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The influence of long-term mercury exposure on selenium availability in tissues: an evaluation of data★

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Abstract

The precise mechanisms of mercury accumulation and retention are still unclear. Generally, the association of mercury with selenium is used to explain these phenomena. It seems that the presence of coaccumulated endogenous Se can protect cells from the harmful effects of Hg. However, as speculated by some authors, this binding of Se to Hg can also result in a relative deficiency of biologically available Se needed for selenoenzyme syntheses. Deriving from the assumption that Hg deposited in tissues is bound to Se in a 1:1 ratio, the quantity of non-Hg bound Se could be calculated by the difference between the molar contents of the two elements (Se_{mol}–Hg_{mol}). In this study we applied such an approach to the data from our previous investigation, where Hg and Se concentrations were determined in autopsy samples of mercury exposed retired Idrija mercury mine workers, Idrija residents living in a Hg contaminated environment and a control group with no known Hg exposure from the environment. Based on these data we tried to estimate the influence of Hg exposure on the physiologically available selenium content in selected tissues, particularly endocrine glands and brain tissues. Comparing the calculated values of (Se_{mol}– Hg_{mol}) it was found that for Idrija residents the values were similar to those of the control group and as expected, diminished values were found in some mercury-loaded organs of retired Idrija miners. It could be speculated that in Idrija residents Hg sequestration of selenium is sufficiently compensated by increased Se levels, but that particularly in active miners and in some organs of retired miners, the activity and/or synthesis of selenoenzymes could be disturbed.

Introduction

The precise mechanisms of mercury accumulation and retention are still unclear. Generally, the association of mercury with the essential element selenium is used to explain these phenomena and a positive correlation between the two elements has been commonly reported (Watanabe 2002). It seems that the presence of accumulated and/or retained endogenous Se can protect cells from the harmful effects of Hg. By 'retained' is meant what was accumulated and latter not excreted. In humans the coaccumulation (and/or coretention) was observed in population groups exposed to mercury vapour occupationally (miners, dental staff) or through a mercury contaminated environment or dental amalgams (Kosta *et al.* 1975; Nylander & Weiner 1989, 1991; Björkman *et al.* 1994; Byrne *et al.* 1995; Drasch *et al.* 1996; Falnoga *et al.* 2000). The highest accumulation and/or retention of both elements, deposited in almost equimolar ratio, was found in thyroid and pituitary glands followed by kidney cortex and some parts of the brain of retired miners and dental staff (Kosta

★Part of the study was presented at the 7th International Conference on Mercury as a Global Pollutant, June 27–July 2, 2004 Ljubljana, Slovenia (Falnoga *et al.* 2000)

et al. 1975; Nylander & Weiner 1989; Falnoga *et al*. 2000). In spite of the very high concentration of Hg, the degree of cell damage in such samples can be quite low (Friberg & Mottet, 1989). Citing Drasch (1996) 'the results suggest the formation of a biologically largely inert 1:1 Hg–Se compound'.

On the cellular level, after tissue homogenisation and centrifugation, the coaccumulation and/or coretention of both elements was found in the cellular particulate phase (pellet) (Björkman *et al*. 1994; Falnoga *et al*. 1997, 1998, 2000), and in the tissues examined by means of electron microscopic X-ray microanalysis most frequently in the lysosomes and nuclei as aggregated electron-dense grains positive for Hg, Se and S (Aoi *et al* 1985). Similar results were also found in animals exposed to mercury through amalgam implantants (Eley 1990; Eley & Cox 1986), and humans after exposure to Hg_2Cl_2 (Davis *et al*. 1974) or methyl mercury (Shirabe 1979). In a few cases it was reported that mercury was found within lipofuscin deposits (Shirabe 1979; Aoi *et al*. 1985; Opitz et al. 1996). Lipofuscin is an intralysosomal, polymeric undegradable substance, primarily composed of crosslinked protein residues (Brunk & Terman 2002).

Interaction of Hg with Se can be explained by the biochemistry of the Hg^{++} ion (and methyl mercury ion) which is dominated by its high affinity for thiols, bisulphides, selenols and selenides (Sugiura *et al*. 1978; Kägi & Hapke 1984). Thiols and selenols in the form of cysteines and selenocysteines represent the constitutive part of many essential enzymes. Regarding the reactivity between mercury and selenium ligands, most important are the selenides. Hydrogen selenide is an intermediate molecule in Se metabolism, very reactive, short-lived and responsible for biosynthesis of selenoproteins and methylated Se excretory products (Ganther *et al*. 1997, Tapiero *et al*. 2003), but also for interactions with transition metal ions, forming metal-Se complexes (Sasakura & Suzuki 1998; Gailer 2002) . This metabolic point is also crucial for selenium bioavailability, because if hydrogen selenide is continuously used up by exogenous metal ions then a blockade (or inhibition) of selenoprotein synthesis could occur. In recent *in vitro* studies it is assumed that an erythrocyte-derived reduced metabolite of Se together with the plasma selenoprotein-P (SelP) may play an important role in Hg sequestration in the bloodstream and consequent accumulation (Suzuki *et al*. 1998). The structural basis is based on the formation of a non-toxic insoluble (Hg–Se)n core (*in vitro* studies, Suzuki *et al*. 1998) or may be a glutathionyl-coated mercuric selenide core $(GS)_5(HgSe)_{core}$ (Gailer 2002) that are later bound to SelP. SelP is the extracellular protein containing most of the Se in plasma (up to 60%) with rapid turnover (Burk *et al*. 2003). It is postulated to function in selenium transport from liver to peripheral tissues such as brain and in protecting endothelial cells from oxidant molecules (Burk *et al*. 2003; Saito *et al*. 2004). The excessive Hg can be also bound to plasma glutathioneperoxidase (pGPx) and plasma albumin, as was verified in a recent study of mercury distribution among serum selenoprotein fractions of mercury vapour exposed miners and local residents living in a heavily Hg-contaminated area near a Hg-mining plant in China (Chen *et al*. 2004a, b). These data support the idea of Yoneda and Suzuki (1997) who claim that 'SelP may be effective primarily against chronic toxicity by consecutive exposure to a low dose of heavy metals', because 'a reduced metabolite of Se which is essential for SelP to sequester selectively, is supplied only in small quantities under normal nutritional conditions'.

Circumstances in other organs and tissues are less well known, although recently expression of SelP has been identified in thyroid (Köhrle 1999). In any case the detoxification process cannot be attributed only to Hg–Se formation related to highly reactive selenides. Inside the cells of different organs are active inducible metal binding molecules like the tripeptide glutathione (GSH) and the cytosolic cysteine-rich metal-binding protein metallothionein (MT), known to form stable complexes with metals, including mercury as was shown for different organs such as kidney (Škreblin *et al*1998; Zalups 2000; Chen *et al*. 2004a, b), liver (Chen *et al*. 2004a, b), thyroid (Falnoga *et al*. 1995) and brain (Falnoga *et al*. 1993) in animal experiments after exposure to mercury vapour or inorganic mercury salts. In the literature it is sometimes supposed that GSH is important during chronic exposure, and MT in particular in acute toxicity. Also there are some data and hypotheses indicating the possibility of the involvement of selenium in MT metal binding (Takatera *et al*. 1994). The degradation endproducts of MT's and other metal binding

compounds (enzymes, selenoproteins) could then contribute to insoluble mercury-selenide or mercury–sulphide complexes found in lysosomes of different tissues (Aoi *et al*. 1985; Li & Zha 2002 cited in Huang *et al*. 2004). The persistence of lysosome and nuclear deposits then depend on lysosomal exocitosis and cellular life-span (Sahagian & Novikoff 1994; Brunk & Terman 2002). The prevailing amount of sulphur or selenium mercury deposits can depend on the type of exposure (acute or chronic).

However, as speculated by some authors (Drasch et al. 1996, 2000; Gailer 2002; Ralston 2003), the binding of Se to Hg could also result in a relative deficiency of biologically available Se (in fact of intermediate hydrogen selenide or other reactive selenium metabolites) needed for selenoenzymes synthesis. That could lead to disturbance of free radical detoxification, thyroid hormone metabolism and production of immune system signal molecules (Tapiero *et al*. 2003; Schweizer *et al*. 2004). Therefore efforts have been made to estimate the amount of non-Hg-associated Se in tissues (Drasch *et al*. 2000). Deriving from the assumption that Hg is almost quantitatively deposited in tissues bound to Se in a 1:1 ratio, the quantity of non-Hg-bound Se can be calculated by the difference between the molar contents of the two elements (Se_{mol}–Hg_{mol}).

In this work we applied this approach to the data from our previous study where Hg and Se concentrations were determined in autopsy samples of mercury exposed and unexposed individuals: retired Idrija mercury mine workers, Idrija residents living in a Hg contaminated environment and a control group with no known Hg exposure from the environment (Falnoga et al. 2000). Hg and Se were determined simultaneously by RNAA. Regarding individual data we tried to estimate the influence of Hg exposure on the physiologically available or Hg-unbound selenium content in selected tissues, but particularly in endocrine glands and brain tissues, known to coaccumulate metals and selenium, but also to preserve selenium levels in periods of insufficient selenium supply (Kyriakopoulos & Behne 2002). Calculations were made on the basis of individual values not presented in the previous article. The results obtained were also compared with data of retired miners from the initial investigations made by Kosta *et al*. (1975) during the highly active mining period in Idrija.

Subjects and methods

Autopsy cases divided into three groups

Thirty-five subjects aged from 33–99 years were collected during 1990–1997 and some data are adapted from our previous work (Falnoga *et al*. 2000). Cases were divided into three groups and basic data are given in detail in Table 1. In the present study we also added one exceptional case not fitting in any of the groups, a 30-year old active miner exposed to mercury for 3 years before dying in an accident in 1989.

Mercury exposure

The Idrija region, Slovenia, is highly contaminated with mercury (Hg) due to the past mining and smelting activities of the Idrija mercury mine, which was operational from 1508 to 1994. Since 1994 shutdown work is going on. In the past, the air mercury vapour (Hg^o) levels in Idrija town have been intermittently highly elevated, but after 1994 the levels were much lower although still elevated. Recent average air levels in different

Table 1. Groups of cases with separately added one case of active miner.

Group	No. of individuals (men + women)	Average age (range)	Occupational exposure period/Retirement period (years)
Control	22 (6 + 16)	68 (33–99)	–
Idrija residents	9 (2 + 7)	55 (41–76)	–
Retired miners	4 (4 + 0)	64 (61–68)	18 to 22/14 to 22
Active miner	1 (1 + 0)	30	3/0

parts are 0.009–0.119 μg Hg/m^3 (Dizdarevič 2001), but in the area near the abandoned smeltery and ventilating shafts the values could reach 2.3–4.0μg/m3 (Jereb 1998). In noncontaminated areas the average levels of mercury are between 0.003 and 0.005 μg/m^3 (Horvat *et al.* 2003).

In the mine the Hg^o concentrations varied, depending on the specific workplace, from 0.05 to over 1 mg/m^3, according to data collected from 1959 to 2000 (Kobal *et al.* 2004). Certainly miners used protective equipment and an approximate assessment of their exposure can be obtained from the calculations recently made for a group of 21 retired miners from the same mine (Kobal *et al.* 2004).

Beside exposure to atmospheric Hg^o, the inhabitants were also exposed to inorganic mercury through locally produced food but only to a small amount (Falnoga *et al.* 2003; Horvat *et al.* 2003).

No allowance was made for the presence of dental amalgams in either of the groups.

Se levels in the Idrija region

Se levels in soil found for individual domestic gardens in Idrija are 0.2 to 0.9 mg/kg per dry weight, which are typical values for soil in Slovenia (0–1 mg/kg; unpublished data 2004) or for Se uncontaminated soil in general (0.1–2 mg/kg; Combs *et al.* 2001). As the former smeltery and mine are positioned inside the town it is obvious that release of possibly volatile selenium compounds from smelting activities during the 500 hundred-year period was low, otherwise selenium levels would be higher. Selenium levels in locally produced vegetables are also in the normal range (Falnoga *et al.* 2003), so the phenomenon of selenium increase can be attributed to the influence of mercury exposure alone.

Autopsy samples, mercury and selenium determination

Samples of endocrine glands (pituitary, thyroid, pineal gland, adrenal gland), kidney cortex and brain tissues (hippocampus, nucleus dentatus, cortex cerebellum) were obtained, but not all from every subject. Average concentration data (wet weight) with standard deviations and ranges, except for adrenal gland, were already published in a previous article dealing with Hg, MeHg, Se and Cd in autopsy samples of mercury exposed population; the presence of MeHg was below 7% of total Hg (Falnoga *et al.* 2000). In this study we used *the individual data* of simultaneously determined Hg and Se and calculated the difference in the molar concentration of Se and Hg, ($Se_{nmol/g}$–$Hg_{nmol/g}$) to obtain a theoretical estimation of non-Hg-associated Se.

The tissue samples from the one active miner included hair, kidney cortex, thyroid and medulla oblongata (brain tissue). Hg and Se were analysed simultaneously using radiochemical activation analysis (RNAA) (Byrne & Kosta 1974).

Results

Table 2 summarises the basic ranges of mercury and selenium found in autopsy samples of different groups in different organs. Data are adapted from a previous article (Falnoga *et al.* 2000) and given in ng/g and nmol/g. Observing the molar concentrations it is evident that increased mercury values are accompanied by increased selenium and the deposition of both element approaches an equimolar ratio. Individual Hg/Se molar ratios for thyroid, pituitary, kidney cortex and nucleus dentatus samples were already presented in a previous article and it was seen that in samples with Hg concentrations above 2000 ng/g (10 nmol/g)

Table 2. Ranges of Hg and Se levels found in autopsy samples of different groups (adapted from Falnoga *et al.* 2000).

Group/N	Concentration levels in organs and tissues	
	Hg [ng/g (nmol/g)*]	Se [ng/g (nmol/g)*]
Control/22	0–281 (0.00–1.4)	43–859 (0.54–10.9)
Idrija residents/9	2–7120 (0.01–35.5)	52–3920 (0.66–49.8)
Retired miners/4	43–39100 (2.14–194.9)	170–15800 (2.15–200.0)

N, Number of cases; *wet weight

the Hg/Se molar ratio was about 1 and only in a few cases higher, near 2 (Falnoga *et al*. 2000). In such cases the excess mercury must be bound to other ligands like sulphides or thiols.

Following the assumption that Hg in tissues is almost quantitatively bound to Se in a 1:1 ratio, even in cases where at the tissue level this is not evident, we calculated the values of non-Hg-bound selenium. Table 3 presents the results. The data are given as an average value ($Se_{nmol/g}$–$Hg_{nmol/g}$) with standard deviation and the number of samples of each organ for each group. In some cases

Table 3. Average values of 'non-Hg-associated Se' ($Se_{nmol/g}$–$Hg_{nmol/g,\ wet\ weight}$) in endocrine glands, kidney cortex and brain tissues of mercury exposed and nonexposed individuals (1990–1997).

Tissues	Control	Idrija residents	*Subgroup	Retired miners	**Drasch *et al*. (2000)
Pituitary					
Average	4.0 ± 1.7	4.3 ± 1.4	7.4; 1.3	0′; 5.2	
Range	1.2–8.0	2.4–6.5			
Geom mean	3.6	4.1			6.9
(N)	(18)	(6)	(2)	(2)	(127)
Thyroid					
Average	5.2 ± 2.7	5.0 ± 1.7	4.0; 12.1	1.3 ± 1.7	
Range	1.8–10.9	1.8–7.1		0″–3.6	
Geom mean	4.5	4.6			4.6
(N)	(20)	(7)	(2)	(4)	(126)
Pineal gland					
Average	1.6 ± 1.0	0′		0′	
Range	0.5–4.6				
Geom mean	1.4				
(N)	(16)	(1)		(1)	
Adrenal gland					
Average	1.3; 5.5	3.0 ± 0.4	4.4	2.3 ± 2.3	
Range		2.7–3.4		0.9–5.0	
Geom mean		2.9		1.7	
(N)	(2)	(3)	(1)	(3)	
Kidney cortex					
Average	6.9 ± 2.1	7.1 ± 3.8	7.2	0.2; 2.4	
Range	4.9–9.4	1.7–10.6			
Geom mean	6.6	5.8			7.3
(N)	(4)	(4)	(1)	(2)	(131)
Hippocampus					
Average	1.5 ± 0.3	1.7 ± 0.2		1.0 ± 0.5	
Range	1.1–1.9	1.4–2.0		0.7–1.6	
Geom mean	1.5	1.7		1.0	
(N)	(8)	(5)		(3)	
Cortex cerebellum					
Average	1.8 ± 0.5	2.0 ± 0.4		1.9 ± 1 .0	
Range	1.2–2.8	1.6–2.5		0.9–3.2	
Geom mean	1.7	2.0		1.7	1.4
(N)	(7)	(7)		(4)	(119)
Nucleus dentatus					
Average	1.8 ± 0.9	2.1 ± 1.6		2.2 ± 2.4	
Range	1.0–3.7	0.6–3.6		0.7–5.0	
Geom mean	1.6	1.7		1.5	
(N)	(12)	(3)		(3)	

0′ or 0″ – zero representing one or two result with calculated negative value (see Figure 1); * – Subgroup formed from two individuals who lived near mercury smelting plant; ** – Data estimated for German general population; N – Number of individuals.

accumulated mercury highly exceeded selenium and in the calculation the phenomenon was manifested as a negative selenium value. In calculating the averages these negative values were taken as zeros. Control values are also compared with data obtained by Drasch *et al.* (2000) for the German general population. The results agree well. Among Idrija residents Hg levels for endocrine glands and kidney cortex of two individuals living near the mercury smelting plant were outstandingly high. These data are separated as an Idrija residents subgroup. Their values for brain tissues were similar to other residents and are included in the average of the main group.

Table 4 summarises the measured Hg and Se values, calculated Hg/Se molar ratios and estimated non-Hg-bound Se data for medulla oblongata, thyroid, kidney cortex and hair of an active miner occupationally exposed for three years. The relatively low mercury level in thyroid and the extremely high mercury level in kidney cortex should be emphasized, because these values are opposite to the burden order observed in retired miners (Kosta *et al.* 1975; Falnoga *et al.* 2000). It seems that in thyroid slow mercury accumulation during exposure and slow elimination after the cessation of occupational exposure occurs. A similar phenomenon was also observed in rats during long-term high mercury vapour exposure and during the elimination period (Falnoga *et al.* 1994). Because of the current exposure of this active miner, mercury highly exceeded coaccumulated selenium in kidney cortex and hair and non-Hg bound selenium was diminished in all tissues examined. The high mercury concentration in hair is explained by external contamination.

In Figure 1 data are given individually. Here non-Hg-bound Se is presented as a function of Hg concentration in human organs of different groups and of one active miner. The different degree of Hg excess is evident from the negative Se values. Negative Se values ($Se_{nmol/g}$–$Hg_{nmol/g}$) represent the amount of Se which would be needed to complex the remaining Hg which is most probably bound to protein thiols or as mercury sulphide polymers, (HgS)n (Aoi *et al.* 1985). Few negative values were mostly found in endocrine glands (one thyroid, two pituitaries, two pineal glands) and the highest in the kidney cortex of an active miner.

Discussion

A forerunner of the idea of estimating non-Hg bound Se (Drasch *et al.* 2000) was described by Kosta *et al.* (1975) where it was stated that in the case of subjects with low Hg contents, the ratio of mercury to selenium (postulated as 1:1 molar) should be calculated by subtracting the normal physiological level of Se in that tissue from the total Se content, and using the difference (i.e., the Hg bound Se) for calculation of the Se:Hg ratio of deposited aggregates. The normal, metabolically controlled, physiological levels should be nearly constant at the same geographical location (Zachara *et al.* 2001). Later the new findings on selenium metabolism and its interactions with metals (Sasakura & Suzuki 1998; Yoneda & Suzuki 1997; Suzuki *et al.* 1998; Gailer 2002; Tapiero *et al.* 2003; Schweizer *et al.* 2004) gave rise to concerns that mercury sequestration by selenium can also decrease the metabolically active pool of Se needed for selenoprotein synthesis. Following this trend Drasch *et al.* (2000) radicalised their view and tried to estimate the non-Hg-bound selenium simply by the difference between the molar contents of the two elements.

In spite of reservations stated later in the discussion, we tentatively used the same approach in the present study. We tried to estimate the maximal possible influence of Hg exposure on the non-Hg bound selenium in selected tissues of individuals exposed to increased levels of mercury

Table 4. Hg and Se in organs of an active miner after three years of occupational exposure.

Active miner	Hg (nmol/g)	Se (nmol/g)	Hg/Se	Se–Hg
Medulla Oblongata	0.55	1.4	0.39	0.85
Thyroid	2.54	3.52	0.72	0.98
Kidney cortex	217.9	27.48	7.32	−190
Hair	62.5	8.61	7.26	−53.9

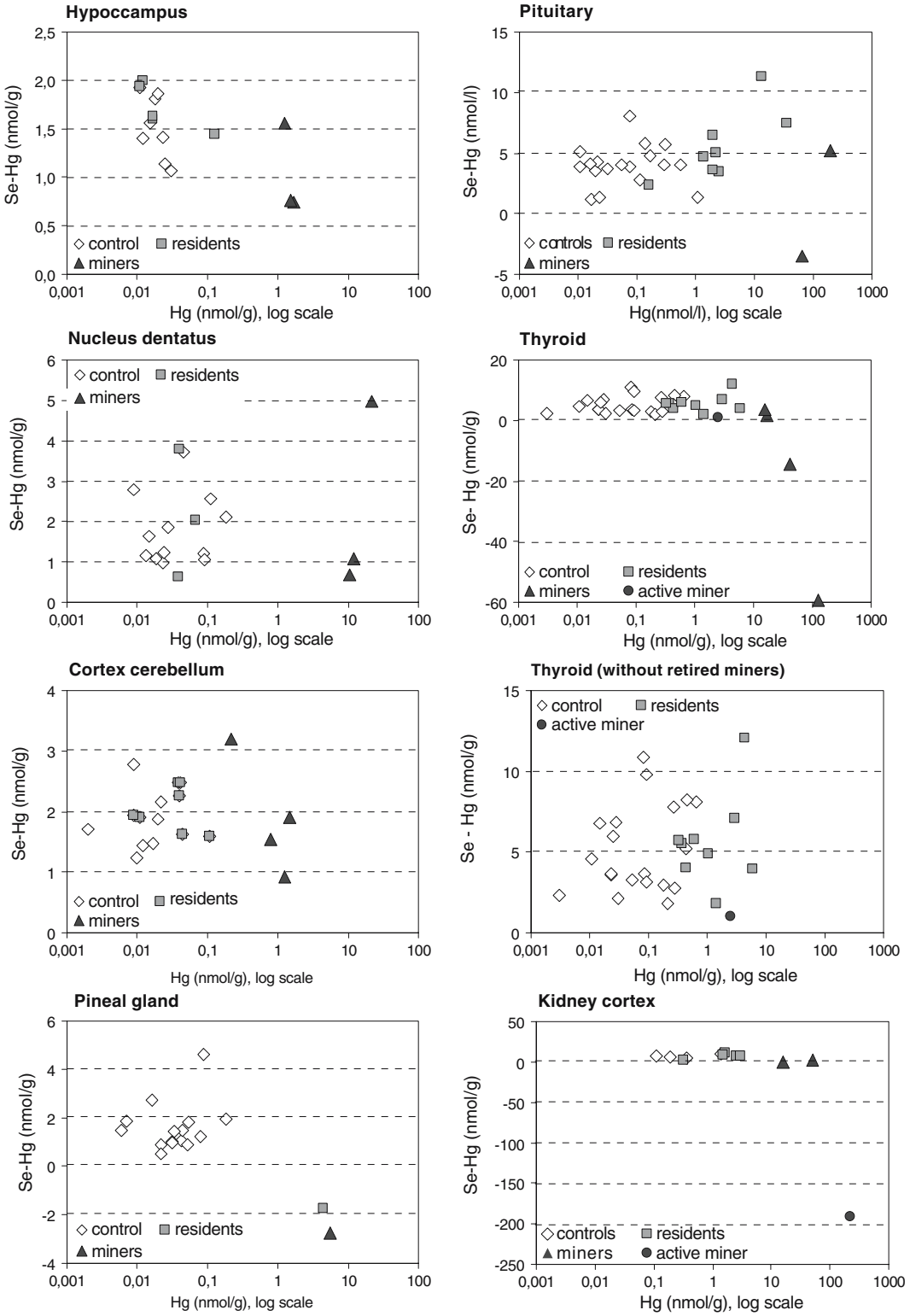

Figure 1. Non-Hg bound Se as a function of Hg concentration in individual human organs of control and mercury exposed groups (Idrija residents, retired Hg miners) between 1990 and 1997. Kidney and thyroid sample of one active miner from 1989 are included as well. (◇ control; ■ Idrija resident; ▲retired miner; ● active miner).

vapour occupationally (retired miners) or in their living environment (residents). Organs of interest were some endocrine glands, kidney cortex and some parts of the brain, all of them supposed to accumulate or retain mercury and selenium to a high extent.

Mercury levels for the control group were negligible (Table 2) and so non-Hg bound Se levels did not differ appreciably from measured selenium levels. Relatively high standard deviations (and wide ranges) found for levels in almost all organs (Table 3) point to high individual variations, which is in general to be expected when we are dealing with a human population. Average control levels were in agreement with data obtained by Drasch *et al.* (2000) for thyroid, pituitary, kidney and cortex cerebellum for the German general population sampled with about 100 individuals (data are included in Table 3). Tissue data for the general population from various countries can also be dissimilar, depending on geographical location, as shown in the article of Zachara *et al.* (2001). In this are cited literature data for kidney levels for different countries; data in nmol/g are: 5.9 ($n = 46$, SD = 1.7) for Poland, 6.1 for Italy ($n = 13$), 9.8 ($n = 13$) Germany, 8.7 ($n = 22$) for Bulgaria, 11.3 for USA ($n = 20$) and 14.2 ($n = 45$) for Japan. So it is obvious that when we are dealing with tissue levels, the only reliable control group should be a local one.

Comparing the calculated values of non-Hg bound selenium (Se_{mol}–Hg_{mol}) among the groups, it was found that for Idrija residents the average values were similar to those of the control group (Table 3). For some tissues it was possible to see a weak upward trend. Individually the levels of non-Hg-bound Se were unchanged, slightly decreased or even elevated, but the differences are not marked (Figure 1). Surprising exceptions were high individual elevations found in one thyroid and one pituitary of residents who lived near the mercury smelting plant, representing one of the most polluted areas (Table 3 and Figure 1; subgroup of Idrija residents). These exceptions point to the possibility of extreme selenium compensation (composed of high mercury bound selenium and increased mercury unbound selenium). In this regard the concerns (Drasch *et al.* 2000; Gailer 2002, Ralston 2003) of diminished bioavailability of selenium after long-term mercury exposure might be overestimated, at least at moderate exposure (such as Idrija residents or the population with amalgams), if selenium intake is sufficient. At this point it is important to mention that soil and vegetable (Falnoga *et al.* 2003) selenium levels in the Idrija region are in the same range as in other parts of Slovenia, so Idrija inhabitants are not protected due to the increased presence of selenium in their environment, but due to increased absorption of Se from food or most probably due to decreased excretion from the organism. Selenium is homeostatically regulated mainly by renal excretion (Windisch 2002; King 2001).

As expected, diminished average non-mercury bound Se values were found in some mercury-loaded organs of retired Idrija miners like the thyroid and kidney cortex as shown in Table 3. Actually, among individuals the values in organs varied from diminished to elevated levels (Figure 1). Selenium compensation or interestingly even 'overcompensation' was found in some individual organs and that is in accordance with calculations made on the basis of data on Idrija retired miners published by Kosta *et al.* (1975) (Figure 2). As the mine was still active at that time, is not surprising that among those five cases the upper (individual) levels of non-Hg bound Se found in thyroid and pituitary are even higher 23 nmol/g, 30 nmol/g) than the highest found in our study for two residents living near the smelting plant (12 nmol/g, 13 nmol/g; Table 3).

Regarding the recent pilot study performed on a small group of living retired miners, it appears that plasma mercury and selenium levels and the percentage of selenium bound to plasma SelP are also in the ranges of control values (Falnoga *et al.* 2004; Kobal *et al.* 2004), but some other factors like decreased urine and increased blood melatonine indicate some changes in oxidative defence mechanisms (Kobal *et al.* 2004). Increased blood and decreased urine melatonin with its antioxidative function (Reiter *et al.* 2000, Sener *et al.* 2003) could represent compensation for a possible depletion of metabolically active selenium in pineal glands (Figure 1; pineal gland), or represent a common response after long-term past exposure to elemental mercury.

A downward trend in available Se was observed in the case of the active miner after working in the mercury mine for three years (Table 4 and Figure 1). The differences in mercury

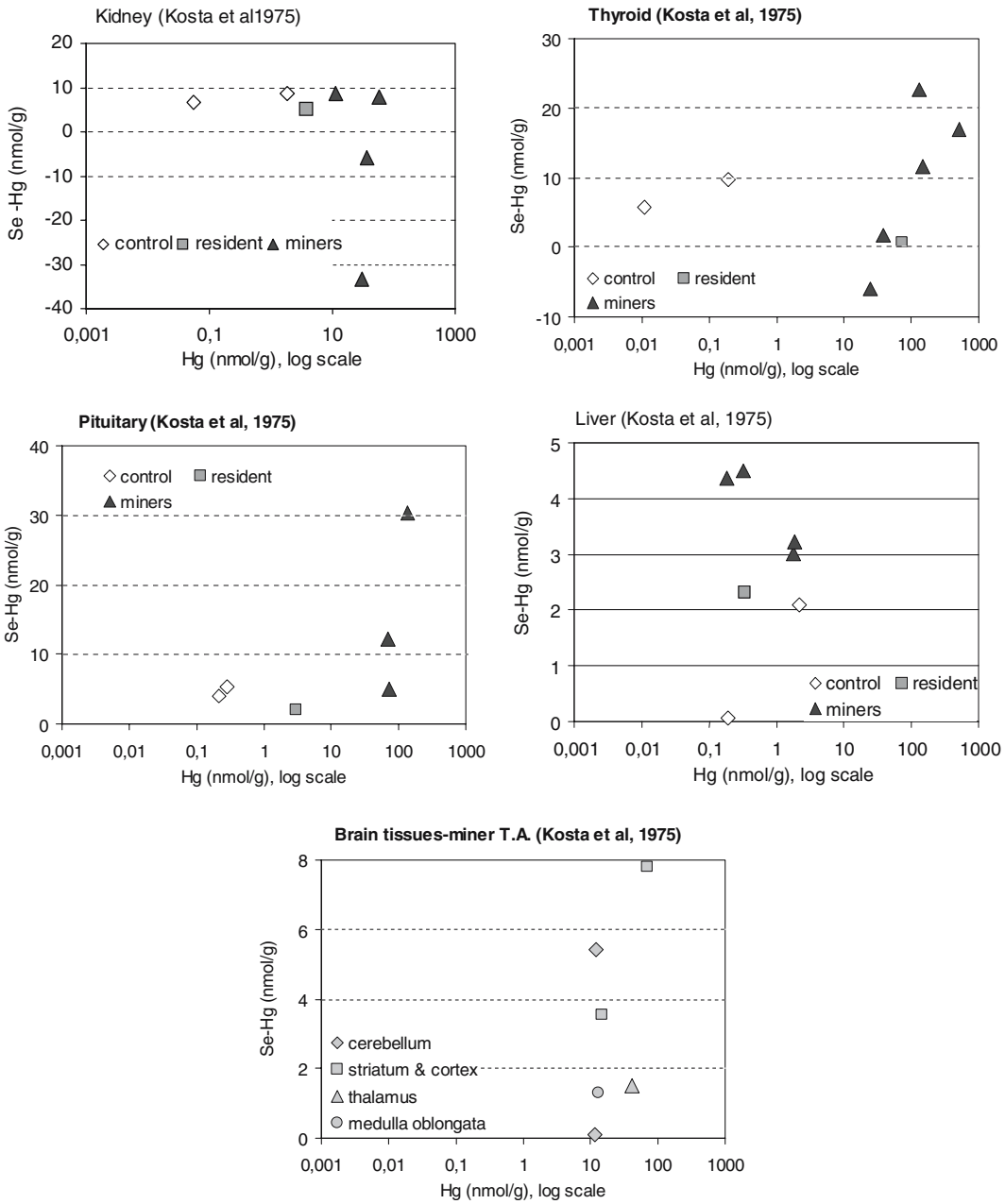

Figure 2. Non-Hg bound Se as a function of Hg concentration in individual human organs in some cases of control group, Idrija residents and retired Hg miners. Brain samples belonging to one retired miner are presented separately. All cases are from before 1975 and data are calculated from Kosta *et al.* 1975. Retired miners were exposed to Hg for 18–22 or 17–33 years and after that retired for 14–22 or 5–16 years. (◇ control; ■ Idrija resident; ▲ retired miner).

and selenium loading between this active miner and retired miners were commented on in a previous section, though some caution is necessary since only one subject is involved. Certainly the repeated intermittent type of exposure to high Hg levels in the mine could be most threatening for physiologically active selenium (inhibition of seleno-enzymes, depletion of metabolically active intermediates like S^{-2}), because there is not enough time and/or amount of nutritional selenium

for sufficient compensation. In such cases we can speak of a deficiency of bioavailable selenium, which should be checked by more specific indicators like the activity of different selenoenzymes. Regarding blood it is possible to follow plasma or erythrocyte GPx activity and plasma SelP activity, but regarding other organs we are still limited to tissue levels and theoretical estimations such as the one used in the present study.

However it should be considered that by such an estimation the results for non-Hg-bound Se can be somewhat low and the problem overestimated. It would be imprudent to claim that all tissue Hg is bound to Se on every occasion, even in cases when non-bound Se is negative. Although Hg affinity for selenium ligands is higher than for sulphur ligands *in vitro*, the situation *in vivo* can be different, influenced by many variables such as the spatial conformation of a macromolecule protecting its binding sites, changeable pH, relative concentrations of sulphur and selenium ligands/binding sites, etc. Tissue levels sometimes mask the situation inside the cells, as can be seen from investigations of the subcelullar distribution of both elements in human kidneys, where the cytosol mercury was bound to selenoproteins in a ratio lower than one to one and in higher amount to metallothioneins (Björkman *et al*. 1994) which is known to have twenty cysteine ligands. This latter study included five individuals, one with known occupational mercury exposure (dentist). It is also interesting that the thyroid of a retired miner, included in our study, where tissue non-Hg bound selenium was negative (−17.5 nmol/g), the amount found in tissue cytosol was positive (0.296 nmol/g), showing that not all selenium was bound to mercury. In molar ratio form (Hg/Se) the results have been published in Falnoga *et al*. 2000. Both investigations dealt with chronic long-term mercury exposure.

Concluding remarks

From the assumption that Hg is almost quantitatively deposited in tissues bound to Se in a 1:1 ratio, Drasch *et al*. (2000) concluded that the quantity of non-Hg-bound Se can be approximately estimated simply by the difference between the molar contents of the two elements (Se_{mol}–Hg_{mol}). But experimental data (Björkman *et al*. 1994; Falnoga *et al*. 2000) do not support the idea that tissue mercury is preferentially bound on all selenium bioligands in all circumstances (regardless of the conditions of previous or recent exposure (s)), or in other words not all tissue selenium is expended in Hg sequestration on every occasion. So according to this estimation the results for non-Hg-bound Se can be somewhat low and the problem of selenium depletion overestimated.

In spite of that we considered that it could be interesting to use the same approach with the data from our previous investigation, where Hg and Se concentrations were determined in autopsy samples of retired Idrija mercury mine workers and Idrija residents living in a mercury contaminated environment. Taking into account the risk of underestimation, we expected evidently/convincingly diminished levels of Hg-nonbound selenium in comparison with the control group. On the contrary, it was found that after long-term exposure to moderately increased mercury levels (Idrija residents) non-Hg bound selenium levels were not only maintained but in some cases even elevated. Diminished values were found in some mercury-loaded organs of retired Idrija miners.

Following these estimated data and the proven possibility that in reality the levels of Hg-nonbound selenium can be even higher, it could be speculated that in Idrija residents Hg sequestration of selenium is sufficiently compensated, while particularly in active miners, the activity of selenoenzymes could be inhibited and the amount of Se necessary for synthesis of selenoezymes insufficient, especially in cases of intoxication. In that context the concerns of some authors (Drasch *et al*. (2000; Gailer 2002; Ralston 2003) that mercury sequestration by selenium can affect (diminish) the metabolically active selenium pool needed for selenoprotein synthesis should be limited to occupational exposures or situations with low selenium intakes.

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