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Whole-body retention, and urinary and fecal excretion of mercury after subchronic oral exposure to mercuric chloride in rats

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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 observed. The animals were exposed to mercuric chloride labeled with ^{203}Hg via drinking water for 8 weeks (5, 50 and 500 μM Hg). ^{203}Hg in urine, feces and blood was quantified. The blood mercury concentration did not keep a linear relationship with the increasing dose. The percentage of the total amount of mercury intake which is excreted by the fecal route in rats exposed to 500 μM Hg was significantly lower than in those exposed to 5 and 50 μM. The daily dose percentage of mercury excreted in urine increased with dose size. The results show that the absorption fraction of mercury through the gastrointestinal tract (30–40%) was higher than values previously reported.

Keywords: mercury, urine, feces, gastrointestinal absorption

Introduction

Mercury exists in different forms, including elemental mercury, inorganic mercury and organic mercury compounds. They have some properties in common but differ in their metabolism and toxicity. The distribution of mercury within the body and specific organs varies with the dose and time after exposure (Cember 1962, Berlin 1986). Furthermore, the route of administration affects the organ distribution of absorbed mercury (Nielsen & Andersen 1989, 1990).

Although the use of inorganic mercury compounds has decreased in recent years and precautions against industrial emissions have improved, future human exposure to inorganic mercury will probably lead to few cases of occupationally highly exposed people and larger populations exposed to low or very low levels from dental fillings or from food items containing mercury (Nielsen 1992). Most toxicokinetic studies of mercuric compounds have used either single dose administration or short-term exposures, although most humans are chronically or subchronically exposed, and doses of mercury much higher than the human exposure situation. In addition, the parenteral administration of a soluble mercury salt has been the most commonly used exposure route, while human mercury exposure is often via the oral route (Nielsen & Andersen 1989). Nielsen (1992) has suggested that an investigation of the toxicokinetics of mercuric compounds after chronic or subchronic exposure via the oral route at very low levels in animal experiments would be useful for a risk evaluation in relation to human exposure.

It has been described that absorption of inorganic compounds of mercury from the gastrointestinal tract is about 7% (Von Burg & Greenwood 1991). However, the re-excretion of absorbed mercury during the experimental period was not considered in these estimates. Nielsen (1992) has calculated that an estimated 'true absorption' of a single dose of $HgCl_2$ is 20 25% in mice. Inorganic mercury is excreted by the kidney, the fecal route, and sweat, lacrimal, mammary and salivary glands (Nordlind 1990). The major part of absorbed inorganic mercury is excreted into the urine and feces. Partition between these two routes is dose-dependent, and the data suggest a larger fraction being excreted into the urine upon administration of higher doses (Berlin 1986). However, the present knowledge of the effect of dose on the excretion of inorganic mercury is scarce.

The present study describes the effects of long-term daily intake of mercury on urinary and fecal excretion, whole-body retention, and blood concentration, as well as on some indicators of chronic mercury toxicity (decreased growth rate and proteinuria), in rats exposed to low and high levels of mercuric chloride.

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Materials and methods

Animals

Twenty-four male Sprague-Dawley rats weighing about 130 g were obtained from CIJISA (Madrid, Spain). After 3 days conditioning in a temperature/light controlled environment, they were randomly assigned to four experimental groups of six animals. Each animal was marked, weighed and housed individually in metabolic cages to facilitate the separate collection of urine and feces. One of the groups served as control (Group I). At the beginning of 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 weighed weekly during the study; this amount was gradually increased until 20 g day^{-1} with the purpose to avoid excessive weight of the animals at the end of exposure period. They were then given 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}). The mercury level was 5 (Group II), 50 (Group III) and 500 μM (Group IV). Rats exposed to 500 μM Hg neither drank nor ate because of an excessive salty taste in the water; therefore, to avoid this problem drinking water containing 500 μM Hg was supplemented with 5% sugar.

Sample collection

Urine and feces samples were collected daily and subsequently frozen. Blood samples (1 ml) were taken weekly from the caudal vein, under light ether anesthesia.

Determination of urinary protein

Protein concentration in the urine was measured by a modification of the Biuret method with bovine serum albumin as standard (Piscator 1962).

Radioactivity determination

The amount of radioactivity in blood, urine and feces samples was determined by solid scintillation counting in a gamma spectrometer (LKB Model 1275). The isotope decay causes a decrease on the specific activity which must be taken into account through the exposure period. Thus, a ^{203}Hg standard (volume of 1 ml) with a known initial amount of ^{203}Hg was counted at the beginning of each counting session. The gamma counter background was below 700 c.p.m. As the different samples varied in size, it was essential to evaluate 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 approximately 40–50%. Thus, the countings (c.p.m.) were converted into d.p.m. after correction for background and sample volume.

Statistical analysis

Statistical analyses of the experimental results were performed by a one-way analysis of variance (ANOVA) using the statistical package of CSS: STATISTICA from Statsoft. Appropriate individual comparisons between means were done with the Scheffé test. The level for rejection of the H_0 hypothesis was set a 0.02. Data were subjected to logarithmic transformation when necessary to achieve homogeneity of variances. When necessary, a non-parametric statistical analysis, such as the Mann–Whitney rank-sum test, was used.

Results

Exposure level and body burden

Means of daily water consumptions for Groups I, II and III and IV were 28, 24, 21 and 14 ml, respectively. A dose-dependent and highly significant decrease of water consumption was observed. Means of daily mercury intakes were about 21, 212 and 1526 μg for Groups II, III and IV, respectively (Table 1).

The body burden of mercury at any particular time is equal to the daily accumulated ingestion minus the daily accumulated loss due to excretion (fecal and urinary) and non-absorbed mercury. The whole-body retention of mercury increased exponentially with the exposure time in all groups of animals during the experimental period and a steady-state was not achieved (Figure 1). The retained fraction of mercury (the total body burden divided by the total amount of mercury intake) in all groups was constant and independent of exposure level (Table 1). This implies that, for the dose range used in this study, the body burden of mercury was linear with regard to the exposure level.

Effect of mercury on growth rate and urinary protein

The body weight of the animals increased considerably during the experimental period. Comparable rates of growth for the control and Groups II and III were observed (Figure 2). Growth rate in the Group IV was significantly lower than in the control group from week 4 until the end of exposure.

The daily amounts of protein excreted in urine are depicted in Figure 3. In the control group, the amount of protein excreted in urine was about 2 mg day^{-1} during the first weeks of exposure, a similar value to that calculated by other authors from non-treated Sprague-Dawley rats (Mitane & Tohyama 1987). However, throughout exposure, a light increase in urinary protein was observed. This increase is reasonable since the amount of the daily diet was gradually increased during the exposure period. Moreover, the urinary protein was about 7 mg day^{-1} during the last weeks of exposure, a similar value to that calculated from Sprague-Dawley rats maintained with a diet containing normal dietary protein (Andrews & Chung 1992). As compared with the control group, proteinuria was detected in all groups exposed to mercury during the first 3 weeks

Table 1. Retention of mercury in rats exposed to $HgCl_2$

Group	Mercury dose level		Retention fraction[a]	Total excreted amount			
				feces		urine	
	$\mu g\ day^{-1}$	$mg\ kg^{-1}\ day^{-1}$		μg	%[b]	μg	%[b]
II	21 (18–24)	0.1 (0.09–0.11)	0.153 (0.089–0.210)	0.99 (0.89–1.14)	83.9 (77.9–90.3)	0.010 (0.007–0.013)	0.8 (0.7–1.2)
III	212 (189–235)	1.0 (0.9–1.1)	0.135 (0.112–0.166)	10.03 (8.90–11.25)	85.2 (82.1–87.0)	0.163 (0.123–0.204)	1.4[d] (1.1–1.9)
IV	1526 (1307–1546)	7.3 (6.8–7.8)	0.177 (0.149–0.204)	60.83 (54.80–65.84)	76.3[c,d] (73.2–79.4)	4.861 (4.236–5.356)	6.1[c,e] (5.8–6.4)

Values are expressed as means. Data in parentheses show the 95% confidence interval.
[a] Retention fraction = body burden intake.
[b] Percentage of total amount mercury intake.
[c] $P < 0.001$ compared with Group III.
[d] $P < 0.01$ compared with Group II.
[e] $P < 0.001$ compared with Group II.

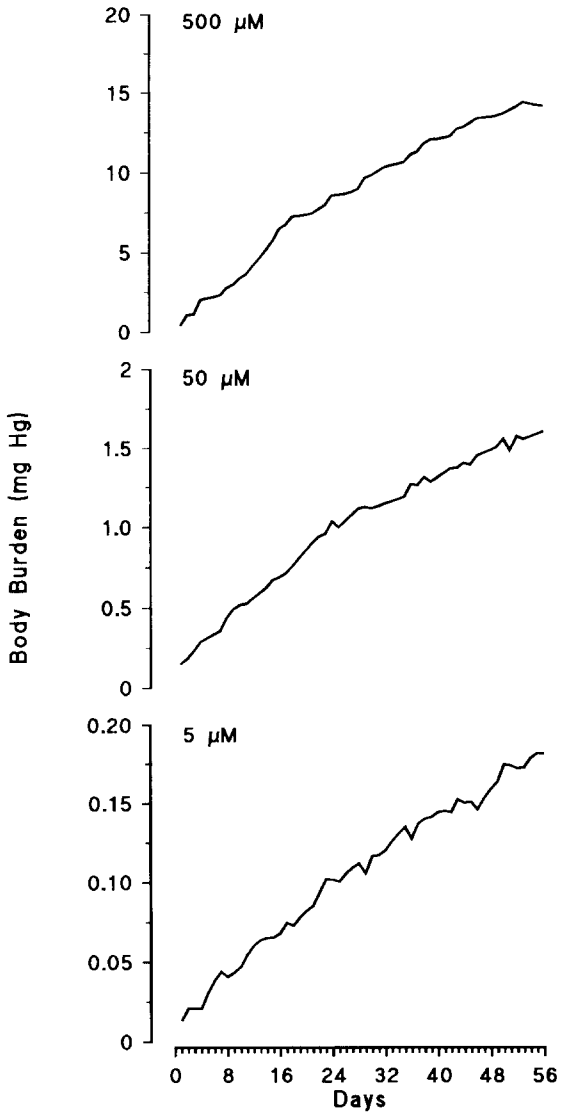

Figure 1. Body burden of mercury in rats exposed to 5, 50 and 500 μM Hg (as $HgCl_2$). Daily values are presented as means.

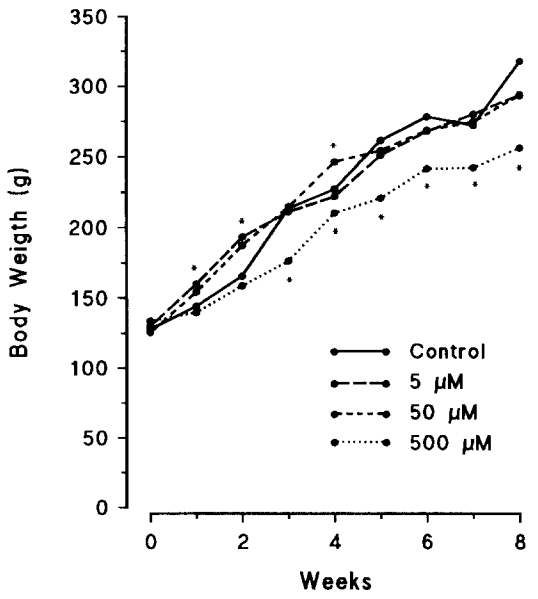

Figure 2. Growth curves for rats exposed to 5, 50 and 500 μM Hg (as $HgCl_2$). Values are presented as means. * $P < 0.02$ compared with the control group.

of exposure and a significant increase of protein excreted in urine was only observed for the animals of Group IV in the last weeks of exposure.

Mercury blood concentration

Blood mercury concentrations during the exposure period are summarized in Table 2. During this period for Groups III and IV the concentrations remained almost unchanged and the mean concentration value (steady-state blood concentration) was about 62 and 273 $\mu g\ l^{-1}$, respectively. For Group II, a steady-state blood concentration was maintained until week 5 (about 10 $\mu g\ l^{-1}$) and starting from this week onwards, the blood mercury level decreased significantly until the end of exposure. A

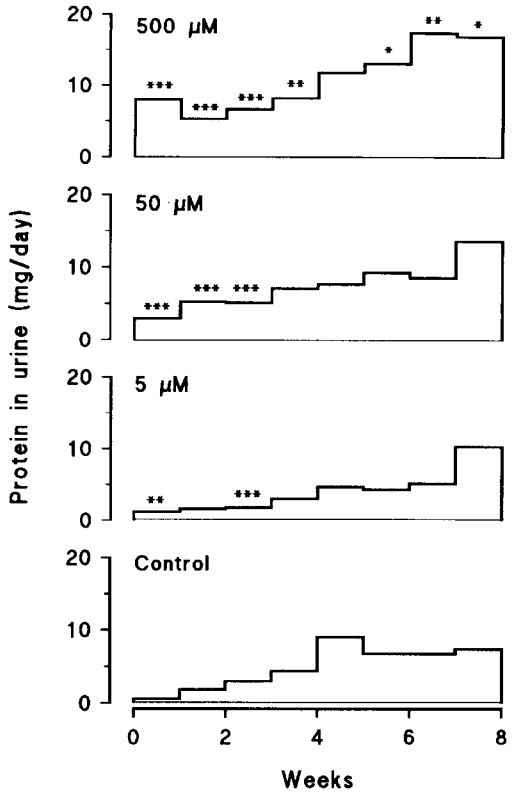

Figure 3. Daily amount of protein excreted in urine for rats exposed to 5, 50 and 500 μM Hg (as $HgCl_2$). Values are presented as geometric means. * $P<0.02$, ** $P<0.01$ and *** $P<0.001$ (rank-sum test) compared with control group.

Table 2. Blood concentration of mercury in rats exposed to $HgCl_2$

Day	Blood concentration (μg Hg l^{-1})		
	Group II	Group III	Group IV
7	8.48 ± 0.96	71.67 ± 17.38	298.92 ± 107.42
14	10.66 ± 1.47	54.71 ± 9.16	185.20 ± 67.21
21	10.45 ± 1.04	63.94 ± 11.78	215.52 ± 62.63
28	10.70 ± 1.21	60.63 ± 7.68	347.76 ± 51.35
35	11.27 ± 1.43	49.59 ± 7.52	283.96 ± 47.66
42	8.11 ± 0.94[a]	63.75 ± 5.99	324.97 ± 78.45
49	6.59 ± 1.07[b]	69.78 ± 19.00	273.80 ± 84.70
56	5.25 ± 0.28[b]	58.74 ± 8.72	257.56 ± 50.03

Values are presented as mean ± SD.
[a] $P<0.01$ and [b] $P<0.001$ compared with the mean blood concentration estimated since the start of exposure until day 35 for Group II.

blood level/dose ratio was calculated (blood level expressed as μg Hg l^{-1} and mercury dose as μg of daily Hg intake) giving 0.426, 0.292 and 0.192 for Groups II, III and IV, respectively. Therefore, a decreased ratio was observed.

Urinary and fecal excretion of mercury

The mean daily volume of excreted urine was 11, 10, 7 and 4 ml for Groups I, II, III and IV, respectively. A dose-dependent decrease was observed; however, a statistically significant correlation between mean daily volume of water ingested and excreted urine was found. This relationship can be expressed by the equation $Vol.urine = -3.278 + 0.537 \times Vol.water$ ($r = 0.964$, $P < 0.001$), where both daily volumes of excreted urine and ingested water are expressed in ml.

The mean cumulative excretion data of mercury in urine during the exposure period are shown in Figure 4(a). The plot of these values versus time was curvilinear until about day 15 for Group II, day 30 for Group III and day 5 for Group IV, and from this moment onwards, a linear relationship between the accumulated amount of mercury in urine and time was found. The slope of this straight line corresponds to the daily amount of mercury excreted into the urine: 0.23, 5.4 and 96 μg day^{-1} for Groups II, III and IV (about 1.1, 2.5 and 6.3% of daily mercury dose), respectively. At the end of exposure, 0.8, 1.4 and 6.1% of the total amount of mercury intake were recovered in the urine of Groups II, III and IV respectively (Table 1). A highly significant and dose-dependent increase of mercury excreted into the urine was observed. The mean cumulative excretion data of mercury in feces during the study period are shown in Figure 4(b). Almost at the start of the exposure period a linear relationship between the cumulative amount of mercury in feces and time was found. The slope of this straight line was 18, 187 and 1115 μg day^{-1} for Groups II, III and IV (about 85, 87 and 76% of daily mercury dose), respectively. The percentage of the total dose of mercury excreted in feces by the rats of Group IV was significantly lower in comparison with the other groups exposed (Table 1). The ratio between the amount of mercury excreted in feces and urine was 100, 62 and 13 for Groups II, III and IV, respectively.

Discussion

Most researchers recognize at least three different phases in half-time excretion rates of inorganic mercury. Thus, when a single subtoxic dose of mecuric chloride is intravenously administered to rats and excretion is determined by whole-body counting (Rothsein & Hayes 1960), the initial rapid phase involves 35% of the administered dose and declines during the first days. Then a slower phase (half-time 30 days) follows, involving 50% of the administered dose, and finally there is a slow phase, accounting for the remaining 15% of the administered dose and characterized by a half-time of 100 days. Clearly this slow component would play a predominant role in determining the cumulative body burden in exposed animals. These findings suggest that rats exposed daily to $HgCl_2$ would gradually accumulate the metal throughout their life spans. The values shown in Figure 1 are compatible with this suggestion, where a steady-state could not be attained after 56 days of exposure to inorganic mercury. The parameters calculated by Rothsein & Hayes (1960) can be used to discuss the results of the present paper in terms of the simple biokinetic model shown in Figure 5. A fraction F of ingested mercury

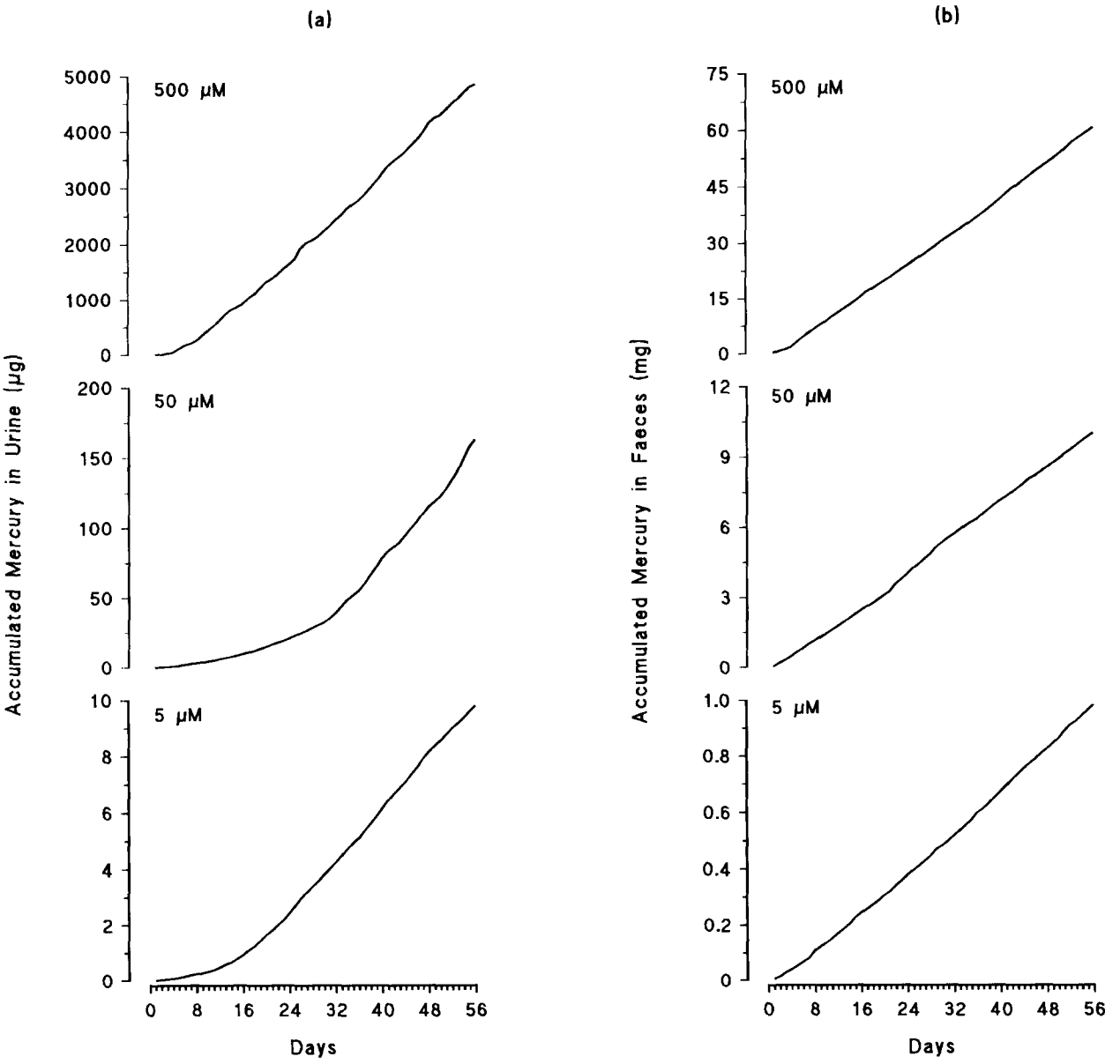

Figure 4. Accumulated amount of mercury excreted in urine (**a**) and feces (**b**) for rats exposed to 5, 50 and 500 µM Hg (as $HgCl_2$). Daily values are presented as means.

is absorbed from the gastrointestinal tract into the bloodstream. The remainder of the mercury is excreted in the feces. The time spent to reach the bloodstream is assumed to be very short compared with the 56 days duration of exposure and is not given further consideration. Of the amount that enters the bloodstream, a fraction f_1 is promptly excreted and a fraction f_n is deposited in compartment n, where it is retained with a half-time T_n. The accumulated amount A_n of the total of mercury ingested and retained in compartment n after an exposure time t can be expressed mathematically as:

$$A_n = \frac{a_n}{b_n \times t}[1 - \exp^{(-b_n \times t)}] \quad (1)$$

where a_n is the fraction of daily intake taken up by compartment n (is $F \times f_n$) and b_n is the elimination constant ($\ln 2/T_n$)

$$A_n = \frac{F \times f_n}{b_n \times t}[1 - \exp^{(-b_n \times t)}] \quad (2)$$

if

$$R_n = \frac{f_n}{b_n \times t}[1 - \exp^{(-b_n \times t)}] \quad (3)$$

then

$$A_n = F \times R_n \quad (4)$$

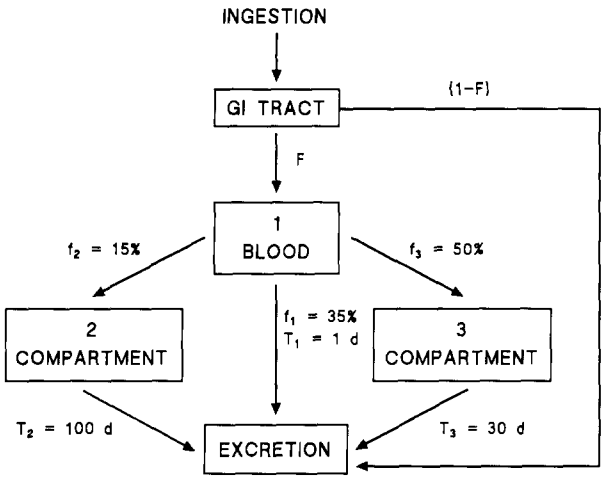

Figure 5. Simple biokinetic model for the uptake and retention of mercury. F is the fraction absorbed by the gastrointestinal tract, f_n is the fraction of the absorbed mercury that is deposited in compartment n and T_n is the retention half-time in compartment n.

So, the expression for the accumulated amount in the whole body is

$$A = A_1 + A_2 + A_3 \quad (5)$$

where A_1, A_2 and A_3 express the accumulated amount in blood, compartment 2 and compartment 3, respectively (Figure 5). Then

$$A = F \times (R_1 + R_2 + R_3) \quad (6)$$

The parameters calculated by Rothsein & Hayes (1960) and presented in Figure 5 can be substituted in equation (3) to obtain R_n for each compartment. When these parameters are substituted in equation (3) for $t = 56$ days, then the following R_n's are obtained: $R_1 = 9.019 \times 10^{-3}$, $R_2 = 0.1243$ and $R_3 = 0.2805$. When these values are substituted in equation (6), the following fractional retention in the body is obtained:

$$A = F \times 0.4138 \quad (7)$$

The whole-body fractional retention for Groups II, III and IV was estimated to be 0.153, 0.130 and 0.180, respectively (Table 1). Thus, the fraction F of ingested mercury that is absorbed from the gastrointestinal tract into the bloodstream can be estimated using equation (7). The estimated fraction F in the three groups exposed varied between 31 and 43%. Rothsein & Hayes (1964) have concluded that the kinetics of excretion following a single dose may be used to estimate the rates of excretion after multiple dosing. Figure 6 shows the time course of the cumulative body burden predicted by means of the parameters calculated by Rothsein & Hayes (1960) taking in account that the daily intake is 21 μg day^{-1} (equal to the rats exposed to 5 μM Hg) and the fraction absorbed through gastrointestinal tract is between 20 and 50% of the administered dose. As it can be observed, the curve of body burden measured for the rats exposed to 5 μM Hg is very similar to the predicted curve for $F = 37\%$, a value equal to the one previously calculated using equation (7) for Group II. Experimental studies indicate that less than 10% of ingested mercuric chloride is absorbed after oral administration (Rahola *et al.* 1973, Elinder *et al.* 1988). The re-excretion of absorbed mercury during the experimental period was not taken in account in these estimates since they were based on the total amounts of excreted mercury. Using whole-body retention data after oral and intraperitoneal administration, Nielsen (1992) has estimated that absorption of a single oral dose of $HgCl_2$ (0.2 and 1 mg Hg kg^{-1}) may be about 20–25% in mice. However, the procedure used by Nielsen is correct if the elimination rate constant is independent of the route of administration (Gibaldi & Perrier, 1975). As the apparent half-time of retained mercury is shorter in orally than in intraperitoneally exposed mice, the gastrointestinal absorption estimated by Nielsen (1992) can be smaller than the true absorption. In the present study, the fraction of mercury absorbed through the gastrointestinal tract was calculated to be higher (about 30–40%) than the value described by this author. It has been described that after a high intake, the corrosive action of mercuric chloride could change the permeability of the gastrointestinal tract, by that enhancing absorption (Nordlind 1990). Although we have not found any statistical significance in the differences between groups for the calculated absorption fraction, the F estimated for the rats exposed to 500 μM Hg was slightly higher than for those exposed to 50 μM Hg. Moreover, the percentage of total mercury intake excreted by the fecal route in rats exposed to 500 μM Hg was significantly lower than in those exposed to 5 and 50 μM (Table 1). Therefore, it is possible that at a high intake of mercuric chloride, an increase in the absorption

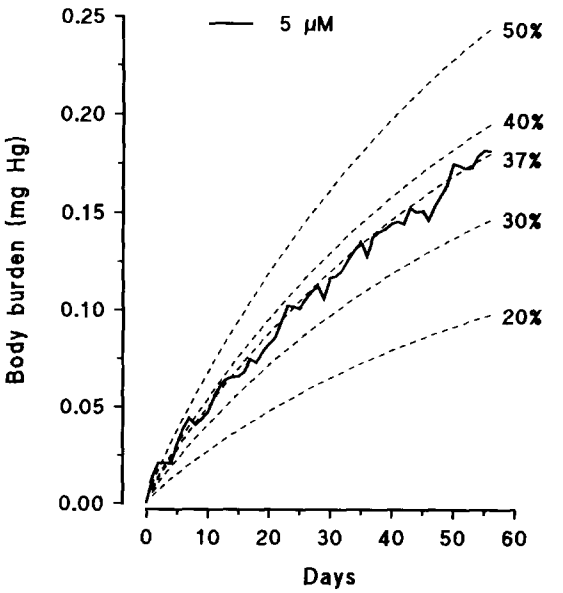

Figure 6. Predicted (– – –) and measured (— – —) body burden of $HgCl_2$ in rats exposed to 5 μM Hg. The predicted curves are presented for different values of the absorption fraction.

fraction could be produced. Nielsen & Andersen (1990) determined that the fractional whole-body retention of mercury 14 days after oral or intraperitoneal dosage was inversely related to the dose size, probably due to the damage in the kidneys responsible for the loss of mercury in urine at the highest dose levels. Although in the present study it is shown that the percentage of daily dose of mercury excreted in urine increases with dose (Table 1), the percentage of whole-body retained mercury in rats exposed to 500 μM is higher than in those exposed to 50 μM, because the percentage of mercury excreted in feces is significantly higher in the last group. The discrepancies between our results and those of Nielsen & Andersen are probably due to treatment. They administered a single dose of $HgCl_2$ to mice and, in this study, the rats were chronically exposed to $HgCl_2$ for 8 weeks.

The acute and long-term action of mercury salts is likely to be due to gastrointestinal disturbance and renal damage (Berlin 1986). Two types of renal damage can occur. First, an autoimmune reaction is induced due to the formation of antigen against the glomerular tissue, and a nephrotic syndrome develops with proteinuria and the classical signs of glomerular nephritis. However, this glomerular injury is partially subject to genetic regulation (WHO 1991). We have not found information about this effect on Sprague-Dawley rats in the reviewed studies. The second type of renal injury is tubular damage causing necrosis and damage of the distal and middle portion of the proximal tubule. Following subchronic exposure of Sprague-Dawley rats to a very low non-toxic level of mercury, Nolan & Shaikh (1987) have found induction of metallothionein in kidney and accumulation of zinc and copper in this tissue. We have reported that renal metallothionein is induced in Sprague-Dawley rats exposed to 50 and 500 μM Hg for 8 weeks but not in those exposed to 5 μM (Morcillo & Santamaria 1993). Moreover, the level of copper in the kidney of rats exposed to 50 and 500 μM Hg was high (Morcillo 1992). On the other hand, indicators of chronic mercury toxicity in rats include, among others, a decreased growth rate and proteinuria (Daston *et al.* 1983). In the present study, a decreased growth rate was only observed in the group of rats exposed to 500 μM Hg (Figure 2), which also presented a significant proteinuria (Figure 3). Although the present study did not include another marker for renal damage, an increase of kidney weight from rats exposed to 500 μM Hg as compared with the control group, a decreased relative deposition of mercury in kidneys with increasing dose, and a different intra-renal distribution of mercury between controls and rats exposed to 500 μM, was observed (unpublished data). Therefore, the rats exposed to 500 μM Hg possibly succumbed to the toxic effects of mercury. The weight of evidence suggests that glomerular filtration of diffusible mercury may not make an important contribution to urinary excretion (Berlin & Gibson 1963). Experimental animal data indicate that mercury is transported from blood to cells of the proximal tubule and transferred to the tubular lumen. In the kidney, mercury is mainly associated with metallothionein (Jakubowski *et al.* 1970, Nolan & Shaikh 1987, Morcillo *et al.* 1992) and, consequently, we suggest that the rate of removal of Hg^{2+} from this protein be the limiting step for their urinary excretion. When the gain and loss from kidney are approximately equal, i.e. when a steady-state is achieved, a linear relationship between the accumulated amount of mercury excreted in urine and time exists. This steady-state was reached after 15 days of exposure for the rats exposed to 5 μM (Figure 4a). For the group of rats exposed to 50 μM Hg the concentration of renal metallothionein was significantly higher (Morcillo & Santamaria 1993) and the steady-state was attained later (about 30 days). Nevertheless, the accumulating capacity of the kidney for mercury is limited. In fact, in rats exposed to 500 μM, which have shown signs of renal toxicity, an impairment in the biochemical process involved in the retention of mercury could happen following a higher and earlier excretion of the metal in urine (day 5, Figure 4a).

On the other hand, the exposure dose/blood concentration ratio decreased in a dose-dependent manner. As previously mentioned, a steady-state for mercury deposition in the body could not be attained. On the contrary, the blood concentration remained relatively constant during exposure for all animals exposed to mercury. This indicates that the apparent terminal elimination half-life for blood mercury is much lower than for body mercury. However, in the rats exposed to 5 μM Hg, and from day 35 of exposure, a statistically significant decrease of blood concentration was observed. This fact was neither correlated with the increase of mercury excreted in urine nor with the content of mercury in feces, therefore, it could suggest that a redistribution of systemic mercury be happening. This is a subject proposed for further studies.

Acknowledgments

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