MT (50–70 fractions) was eluted. Because the ^{203}Hg content in the liver MT peak was low and near to the detection limit of the gamma counter, the amount of mercury bound to liver MT was not calculated. Figure 3 also shows the elution profiles of the soluble cytoplasmic fraction of rat kidney. The mean recovery of the eluted cytosolic ^{203}Hg associated with MT was 66% at lower dose levels, i.e. 5 and 50 μM Hg. At the highest exposure dose level studied, i.e. 500 μM Hg, the mean recovery of the eluted radioactivity detected in association with MT was 84%. The mean recovery from the total elution of renal cytosolic radioactivity from the Sephadex G-75 column was about 98%. The percentage of renal ^{203}Hg associated with MT

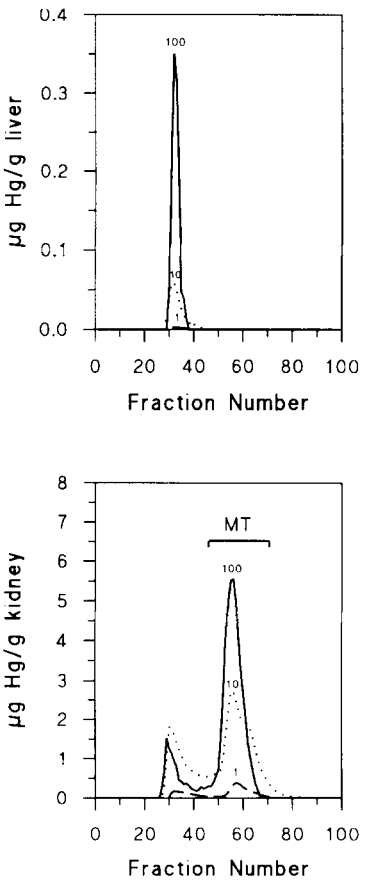

Figure 3. Gel filtration on Sephadex G-75 chromatograms of ^{203}Hg-labelled liver (top) and kidney (bottom) cytosols of rats exposed to 5, 50 and 500 μM Hg mercury.

Table 3. Binding of mercury to renal MT in rats exposed to mercuric chloride.

Exposure level (μM Hg)	Renal ^{203}Hg associated to MT (%)	Hg bound to MT (μg Hg g^{-1} tissue)	Renal MT (μg MT g^{-1} tissue)	Hg/MT
5	45.3±2.3	3.88±0.19	72.26±9.10	1.5±0.1
50	40.2±2.1	35.25±3.62[a]	196.23±27.47[a]	4.8±0.5[a]
500	48.0±1.4[b]	45.19±2.60[a]	283.72±5.35[a,b]	4.7±0.2[a]

[a]Statistically significant differences with the rats exposed to 5 μM ($P<0.001$).
[b]Statistically significant differences with the rats exposed to 50 μM ($P<0.05$).

was calculated and is shown in Table 3. Although the percentage of mercury retained in the kidney associated with MT was significantly higher in the group exposed to 500 μM than in the group exposed to 50 μM, almost 50% the total renal ^{203}Hg was detected in association with MT in rats exposed to both low and high mercuric chloride levels.

The amount of mercury bound to MT in the kidney has been calculated in terms of micrograms of mercury bound to MT per gram of unfractionated wet tissue and the results are presented in Table 3. As can be observed, although the amount of mercury bound to MT increases gradually with the exposure level, no statistically significant differences between rats exposed to 50 and 500 μM Hg were found. A statistically significant correlation between mercury concentration in kidney and concentration of mercury bound to MT was calculated. This linear relationship can be expressed as:

$$[Hg]_{MT} = -0.283 + 0.451[Hg]_{KIDNEY}$$

$$r = 0.986 \ (P < 0.0001)$$

Where $[Hg]_{MT}$ and $[Hg]_{KIDNEY}$ are the concentrations of mercury bound to the renal MT and the whole kidney, respectively.

MT determination

An increase of the -SH content in renal MT peak with increasing exposure level was observed. Thus, renal MT levels in rats exposed to 50 and 500 μM were 2.7 and 3.9 times higher than in rats exposed to 5 μM (Table 3). Similar renal MT levels were observed by indirect determination via the -SH content and chromatographic methods in rats exposed to 5, 50 and 500 μM Hg (Morcillo 1992). Molar ratios of Hg/MT were calculated from the data of -SH groups and ^{203}Hg content in all fractions of the MT peak (Table 3), assuming that the amount of SH-containing compounds other than MT in the MT peak was negligible. The molar ratio was 1.5 mol Hg (mol MT)$^{-1}$ in rats exposed to 5 μM and almost 5 mol Hg (mol MT)$^{-1}$ in both groups exposed to 50 and 500 μM Hg. These ratios suggest that renal MT has a limited capacity to bind mercury since this limit is reached in rats exposed to 50 μM Hg. On the other hand, the -SH content in the liver MT peak was also determined and no statistically significant differences between groups were found.

Discussion

Following chronic exposure of animals to inorganic mercury, the highest concentration of the metal is found in the kidney (Berlin 1986). The results in the present study corroborate this conclusion. Nevertheless, the accumulating capacity of the kidneys for mercury is probably limited; in fact, the concentration of mercury in the kidneys of rats exposed to 50 and 500 μM Hg was similar (Table 3). In agreement with this result, it has been described that the relative deposition of mercury in the kidneys of rats orally exposed to $HgCl_2$ decreases according to the increase in the dose (Nielsen & Andersen 1990, Nielsen 1992). The pattern of intrarenal distribution of mercury was characterized by a deposit in the outer stripe of the outer medulla and, to a minor degree, in the renal cortex (Figure 2). In concordance with our findings, it has been described that the renal accumulation of inorganic mercury increases significantly in rats treated with a non-toxic dose of mercuric chloride after unilateral nephrectomy and compensatory renal growth due to a specific increase in the accumulation of inorganic mercury in the outer medulla (Zalups & Diamond 1987, Zalups *et al.* 1987), specifically in the outer stripe of the outer medulla (Zalups & Barfuss 1990). Recent histochemical evidence shows that the increased accumulation of inorganic mercury in the renal outer stripe of the outer medulla is due to a specific increase in the accumulation of the metal in the pars recta of the proximal tubules (Zalups 1991). Moreover, we have observed that the difference in the mercury concentration between the outer stripe of the outer medulla and the renal cortex was more remarkable in rats exposed to 50 and 500 μM than in those rats exposed to 5 μM Hg, suggesting that renal damage might be produced at levels from 50 to 500 μM Hg. We have previously observed that rats exposed to 500 μM Hg presented several signs of nephrotoxicity, e.g. proteinuria and a decrease of the growth rate (Morcillo & Santamaria 1995). Moreover, in the present study an increase of kidney weight in the animals exposed to 50 and 500 μM was observed (Table 1), which is in agreement with previous data (Bodgen *et al.* 1980, Mengel & Karlog 1980, Blanusa *et al.* 1994). An increase in the kidney weight is an indicator of the chronic toxicity of mercury (Daston *et al.* 1983). In addition, the mean mercury concentrations in the kidneys of rats exposed to 50 and 500 μM (about 88 and 94 μg g^{-1} tissue) were higher than the renal levels of mercury associated with changes in the renal

function (40 $\mu g\ g^{-1}$ tissue) as described Clarkson & Magos (1966). Therefore, subchronic exposure to at least 50 μM Hg may be associated with nephrotoxic effects.

Tissue fractionation studies have shown that mercury is enriched in the renal soluble fraction where it is mainly associated with MT (Jakubowski *et al.* 1970, Piotrowski *et al.* 1974, Zelazowski & Piotrowski 1980, Morcillo & Santamaria 1992). The results of the binding of mercury in the renal cytosol, which showed that almost 50% the renal mercury was associated with MT, confirm the observations mentioned above (Table 3). We have previously shown that after exposure to 0.5 and 5 μM Hg for 8 weeks, the level of MT in the rat kidney did not increase (Morcillo 1992). In the present study, the mean renal MT concentration was 72 $\mu g\ g^{-1}$ tissue in rats exposed to 5 μM of mercury, a similar value to that calculated by radioimmunoassay from non-exposed Sprague-Dawley rats (Nolan & Shaikh 1987, Mitane & Tohyama 1987). Although an increase of the MT concentration in rat kidney was observed with increasing doses, the binding capacity of renal MT for mercury tends to saturate (Table 3). Planas-Bohne *et al.* (1985) have observed such saturation of the MT-binding capacity for mercury; however, they observed a lower mercury content in the renal MT-containing fractions, about 8 $\mu g\ g^{-1}$ tissue. The reason for this discrepancy may be related to treatment differences; they administered a single i.v. dose of 1 mg Hg kg^{-1} while, in this study, the rats are subchronically exposed to mercuric chloride by the oral route. The mean concentration found in our study in rats exposed to 500 μM (45 μg Hg g^{-1} tissue) was similar to that found in rats exposed to 500 μM of mercury in the diet as mercuric chloride for 6 weeks, 39 μg Hg g^{-1} tissue (Whanger & Deagen 1983). A protective role of MT would explain that, during chronic exposure to mercury compounds, the kidneys can accumulate levels of inorganic mercury much higher than the toxic level observed after a single dose. It suggests a close association of renal injury with induction of renal MT, the increased urinary excretion of MT (Mitane & Tohyama 1987), and the increased excretion in urine of zinc and copper (Liu *et al.* 1992, Morcillo 1992). Besides the increase in the amount of mercury bound by mole of MT with increasing doses of $HgCl_2$, we have previously observed that exposure to mercury increases also the Cu/renal MT molar ratio (Morcillo 1992); subsequently, mercury exposure seems to modify the metal content of the renal MT. Possibly, this alteration can produce a change in the conformation of the native renal MT. This hypothesis is in line with the observations of Sokolowski *et al.* (1974), who found drastic changes in the conformation of MT after substitution of cadmium and zinc by mercury, and with the data of Nordberg *et al.* (1974), who reported that after exposing Cd–MT to Hg^{2+} *in vitro*, a change of isoelectric point of MT to higher values was obtained. We have also described that renal MT with high mercury and copper content is more positively charged than the native protein (Morcillo *et al.* 1992). The change in the conformation of renal MT with increasing doses of mercury could disturb the role that the MT plays in the homeostatic control of zinc and copper renal metabolism. With regard to this, it has been also described that increased urinary zinc and copper excretion observed after $HgCl_2$ administration might be a reflection of a disturbance in the homeostasis of essential elements (Liu *et al.* 1992, Morcillo *et al.* 1993). Such alteration might be, in turn, a mechanism of toxic effects of mercury in the kidney. This is not concordant with the hypothesis that the metal-thionein complex is protective for the kidney. It seems more probable that MT synthesis in the kidneys after mercury exposure is probably accidental and reflects the chemical similarities of mercury, copper and zinc. A similar argument has been suggested concerning cadmium and MT (Bremner & Beattie 1990).

With regard to the remaining organs, besides the high concentration of radioactivity found in the oesophagus and the buccal cavity, which suggest that the upper gastrointestinal tract may be an important place for the deposition of oral mercury, the increase of mercury levels in brain with increasing dose is noteworthy for its neurotoxic effect. However, the mean mercury concentration in brains of rats exposed to 500 μM, 1.2 $\mu g\ g^{-1}$ tissue (Table 2), was lower than that associated with behavioural changes in rats, revealed by studies of conditioned avoidance reflexes, of 10 $\mu g\ g^{-1}$ brain tissue (Kishi *et al.* 1978).

The present study demonstrates that the greatest concentrations of mercury are found in the kidneys, buccal cavity and oesophagus after oral subchronic exposure of rats at low and high levels of mercuric chloride. Almost 50% the total renal mercury is associated to MT at any dose. The renal MT-binding capacity for mercury is saturated at high doses, which means that the kidney has a limited capacity to accumulate mercury. The nephrotoxic symptoms show up when the renal-binding capacity is saturated.

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