Mercury, selenium, and glutathione peroxidase before and after amalgam removal in man

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In 10 healthy persons all amalgam fillings were replaced with gold inlays. Blood and urinary levels were measured on 10 occasions during a 4-month period before and a 12-month period after amalgam removal. These variables were also measured three times in 10 healthy controls. A strong statistically significant relation was found between plasma mercury values and both the total number of amalgam surfaces ($r = 0.71$, $p = 0.0006$) and the total surface area of the fillings ($r = 0.73$, $p = 0.0004$). In the immediate postremoval phase plasma mercury rose three- to four-fold, whereas the urinary and erythrocyte mercury rose about 50%. These peak values declined to the preremoval level at about 1 month. Twelve months after the removal the plasma and urinary mercury levels were significantly reduced to 50% and 25%, respectively, of the initial values for the experimental group. Apart from the significantly lower plasma selenium values 5 and 10 days after removal no significant differences were found with regard to plasma selenium or erythrocyte glutathione peroxidase either within or between the experimental and the control groups. A large number of supplementary biochemical analyses did not show any influence on organ functions or any differences between the groups before or after the amalgam removal. Amalgam fillings considerably contributed to the plasma and urinary mercury levels. □ *Blood analysis; dental materials; erythrocytes; plasma; urinalysis*

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During the last few decades the question has arisen of whether mercury possibly released from dental amalgam fillings is a human health problem (1–4). This question has been approached in several ways. It has not been possible to correlate subjective symptoms and complaints—ascribed by the patients themselves to mercury release—to the presence of amalgam fillings (5–9).

Mercury vapor concentrations in expired and intraoral air (10–15) and of mercury in urine (16–18), whole blood (19–21), plasma (8, 22) and human brain and kidneys (23) have been measured. The results attained are sometimes conflicting. However, a general conclusion is that mercury is released from amalgam fillings, but the biologic significance of this release is unknown except for some allergic and local hypersensitivity reactions (24–28).

Several studies have demonstrated an interaction between both organic and inorganic mercury and selenium (29–33). With regard to mercury vapor there is no scientific evidence that selenium will protect against a possible toxic effect of this mercury form, but there is evidence that selenium influences the distribution and the retention patterns (34–38). In two earlier studies no correlations were found between either the plasma mercury level or the number of amalgam fillings and the plasma selenium level (8, 22).

One logical approach to the question of whether some biologic variables are influenced by release of mercury from amalgam would be to estimate possible changes in these parameters after removal of amalgam fillings. Thus, the aim of the present investigation was to study mercury, selenium, and the selenium-dependent enzyme glutathione peroxidase (GSHPx) in man before and after

removal of all amalgam fillings. To evaluate any effects on various organ systems, supplementary blood and urinary analyses were carried out.

Materials and methods

Ten healthy persons visiting their dentist for the annual dental examination were asked to participate in the present study. The subjects consisted of five men and five women with a mean age of 35 years (range, 28 to 39) and 32 years (range, 26 to 44), respectively. A control group consisted of five men with a mean age of 35 years (range, 30 to 44) and five women with a mean age of 33 years (range, 25 to 40). With the exception of three women and one man, the controls were spouses of the experimental persons. None of the participants had been occupationally exposed to mercury vapor, and none of them was a consumer of selenium preparations.

The fish consumption pattern was almost identical and the intake rather limited in the two groups. Six persons, three from each group, had one fish meal a week, and the other 14 persons had one fish meal every other week or more seldom.

Only one person belonging to the control group was a smoker. The alcohol consumption reported in the two groups seemed to be limited and quite similar. Two controls and one experimental person did not consume alcohol at all. Fourteen participants took alcohol once a month at the most, and three participants, two experimental and one control, consumed alcohol every week. To our knowledge none of the participants consumed alcohol the day before or the same day as the treatment was performed.

The total number of amalgam surfaces was registered, and the mean number was 19.9 surfaces (range, 8 to 42) for the experimental group and 24.7 surfaces (range, 2–47) for the controls. The total surface area of the amalgam fillings was calculated from stone casts of the teeth of each participant. A tinfoil was punched to cover the amalgam surface of each tooth. By subsequently cutting and enlarging each foil ten times it was possible to transfer the amalgam surface area to a transparent paper. The surface area could then be calculated by using a computerized digitizer (CALCOMP 2000, Series Digitizer).

All amalgam fillings were removed by means of water spray cutting and a vacuum evacuator. The preparation and the impression for gold inlays were carried out at the same time. Temporary restorations (Nobetec® or zinc oxide-eugenol) were applied for about 15 days, whereafter the gold inlays were cemented (Phosphocap®, Ivoclar). A total of 113 teeth were restored with 113 gold inlays in JS C3 gold (AB, John Sjöding, Sweden). The mean number of gold inlays was 11 (range, 6–17) distributed on 19 surfaces (range, 8–37). In one person a third molar with two amalgam surfaces was extracted. Eight buccal and occlusal surfaces were restored with small composite resin fillings. The controls had no treatment performed.

Blood and urine samples

Blood and urine samples were collected at 4 and at 3 months before the removal of the amalgam fillings and then 1, 5, and 10 days and 1, 3, 6, 9, and 12 months after removal of the amalgam fillings. Blood and morning urine samples from the control persons were collected 3 months before and 6 and 12 months after the amalgam removal from the experimental persons. One female test person died within the period between the two last test occasions.

Blood was collected in metal-free tubes (Terumo) from the cubital vein with heparin as anticoagulant for the mercury assays and with ethylenediaminetetraacetic acid (EDTA) as anticoagulant for the selenium and GSHPx assays. Apart from the samples intended for blood cell analyses, the samples were centrifuged at 2000 *g* for 10 min, and the supernatant plasma was drawn off for further analyses. The buffy coat was discarded. Urine was collected in acid-washed polyethylene bottles. Unless the samples were analyzed immediately, they were kept at −80°C until analysis.

Analytical procedures

The mercury content was determined in wet-digested samples by a 'cold vapour' atomic absorption technique using automatic equipment (39). The plasma samples, 1.0 ml, were digested with nitric and perchloric acids at 65°C overnight (40), and the urine samples, 0.5 ml, by potassium permanganate and sulfuric acid at room temperature overnight (41). Both digestion procedures were slightly modified to suit large sample volumes. All samples were analyzed in duplicate. The detection limit was 0.5 nmol/l in plasma and 1.0 nmol/l in urine. The precision, as calculated from the duplicate analyses, was 11% (coefficient of variation) for plasma samples in the range 1.0–9.9 nmol/l (mean, 4.5 nmol/l; $n = 92$) and 3% in the range 9.9–54.8 nmol/l (mean, 24.4 nmol/l; $n = 12$). The accuracy was checked by analyses of reference samples. For the plasma sample (Seronorm®, Nycomed) the reference value was 5.5 nmol/l, and our results from different runs averaged 6.3 nmol/l (SD, 0.17; range, 5.5–7.8; $n = 6$). The reference value of the urine sample (Lanonorm®, Nycomed) was 48.4 nmol/l, and our results averaged 47.2 nmol/l (SD, 0.80; range, 44.4–59.8; $n = 12$).

Erythrocyte glutathione peroxidase (Ery-GSHPx) was determined as follows: 150 µl packed erythrocytes were lysed in 2.5 ml 5 mM sodium *N*-2-hydroxyethyl-piperazine-*N*-1,2-ethanesulfonic acid buffer (pH 7.4) with 50 µM EDTA and then centrifuged. The hemolysates were then treated with 1 mM potassium ferricyanide and 8.7 mM sodium cyanide to inhibit the peroxidase activity of hemoglobin (42). A 25-µl volume of treated hemolysates was added to 500 µl 0.1 mM sodium-*N*-2-hydroxyethylpiperazine-*N*-1,2-ethanesulfonic acid buffer (pH 7.4) with 1 mM EDTA, 2 mM reduced glutathione, 1 U/ml glutathione reductase, 0.16 mM nicotinamide adenine dinucleotide phosphate, reduced form (NADPH), and 0.16 mM *tert*-butylhydroperoxide. The assay was performed at 37°C, and the activity was expressed as µkat/g hemoglobin in the hemolysates. Hemoglobin was determined by a standard cyanomethemoglobin method.

P- and U-selenium was determined by a fluorometric method with diaminonaphthalene as described by Lalonde et al. (43). P-sodium and P-potassium were determined with ion-selective electrodes, P-calcium as a complex with cresolphthalein purple, and P- and U-creatinine with an alkaline picrate reagent. Plasma levels of total and conjugated bilirubin were determined with sodium nitrite and sulfanilic acid, and P-alkaline phosphatase by P-nitrophenyl-phosphate assay. P-γ-glutamyl transpeptidase, aspartate aminotransferase, alanine aminotransferase, lactate dehydrogenase, and creatine kinase were determined with reagent kits from Boehringer Mannheim AG. Albumin, α-1-antitrypsin, orosomucoid, haptoglobin, and C-reactive protein (CRP) in plasma were analyzed by electroimmunoassay. Blood cells and erythrocyte values were analyzed on a Technicon H 6000 apparatus, U-albumin by means of electroimmunoassay, U-protein by the Coomassie Brilliant Blue G-250 procedure (44), U-β_2-microglobulin with a radioimmunoassay kit (β_2-mikro RIA 100) supplied by Pharmacia Diagnostics AB, Uppsala, Sweden, and osmolality of urine with the freezing-point depression method.

Reference intervals (Table 1)

The P-selenium (0.72–1.47 µmol/l) and Ery-GSHPx (0.63–1.82 µkat/g Hb) reference intervals were obtained from analysis at the laboratory of Clinical Chemistry, University of Umeå, of samples from 250 persons aged 30, 40, 50, and 60 years in a health survey study in the county of Västerbotten. The U-selenium (0.010–0.053 µmol/mmol creatinine) reference interval was taken from 130 persons in that study. Data established and used in the laboratory of Clinical Chemistry were used as reference intervals for the other analyses. The reference interval limits presented are in general the 2.5 and 97.5 percentiles.

Statistical methods

A Mann–Whitney U-test was used for comparing the blood and urine values and the number of amalgam surfaces and amal-

Table 1. Mean values and standard deviations of some variables tested. Figures in parentheses refer to the control group

	P-Hg nmol Hg/l		Ery-Hg, nmol Hg/l		U-Hg, nmol Hg/mmol creatinine		P-Se, µmol/l		U-Se, µmol/mmol creatinine		Ery-GSHPx, µkat/g Hb	
Test occasion	$\bar{x}$	SD	$\bar{x}$	SD	$\bar{x}$	SD	$\bar{x}$	SD	$\bar{x}$	SD	$\bar{x}$	SD
Before removal												
4 months	4.3	1.3	21.4	9.0	1.01	0.64	1.08	0.11	0.023	0.007	1.10	0.18
3 months	4.7	1.1	20.1	6.6	1.00	0.71	1.05	0.14	0.021	0.003	1.00	0.16
	(6.2	3.3)	(23.0	10.8)	(1.79	1.45)	(1.09	0.15)	(0.027	0.010)	(1.20	0.20)
After removal												
1 day	16.6	15.2	31.6	16.9	0.99	0.34	1.03	0.12	0.022	0.006	1.05	0.18
5 days	11.8	11.5	27.3	11.0	1.30	0.87	0.98	0.15	0.025	0.005	1.10	0.14
10 days	9.4	10.2	26.5	13.4	1.61	1.20	0.99	0.10	0.025	0.007	1.07	0.16
1 month	6.4	7.5	23.8	12.9	1.42	1.27	1.12	0.18	0.020	0.004	1.11	0.17
3 months	3.7	1.5	20.7	10.3	0.86	0.81	1.14	0.12	0.038	0.039	1.16	0.15
6 months	3.1	0.8	20.8	7.4	0.49	0.37	1.11	0.20	0.020	0.010	1.15	0.18
	(6.7	3.6)	(22.6	8.7)	(1.70	1.48)	(1.14	0.09)	(0.029	0.011)	(1.08	0.14)
9 months	2.8	1.3	20.6	9.1	0.30	0.16	1.17	0.08	0.024	0.004	1.13	0.17
12 months	2.3	0.7	20.0	7.0	0.27	0.52	1.06	0.01	0.025	0.008	1.16	0.18
	(4.4	1.9)	(20.9	7.2)	(1.25	1.12)	(1.11	0.09)	(0.032	0.008)	(1.10	0.19)
Reference interval	—		—		—		0.72–1.47		0.010–0.053		0.63–1.82	

gam surface area between the two groups. A Wilcoxon signed-rank test was used for comparing the blood and urine values within each group. A simple regression analysis was used to compare the P- and U-mercury levels with the number of amalgam surfaces and surface area, respectively, in both groups.

Results

Two individuals in the test group reported subjective symptoms after the amalgam removal procedure. One man experienced severe dizziness about 18 h after the removal. The symptom persisted for approximately 4 h and then slowly disappeared during the day. One woman had vigorous attacks of vomiting about 8 h after the removal. Her vomiting disappeared after a few hours. It was noted that these two persons were those with the largest number of amalgam fillings—42 and 36 surfaces, respectively. No other patients reported any subjective symptoms whatsoever after the amalgam removal.

The mean number of amalgam surfaces and the mean surface area of the fillings were higher among the controls than among the test group. The mean total surface area for the experimental group was 433.3 mm^2 (range, 185–821 mm^2) and for the control group 495.5 mm^2 (range, 77–956 mm^2). The whole procedure was repeated on three stone casts. The error of the method for a single determination was 14.33 mm^2. The differences between the groups were, however, not statistically significant.

The patients had mean P-mercury values of 4.3 nmol/l (range, 2–6) and 4.7 nmol/l (range, 3–6) 4 and 3 months, respectively, before amalgam removal. The controls had a mean value of 6.2 nmol/l (range, 4–15) on their first test occasion. The difference between the groups was not statistically significant (Table 1, Fig. 1). One person in the test group had quite remarkable changes in his P-mercury value at 1 (57 nmol/l), 5 (43 nmol/l), 10 (36 nmol/l) days and 1 month (26 nmol/l) after the amalgam removal. This person had initially 16 amalgam surfaces, which was the fourth lowest value in the group. However, the P-mercury values in the experimental group increased immediately after the amalgam removal and remained increased at least until the measurements

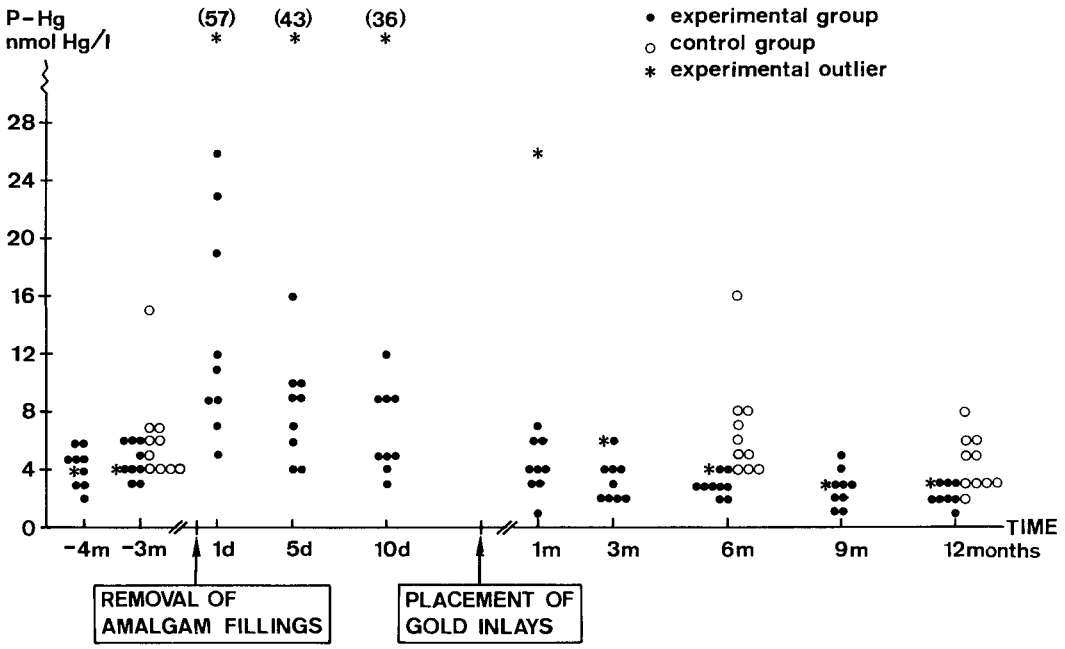

Fig. 1. Each value of plasma mercury before and after amalgam removal in experimental and control groups.

after 10 days (Fig. 1). At the registration 1 month after the removal the mean P-mercury value had returned to the preremoval level. When the outlier was included in the experimental group (asterisk in Fig. 1), the mean P-mercury value increase after amalgam removal was still more pronounced. Three and 6 months after removal the P-mercury levels were lower than before, and 9 and 12 months after the removal they were statistically significantly lower than before ($p < 0.01$). Twelve months after amalgam removal in the experimental group the control group had a statistically significantly higher P-mercury level than the experimental group ($p = 0.007$).

The erythrocyte mercury (Ery-mercury) level showed small deviations before the amalgam removal ($\bar{x} = 21.4$ and 20.1 nmol/l, respectively, at the two samplings) (Table 1, Fig. 2). After the removal the values increased the next day ($\bar{x} = 31.6$ nmol/l). After 3 months the values had returned to the same level as before the removal ($\bar{x} = 20.7$ nmol/l) and thereafter remained constant. The same outlier who deviated in P-mercury had a pronounced rise in Ery-mercury levels 1 and 5 days after removal. Neither his increase in Ery-mercury level nor those of the other patients were correlated to any change in fish consumption. The control group remained at almost the same Ery-mercury level during the whole experimental period. There were no statistically significant differences between the experimental and the control group at the three comparable test occasions.

For the U-mercury the mean values 4 and 3 months, respectively, before the amalgam removal were identical ($\bar{x} = 1.0$ nmol/mmol creatinine) (Table 1, Fig. 3). After the removal the U-mercury increased, at least until the measurements after 10 days ($\bar{x} = 1.61$ nmol/mmol creatinine). At 1, 3, 6, and 9 months the values were decreasing, and 12 months after the amalgam removal the U-mercury was 25% of the preremoval level ($p < 0.01$). Compared with the controls, the experimental group had statistically significantly lower U-mercury levels 6 and 12 months after the removal ($p = 0.01$).

All individual mercury levels in plasma

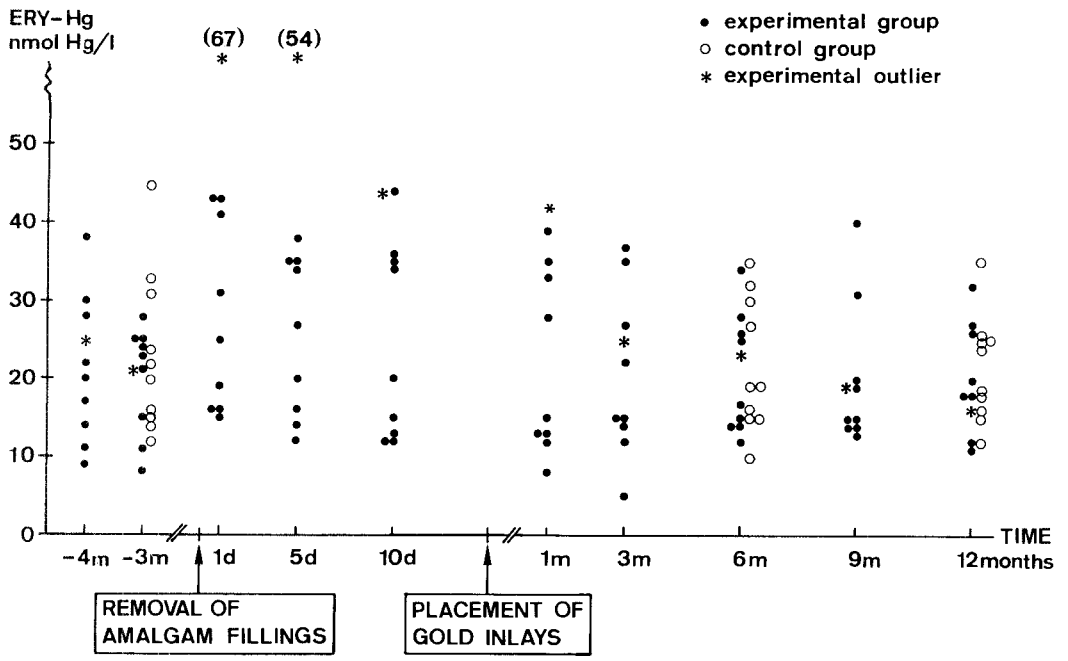

Fig. 2. Each value of erythrocyte mercury before and after amalgam removal in experimental and control groups.

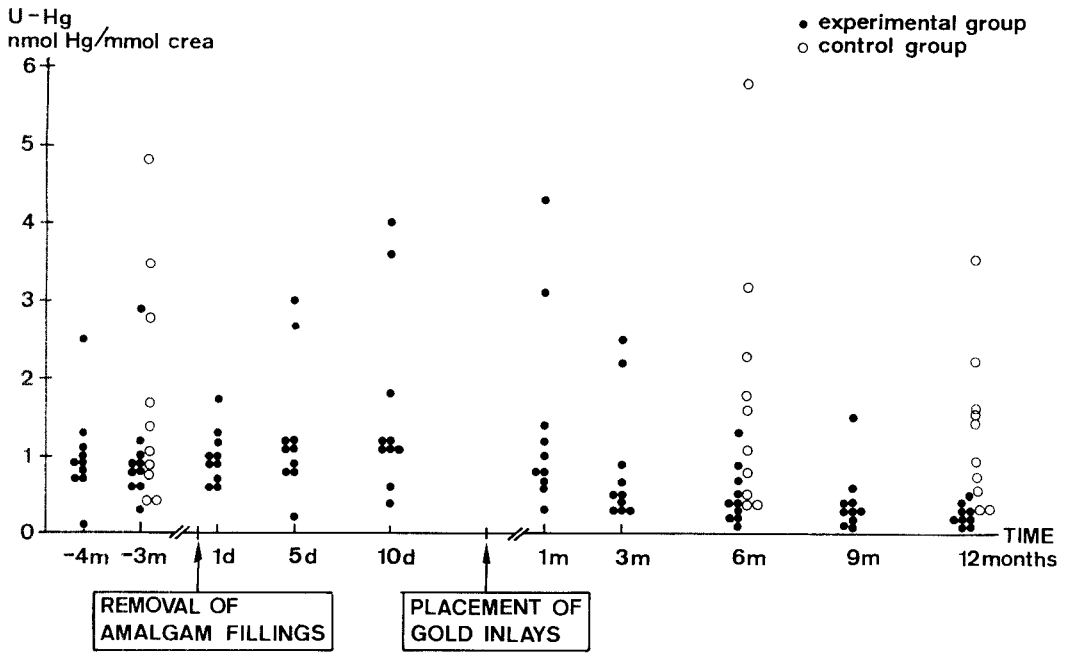

Fig. 3. Each value of urinary mercury before and after amalgam removal in experimental and control groups.

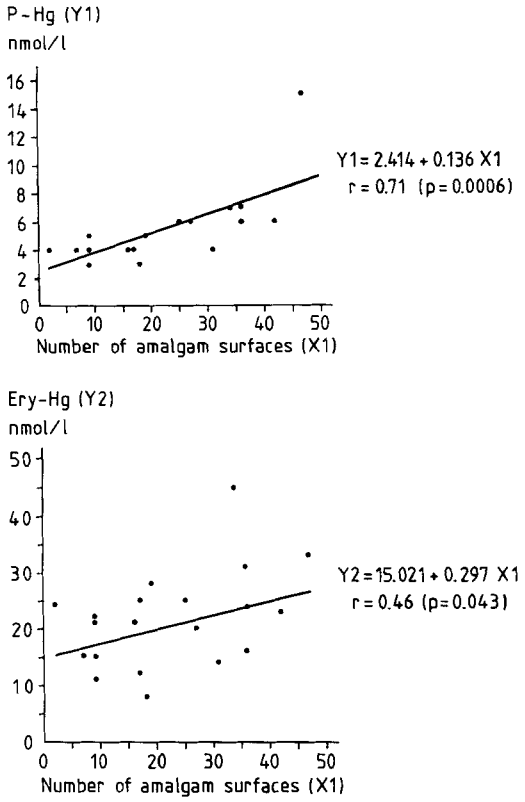

Fig. 4A. Plasma mercury and erythrocyte mercury in relation to the number of amalgam surfaces. Solid line is linear regression for the experimental and control groups.

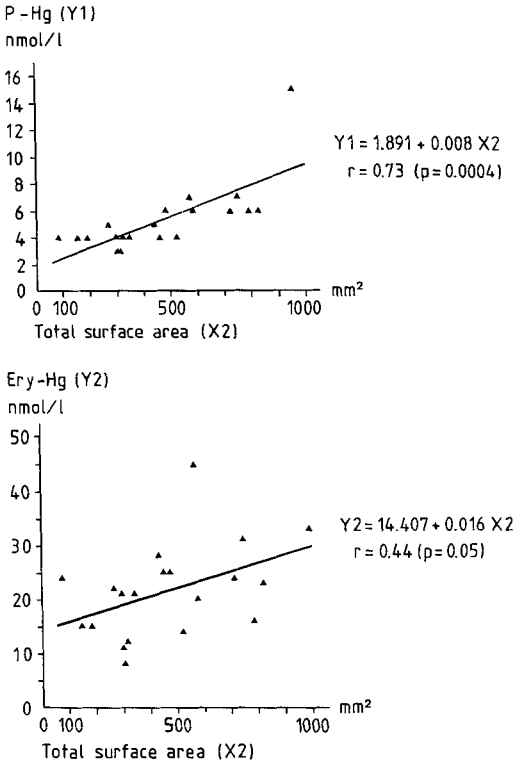

Fig. 4B. Plasma mercury and erythrocyte mercury in relation to the total amalgam surface area. Solid line is linear regression for the experimental and control groups.

and urine were, during the 16-month experimental period, equal to or below the values of the control material reported by Åkesson et al. (45).

A positive, statistically significant relation was found between P-mercury and Ery-mercury, on the one hand, and the total number of amalgam surfaces and total surface area of the amalgam fillings, on the other (Figs. 4A, 4B). With regard to the correlation between U-mercury concentration and the number of amalgam surfaces and the total surface area the tendency was not statistically significant (Fig. 4C).

The P-selenium showed quite similar levels ($\bar{x}$ = 1.08 and 1.05 μmol/l) on the two test occasions before the amalgam removal (Table 1, Fig. 5). On the 5th and 10th day after the removal the P-selenium values were statistically significantly lower than the preremoval selenium values ($p < 0.04$). Thereafter the values returned to the preremoval levels on the following occasions. The values 3 months before and 6 and 12 months after the amalgam removal did not differ from those of the controls. For all the P-selenium values analyzed for both groups, only one value, 0.67 μmol/l, measured in one experimental person at the 6-month control, fell outside the reference interval (0.72–1.47 μmol/l).

During the period from 4 months before until 12 months after the amalgam removal, all except two of the individual values for U-selenium were within the reference interval (Table 1, Fig. 6). Except for 3 months after the amalgam removal all the U-selenium mean values fluctuated between 0.020 and 0.025 μmol/mmol creatinine. The mean

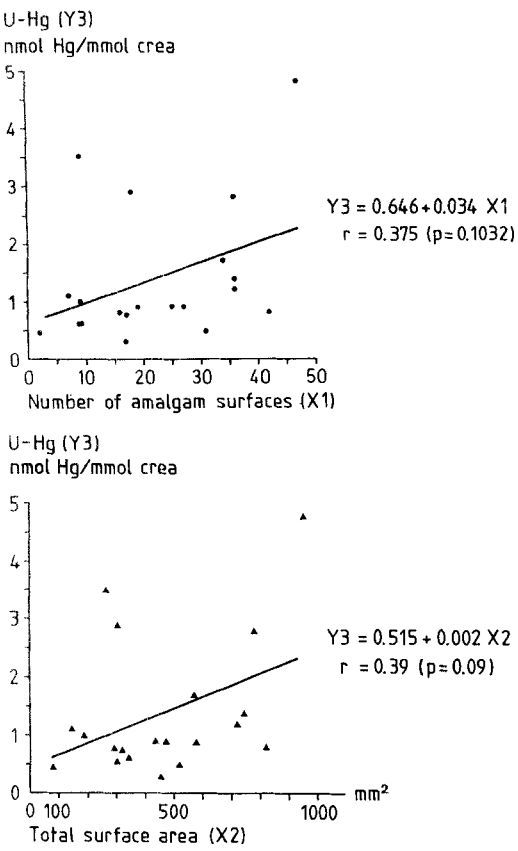

Fig. 4C. Urinary mercury in relation to the number of amalgam surfaces and the total amalgam surface area. Solid line is linear regression for the experimental and control groups.

value at 3 months was 0.038 μmol/mmol creatinine, but this value did not differ statistically significantly from the others. In the control group the mean values varied between 0.027 and 0.032 μmol/mmol creatinine; all individual values were within the reference interval. Neither between nor within the experimental and the control group were any statistically significant differences noted over the time period studied. All individual Ery-GSHPx values except for one control value fell within the reference interval (Table 1, Fig. 7). There were no statistically significant differences between the two groups on any test occasion. Nor was any such difference found within each group when comparisons were made between different occasions.

A large number of supplementary analyses were performed to evaluate organ functions. With the exception of U-albumin there were no statistically significant differences between the pre- and post-removal values within the experimental group for blood cell variables (erythrocyte particle concentration, B-hemoglobin, mean corpuscular volume, leukocyte particle concentration, platelets, neutrophils, lymphocytes, monocytes, eosinophils, and basophils), for plasma electrolytes (P-natrium, P-potassium, and P-phosphate), for analyses assessing liver status (P-total bilirubin, P-conjugated bilirubin, P-alkaline phosphatase, P-γ-glutamyl transpeptidase, P-alanine aminotransferase, P-aspartate aminotransferase, and P-lactate dehydrogenase), for skeletal muscle status (P-creatine kinase, P-aspartate aminotransferase, and P-lactate dehydrogenase), for plasma proteins indicating inflammatory reaction (P-α-1-antitrypsin, P-orosomucoid, P-haptoglobin, and P-CRP), for IgG, or for kidney variables (U-albumin, U-protein, U-β_2-microglobulin, morning U-osmolality, and P-creatinine).

For the U-albumin there was a statistically significant higher mean value ($p < 0.02$) at the measurement 12 months after the amalgam removal as compared with the measurements 4 months before amalgam removal. However, all individual values in both groups fell within the reference intervals. When comparing the mean values between the two groups at the three possible occasions, no significant differences were found.

Almost all 4300 values of the supplementary analyses were within the reference intervals. The 17 deviating values referring to different persons on different occasions occurred in both groups and thus did not show any systematic pattern.

Discussion

In the present study the amalgam removal was extensive and beyond the quantities

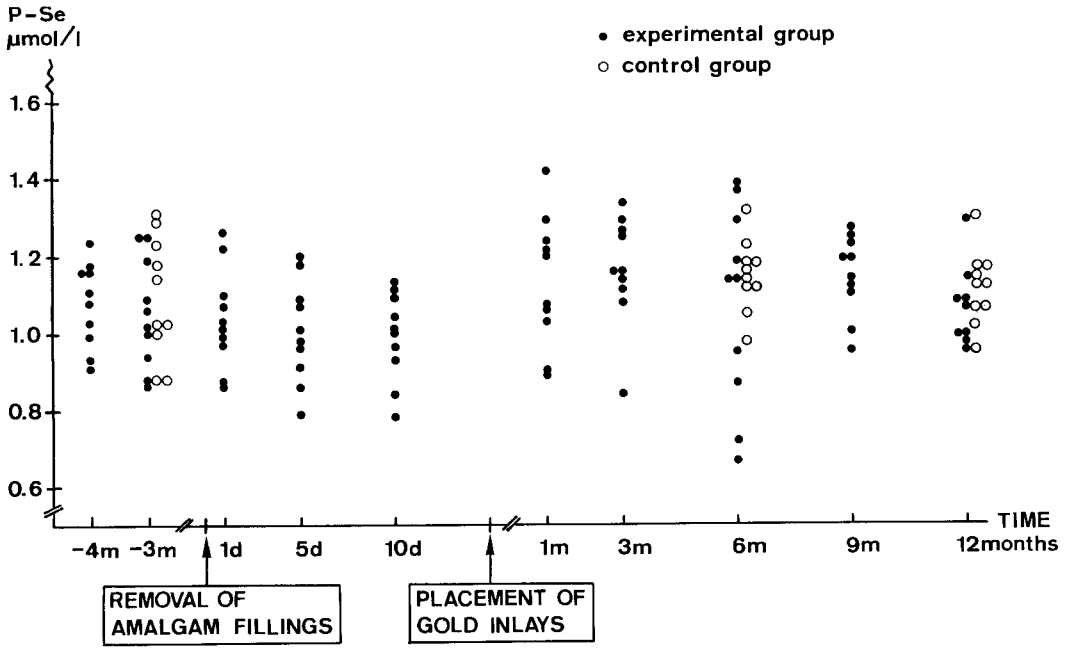

Fig. 5. Each value of plasma selenium before and after amalgam removal in experimental and control groups.

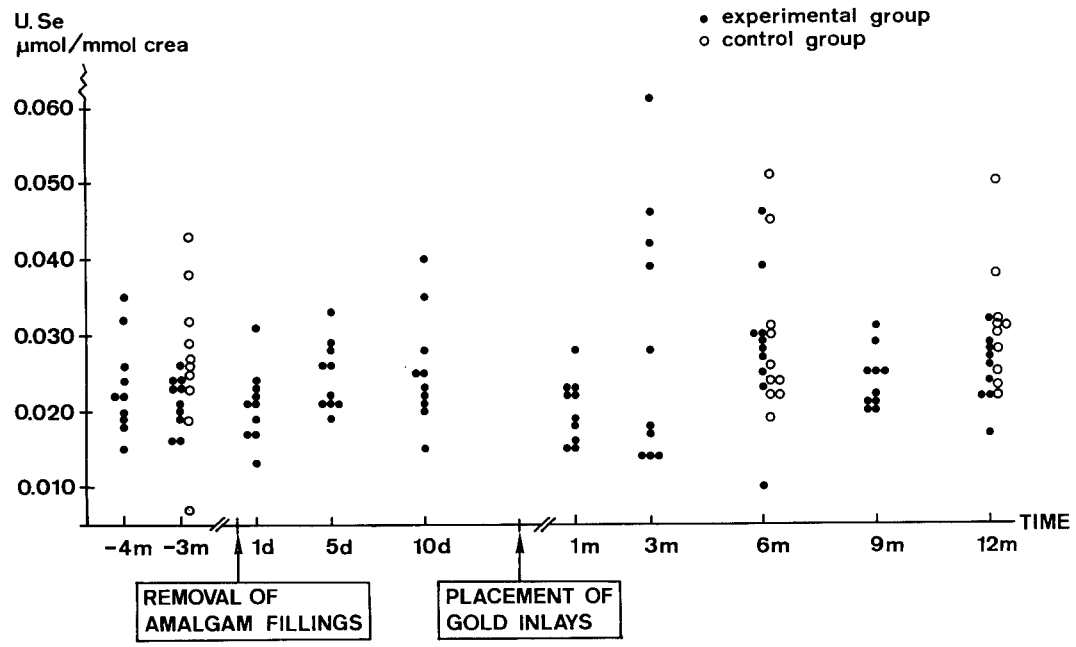

Fig. 6. Each value of urinary selenium before and after amalgam removal in experimental and control groups.

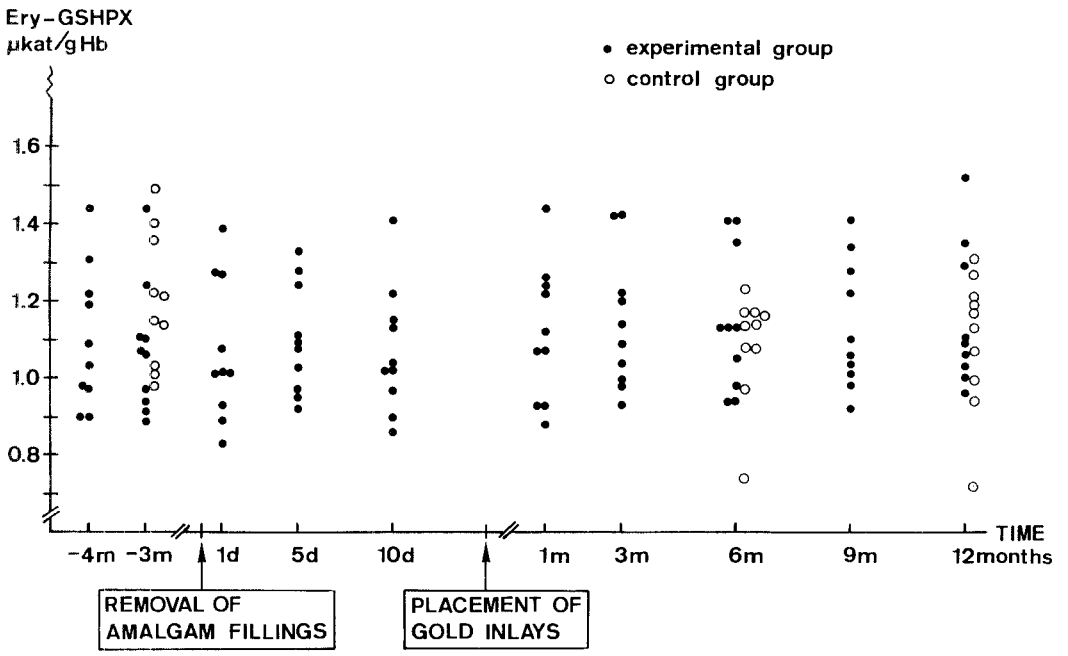

Fig. 7. Each value of erythrocyte glutathione peroxidase before and after amalgam removal in experimental and control groups.

normally removed during one treatment occasion. Although amalgam is removed with water spray cutting and a vacuum evacuator, it is not possible to prevent some metal vapor and dust from becoming available for inhalation. In our study, two patients in the test group reported severe dizziness and nausea, respectively, within 24 h after the amalgam removal. These symptoms were found in the two persons with the highest numbers of amalgam fillings. They cannot be ascribed to acute mercury poisoning, in which the signs are coughing, breathlessness, chest pain, cyanosis, and tremor (46, 47). The symptoms were of short duration and could perhaps be signs of so-called metal fume fever (48).

In hitherto published studies from other groups, mercury measurements have been carried out in whole blood. However, this approach does not enable various sources of mercury exposure to be distinguished. In this study, to facilitate such a differentiation, plasma and erythrocyte mercury measurements were performed. The methyl mercury from fish is found mainly in the erythrocytes (49, 50), whereas the elemental mercury vapor exposure, as from amalgam fillings, is almost equally distributed between plasma and erythrocytes (51). In persons with frequent fish consumption the contribution of mercury from amalgam fillings to the erythrocyte level is camouflaged by that from fish. Mercury levels in urine are relatively unaffected by fish consumption but reflect exposure to metallic mercury vapor well (52).

Several explanations for the initially higher mean plasma and urinary mercury levels in the control group, as compared with the test group, must be considered. For example, higher blood mercury concentrations have been reported among cigarette smokers (53), but the fact that only one person in the two groups was a smoker eliminates this explanation. Ethyl alcohol has also been reported to affect the mercury blood concentration (54, 55). As there was no reported difference between the groups with regard to alcohol consumption, this fac-

tor could hardly explain the difference in plasma mercury level. However, declarations by the individuals themselves concerning their intake of alcohol should be considered with great scepticism. Thus the possibility cannot be excluded that there was a difference between the groups with regard to alcohol consumption, which might possibly explain the differences in the P-mercury concentrations. The fact that the initially higher plasma and urinary mercury levels in the control group correspond both to a higher mean value of amalgam surfaces and to a higher mean surface area in this group is, however, the most probable explanation for the differences, and this is in agreement with our earlier findings (8, 22).

The erythrocyte mercury levels did not differ between the groups. They reflect the fish consumption pattern well. The six persons, three from each group, who reported a weekly fish consumption also had the highest erythrocyte mercury levels, and the person who had fish less than once a month had the lowest erythrocyte mercury level. These findings are in accordance with those of Svensson et al. (56).

Frykholm (16) reported that removal of amalgam fillings in man was accompanied by elevated concentrations of mercury in urine. After 2 weeks the urinary concentrations returned to the pre-removal levels. His group was not followed up for more than 2 weeks. In the present study the experimental group and the control group were followed up for 12 months after amalgam removal. In the beginning we found raised concentrations of mercury in plasma, erythrocytes, and urine. Our results for urine are roughly in agreement with those of Frykholm (16), although the elevation of the urinary mercury level in his study occurred earlier. In our study the 12-month observation period enabled us to follow up the participants over an extended time period, and plasma and urine mercury values were found to decrease significantly compared with the preremoval values in the experimental group. This is in accordance with the fact that nonsignificant differences between the groups before amalgam removal changed to significant differences after 6 months. The erythrocyte mercury values returned to the preremoval levels after 3 months and remained there for the further 9 months. The relation between the erythrocyte mercury values and the number of amalgam surfaces/the total amalgam surface area was weak. These findings indicate that the contribution of mercury from amalgam fillings to the Ery-mercury concentration is relatively small compared with other external mercury sources. The value of using whole blood mercury measurements to analyze mercury release from amalgam fillings is therefore questionable.

It is not surprising that extensive removal of amalgam fillings during one and the same treatment occasion will result in raised mercury levels in blood and urine for a short time. One cannot disregard that operative rooms in a dental clinic have elevated mercury vapor concentrations (57). However, the contributing amount of mercury from this source is most likely small compared with the mercury released during the drilling procedure. Twelve months after extensive amalgam removal the mercury levels in plasma and urine were significantly lower than before. This clearly shows that mercury release from amalgam fillings does contribute to the total mercury content in plasma and urine.

The statistically significant relation found between the plasma mercury values and the number of amalgam surfaces/the total amalgam surface area corroborate our earlier findings (8, 22). Kröncke et al. (19) could not find any correlation between urinary mercury concentrations and the number of amalgam surfaces. These results are contradictory to those from later studies (17, 18). With regard to the very good correlation found between plasma mercury and the number or the area of amalgam surfaces in this study and also the corresponding correlation noted concerning Ery-mercury a similar correlation was expected for urine. The lack of statistically significant correlation between the urinary mercury values and the number or the area of amalgam surfaces in this study may be explained by the small number of participants. In a small material of both sexes the difference in creatinine excretion between the sexes increases the

variation in the urinary/mercury ratio and thus makes it more difficult to discover a possible correlation to amalgam fillings.

It should be noted that the coefficient of correlation between plasma and erythrocyte mercury concentrations and the number of amalgam surfaces or amalgam surface area was about the same. This indicates that the less time-consuming method of using only the number of amalgam surfaces may be sufficient for purposes similar to those of the present study.

As in our previous studies (8, 22), the other plasma selenium and Ery-GSHPx values fell within the reference intervals. Although the possibility cannot be excluded that the small but statistically significantly lower plasma selenium values 5 and 10 days after the amalgam removal was a result of the relatively extensive mercury release due to this removal, the operation did not seem to affect the plasma selenium or the Ery-GSHPx values during the remaining time studied. The only P-selenium value outside the reference interval in one of the patients was not accompanied by a low Ery-GSHPx value and can probably be explained by the fact that this person underwent an extensive bone operation during the period and remained immobilized for a rather long time. This condition generally affects the electrolyte balance and all the plasma values.

Selenium is excreted via the lungs, kidneys, and liver (58). Increased levels of urinary selenium have been reported in workers exposed to mercury vapor (37). Our results, showing no differences in urinary selenium either within the experimental group or when comparisons were made with the control group, before or after the amalgam removal, are in agreement with those of Barregård et al. (38) in industrial workers.

The kidney is one of the organs especially susceptible to inorganic mercury toxicity (59). Occasional increased levels of urinary proteins and/or urinary albumins in persons with increased levels of urinary mercury (60) and mercury in blood have been reported (61). Our study showed statistically significantly higher urinary albumin level 12 months after amalgam removal in the experimental group as compared with the pre-removal values. The possibility cannot be excluded that this increased urinary albumin level is a result of the amalgam removal. However, this needs further confirmation before definite conclusions can be drawn. Also, all values fell well within the reference intervals, and thus the mercury exposure was too low to influence either glomerular or tubular function significantly. None of the other supplementary analyses revealed any influence from mercury of the amalgam fillings, either the short-term release due to the extensive amalgam removal in the experimental group or the low long-term release in the control group.

To conclude, the results of the present study show that mercury from amalgam fillings does contribute significantly to the mercury concentrations in plasma and urine. However, the analyses have not shown any biologic effects that can with certainty be associated with release of mercury from amalgam fillings.

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