Comprehensive medical examination of a group of patients with alleged adverse effects from dental amalgams

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Mercury from dental amalgams does not seem to cause dose-related intoxications. However, animal studies have shown that high-dose exposure to mercury may support various types of immunologic reactions. Ten patients claiming that their symptoms were caused and aggravated by amalgam therapy were selected for a study of the effects of removal of one amalgam restoration followed by placing of a composite filling. Clinical symptoms and the result of laboratory tests were recorded. Six patients had contact allergies to metals, three of them to mercury ammonium chloride. The comparison of pre- and post-experimental test results showed significant reductions in p-IgE and dU-albumin and significant increases in p-C3d and dU-β_2-microglobulin. There was no laboratory evidence of a direct toxic effect by mercury on the patients. The observed response by some of the studied factors to the low acute exposure to amalgam may imply that an activation of the immune system occurred. □ *Allergy; immunology; operative dentistry; restorative materials; toxicology*

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The number of patients who claimed that they have various chronic symptoms, as a result of exposure to mercury released from dental amalgam, has increased during the past decades. Long lists of alleged side effects have been published (1–3), but a relationship between symptoms and mercury levels in blood or urine has not been verified. Low values of serum and urine mercury were found in Scandinavian dental personnel (4, 5). Although the values were higher than the population mean of 3 μg/l (16 nmol) in serum and 4 μg/l (21 nmol) in urine (6), they were well below the occupational safety limits of 30 μg/l and 50 μg/l, respectively, set by the Swedish National Occupational Board of Safety and Health (7).

Contact allergic reactions to dental amalgam are found in a minority of the population (8–10), but there are several reports in animal and in vitro studies on the effects of mercury on the immune system. Thus, mercury chloride may cause non-specific immunosuppression (11, 12) and proliferation of T and B cells (13), which may induce formation of polyclonal activation of the B lymphocytes, requiring T cells (14–16). Mercury salts may also induce antinuclear antibodies (14–19), anti-glomerular basement membrane antibodies, and anti-DNA antibodies (16, 20–22), increases of serum IgE (15, 16, 23) and immune complexes (16, 20–22), and activation of the coagulation system (24). As an expression of immunoactivation, antibody-mediated renal injury, such as autoimmune glomerulonephritis, may occur (15, 17, 19, 20, 22, 25).

Apart from causing deposition of immunoglobulins and immune complexes, mercury may interfere with enzyme systems in the kidney (26, 27). This has been observed in the straight regions of the proximal tubules (22, 28–30). Furthermore, interaction with membrane phospholipids (30) and accumulation of unesterfied fatty acids and lysophospholipids in epithelial kidney cells have

been reported (31). The occurrence of proteinuria and nephrotic syndrome has been related to involvement of the glomerular basement membrane (11, 20, 22).

These findings are based on experiments in which high doses of mercury salts were used. Caution has to be exercised in extrapolating these effects to humans exposed to low doses of mercury continuously released from dental amalgam restorations (32, 33). A temporarily increased exposure occurs during removal and replacement of amalgam restorations.

The aim of this study was to evaluate the principles for claimed side effects by mercury in amalgam induced by removal of one dental amalgam restoration. By laboratory analysis the toxic effect was evaluated in comparison with variables related to immunologic and allergic reactions.

Materials and methods

Patients

Ten individuals, seven women and three men, were selected consecutively from those referred to the University Clinic of Odontology in Umeå for alleged side effects of amalgam restorations. Sex and age distribution are given in Table 1. The participation was voluntary, and the patients were informed about the study before the investigations. Initially, all patients underwent general and oral investigations, performed by the authors, to exclude underlying disease related to their present symptoms. No malignant or inflammatory disease could be diagnosed by the use of regular indicators for such diseases. They all had a long history of unexplained symptoms (cf. Table 4) and now complained of increasing problems related to removal of amalgam restorations.

Table 1. Code, sex, and age in years of each patient

Patient code*	Sex	Age, years
A	Male	34
B	Male	36
C	Female	38
D	Female	38
E	Female	38
F	Male	43
G	Female	48
H	Female	54
I	Female	55
J	Female	59

* Referred to in the text.

Experimental design

No amalgam restorations were made or polished for 1 month before the experiment. After the initial clinical examination, samples of blood, urine, and saliva were collected from all patients. Each patient also recorded food intake for 4 consecutive days. Then, a three-surface amalgam restoration in a first molar was removed, using a high-speed handpiece and a cylindrical diamond bur. Water cooling was used, and a high-efficiency saliva ejector collected water, amalgam dust, and vapor. After removal of the restoration, the tooth was isolated with rubber dam, and a composite restoration (Occlusin®, ICI, Cheshire, U.K.) was made. Forty-eight to 72 h after the change of restoration, a second series of blood and urine samples was collected and analyzed.

Saliva analyses

Samples of unstimulated whole saliva and of whole saliva stimulated by chewing on a lump of paraffin were collected. The secretion rates were calculated, and the concentrations of total protein (34), sialic acid (35), and fucose (36) were determined. The activities of lysozyme (37) and a bacteria-agglutinating glycoprotein (38) were analyzed in unstimulated saliva. In stimulated whole saliva the buffer capacity (39), pH, concentrations of electrolytes (AAS, Varian Techtron AA6, Varian Associates, Instruments Group, Palo Alto, Calif., USA) and hexosamines (40), and the activity of amylase (Phadebas®, Pharmacia, Uppsala, Sweden) were determined.

Exclusion of malignant and inflammatory diseases was based on clinical investigation and laboratory variables.

Blood analyses

Whole blood was analyzed for erythrocyte sedimentation rate (SR), hemoglobin, leukocytes (WBC), differential WBC, and platelets (PTC). Plasma was analyzed for albumin, α_1-antitrypsin, orosomucoid, haptoglobin, C-reactive protein (CRP), IgG, IgA, and IgM.

Antinuclear antibodies (ANA) and antibodies against smooth-muscle fibers and mitochondria were investigated with an immunofluorescence technique by the use of stained antiserum (Dakopatts AB, Stockholm, Sweden). Antibodies against thyroglobulin or microsomal thyroid antigen were analyzed by means of passive hemagglutination (Kronans droghandel, Stockholm, Sweden). The presence of IgE antibodies against milk (Phadebas RAST, Pharmacia Diagnostics, Uppsala, Sweden) and a panel of fungi and domestic animals (Panel RAST, Pharmacia Diagnostics) and IgA antibodies against gluten (Pharmacia Diagnostics) was analyzed. Furthermore, serum values of creatinine, total bilirubin, alkaline phosphatase (ALP) gamma-glutamyl transferase (GT), amylase, creatinine phosphokinase (CK), aspartate aminotransferase (ASAT), alanine aminotransferase (ALAT), lactate dehydrogenase (LD), iron, transferrin, ferritin, vitamin B_{12}, and folic acid were analyzed in serum in accordance with the University Hospital routines. Urine was cultured to exclude urinary tract infections.

Before the removal of the amalgam filling the following tests showed values above reference levels in occasional patients: sedimentation rate, Patient H; WBC, Patient G; platelet concentration, Patient G; ALAT, Patients A and F; CK, Patient F; α_1-antitrypsin, Patient I; orosomucoid, Patient I; haptoglobin, Patient I; and CRP, Patients G and J.

To evaluate possible alterations of the immunologic system or predisposition for allergy, analysis were performed for the percentages of T_4 and T_8 lymphocytes, determined by flow cytometry (Facscan® cytometer, Becton Dickinson AB, Stockholm, Sweden), using the antibodies OKT 4® and OKT 8® (Ortho Diagnostic Systems, Sollentuna, Sweden). Analyses were also performed for IgE, C3, C4, and C3d (41, 42), fibrinogen degradation products (FDP), the presence of antibodies against glomerular basement membrane (anti-GBM) subgroups IgG (Goodpastures Antigen), IgG (none selective), IgM and IgA (BioCarb Diagnostics, Lund, Sweden), and anti-neutrophil cytoplasmic antibodies against cytoplasmic myeloperoxidase (BioCarb Diagnostics, Lund, Sweden) (43).

Immune complexes (IC) in serum were analyzed indirectly as C1q reactivity, and directly by C3 bound to IC (National Bacteriological Laboratory, Stockholm, Sweden).

Urine analyses

Urine was collected and pooled for 24-h periods, 1 day before and on the 2nd or 3rd day after amalgam removal. The concentration of mercury was analyzed by atomic absorption (44, 45). Renal function was assessed by measuring the glomerular filtration rate (creatinine clearance in ml/min per 1.73 m^2), the ability to concentrate urine within the collecting ducts of the nephrons (subcutaneous injection of 4 µg of desmopressin; Ferring, Malmö, Sweden), and by determining glomerular albuminuria by rocket immune electrophoresis of diurnal urine (detection limit > 5 mg/l). The tubular function was further assessed by β_2-microglobulin in serum and urine (Pharmacia Diagnostics).

Epicutaneous patch testing

Nine patients agreed to have a standard epicutaneous patch test against 35 substances used in dental practice (Table 2). The test substances were applied to normal skin on the patient's back for 48 h. Twenty-four hours after removal of the patches, the reactions were read by an experienced dermatologist.

Histologic examination

Biopsy specimens were taken from visible oral mucosal lesions. The specimens were

fixed in 10% buffered neutral formalin, paraffin-embedded, sectioned, and stained with Mayer's hematoxylin–eosin and Wiegert's hematoxylin–van Gieson stains.

Statistical analyses

Pre- and post-experimental data were analyzed individually, using Wilcoxon's paired rank test (46). Two-tailed p values < 0.05 were considered statistically significant.

Results

Preexperimental findings

Patient E used 7.5 mg prednisolone daily for a suspected, but not classified, vasculitis. Patient G had a slight IgA glomerulonephritis, verified by previous renal biopsy. Two men had slightly increased levels of ALAT (A, F), and in one of them (A) a liver biopsy showed changes consistent with moderate fatty liver. Patient I was treated for asymptomatic bacteriuria 2 weeks before the experiment. Six patients had been completely unable to work for the past year, and another patient had been partly off work (1 month during the past year), owing to symptoms that they related to mercury in their amalgam restorations. According to their own statements, none of the patients abused alcohol or drugs.

The patients had an average of 31 ± 8

Table 2. Materials included in the dental screening test. Substances were obtained from Chemotechnique Diagnostic AB (Malmö, Sweden) and Trolab (Hellerup, Denmark)

1.	Dibutyl phthalate	5%
2.	Dimethyl phthalate	5%
3.	*N*,*N*-dimethyl-4-toluidine	2%
4.	BIS-MA	2%
5.	Triethylene glycol dimethacrylate (TREGDMA)	2%
6.	Urethane dimethacrylate (UEDMA)	2%
7.	Ethylene glycol dimethacrylate (EGDMA)	2%
8.	BIS-GMA	2%
9.	2-Hydroxy-4-methoxy-benzophenone	2%
10.	Methyl dichlorobenzene sulfonate	0.1%
11.	*N*-ethyl-4-toluenesulfonamide	0.1%
12.	4-Tolyldiethanolamine	2%
13.	Eugenol	1%
14.	Balsam of Peru	25%
15.	Colophony	20%
16.	Butyl methacrylate	2%
17.	Cyanoacrylate	2%
18.	Ethyl acrylate	0.1%
19.	2-Ethyl hexylacrylate	0.1%
20.	Methyl methacrylate	2%
21.	Hydroquinone	1%
22.	Benzoic acid	5%
23.	BHT	2%
24.	Formaldehyde	1%
25.	Benzoyl peroxide	1%
26.	Potassium dichromate	0.5%
27.	Cobalt chloride	0.5%
28.	Mercury ammonium chloride	1%
29.	Nickel sulphate	5%
30.	Copper sulphate	1%
31.	Potassium dicyanoaurate	0.002%
32.	Silver nitrate	0.1%
33.	Potassium thiosulphatoaurate	0.5%
34.	Ammonium tetrachloroplatinate	0.25%
35.	Palladium chloride	1%

Table 3. Various immunologic variables and indicators for coagulation process (FDP) before (top line) and 2–3 days after (lower line) amalgam removal in 10 patients (A–J). T-lymphocyte subtype and percentage of T_4 versus T_8 ratios (T_4/T_8) are given

Patient	T_4/T_8	ANA	S-FDP, mg/l	Anti-GBM units	C3-IC	C1q binding ability	Epicutaneous patch test
A	47/23	Neg.	8–16	Neg.	Neg.	+	Neg.
	44/27	Neg.	32–64		Neg.	Neg.	
B	36/46	Neg.	<8	Neg.	Neg.	+	Neg.
	45/40	Neg.	<8	Neg.	+	Neg.	
C	*	Neg.	8–16	Neg.	Neg.	+	
	50/28	Neg.	<8	Neg.	+	+	†
D	46/45	Neg.	64–128	Neg.	Neg.	Neg.	$HgNH_2Cl$
	43/45	Neg.	16–32	Neg.	Neg.	Neg.	
E	35/20	Neg.	<8	6.6	Neg.	Neg.	$CoCl_2$
	54/34	Neg.	<8	3.7	Neg.	Neg.	$NiSO_4$
F	43/21	Neg.	<8	6.7	Neg.	Neg.	$HgNH_2Cl$
	40/20	Neg.	<8	3.7	Neg.	++	
G	31/18	Neg.	<8	+	Neg.	Neg.	$NiSO_4$, $CoCl_2$
	56/39	Neg.	<8	4.0	+	+	$AuK_2S_2O_3$
H	54/31	+	<8	Neg.	Neg.	Neg.	Neg.
	51/25	+	<8	Neg.	Neg.	Neg.	
I	32/35	+	<8	Neg.	Neg.	Neg.	$HgNH_2Cl$
	40/20	Neg.	<8	3.9	Neg.	+	$NiSO_4$
J	55/28	Neg.	<8	Neg.	Neg.	Neg.	Neg.
	46/20	Neg.	<8	Neg.‡	+	Neg.	

+, ++ = Pathologically less (+) or more (++) increased values or titers; Neg. = value or titer within normal range.

* Data missing.

† Refused epicutaneous test. Anamnestic reaction against nickel on contact.

‡ Elevated level of IgG antibodies against myeloperoxidase (9.4; ref. value < 8.0).

(mean ± SD) surfaces filled with dental amalgam. Other restorations were made of gold, composite, or porcelain.

For immunologic tests and T_4/T_8 ratio, see Table 3. Nine of the patients had symptoms (Table 4) and test values that could not be expressed in the diagnosis of a disease.

The ability to concentrate urine was below the reference value and below 793 mosm/kg in 8 of 10 patients (not Patients B and D). Some of the patients had an elevated value for dU-β_2-microglobulin (Patient F) or dU-albumin (Patients C, E, G, H, I, and J) and/or a creatinine clearance below the reference value and below 84 ml/min · 1.73 m^2 (Patients B, I, and J). In three of the patients increased levels of anti-GBM antibodies of subtype IgM were found (Table 3). Three individuals (A, C, and D) had elevated levels of plasma FDP, and two (H, I) had positive ANA (Table 3) titers.

Large interindividual variations were found in saliva secretion rate and in all variables analyzed. No obvious pattern of deviation from reference values could be found. A low secretion rate for stimulated saliva was seen only in Patient A.

The results of the epicutaneous patch tests are shown in Table 3. Five patients showed reaction to one or more of the metal salts, and one patient claimed to have nickel allergy.

Histologic examination of four biopsy specimens from Patients D, F, and I showed a picture consistent with oral lichen planus in Patients D and F. In the third patient (I), two specimens from the buccal mucosa and the lateral border of the tongue showed benign hyperparakeratosis together with chronic inflammatory cell infiltrate with an abundant occurrence of eosinophilic granulocytes (Fig. 1).

All 10 individuals had a regular meal pattern and ate an ordinary Swedish mixed diet

Table 4. Patients with preexperimental symptoms and *additional* or *increased* symptoms in connection with the removal of one amalgam restoration. Patients referred to by the code presented in Table 1

	Preexperimental symptoms	Postexperimental symptoms
Headache	D, I	D, I
Edema	I	
Muscular weakness	A, C, F, H, I	F, I
Muscular tenderness	F, J	F, I
Paresthesia		D, I
Cardiac sensation	A, E, J	C, I
Joint pain	C, D, E, F, G, I, J	D, F, G
Blurred vision	A, C, J	
Gastrointestinal discomfort	A, F	
Polyuria	B	A, I
Dysgeusia	A, J	
Tinnitus	H	I
Dizziness	A, H	C, E, I
Oral lichenoid reaction	D, F, I	
Fatigue	C, F	D, F, I
Eye pain		D
Depression		D
Dermatologic disease (eczema, erythema, ulcers)	C, G	F
Oral enanthema		D
Abdominal pain	H	H
Diarrhea		H
Tremor		H
Malaise		A, H
Mucous membrane dysfunction	C, G	
Burning mouth	A	
Dyspnea		I

including milk and meat products. Fish was not consumed more than once a week, and patient A never ate fish. Five of the patients took supplementary multivitamin tablets and/or minerals.

Experimental findings

After the experimental removal of an amalgam restoration, eight patients complained of increased and/or additional side effects (Table 4). In Patient G an aseptic arthritis of the right ankle developed. In Patient I the amalgam removal was followed by progressive hypoxemia, tachypnea, and expiratory stridor, as seen in asthmatic patients. This patient also had an extensive infiltration of eosinophilic granulocytes in the oral mucosa. Treating this as an allergic reaction by the administration of steroids clearly improved her condition. Patient D, also allergic to mercury, showed enanthema of the palatinal mucosa after the experimental procedure.

The only significant changes in the biochemical and serologic variables which occurred postexperimentally after amalgam removal were a reduction in p-IgE ($p = 0.026$) and diurnal urinary albumin excretion ($p = 0.006$) and an increase in p-C3d ($p = 0.010$) and diurnal urinary β_2-microglobulin ($p = 0.019$) (Fig. 2).

There was no difference in the 24-h volume of urine before and after the amalgam removal. No significant difference in mercury excretion was found between the samples collected before and after the experiment. Diurnal urinary mercury was less than 4.6 μg both pre- and post-experimentally.

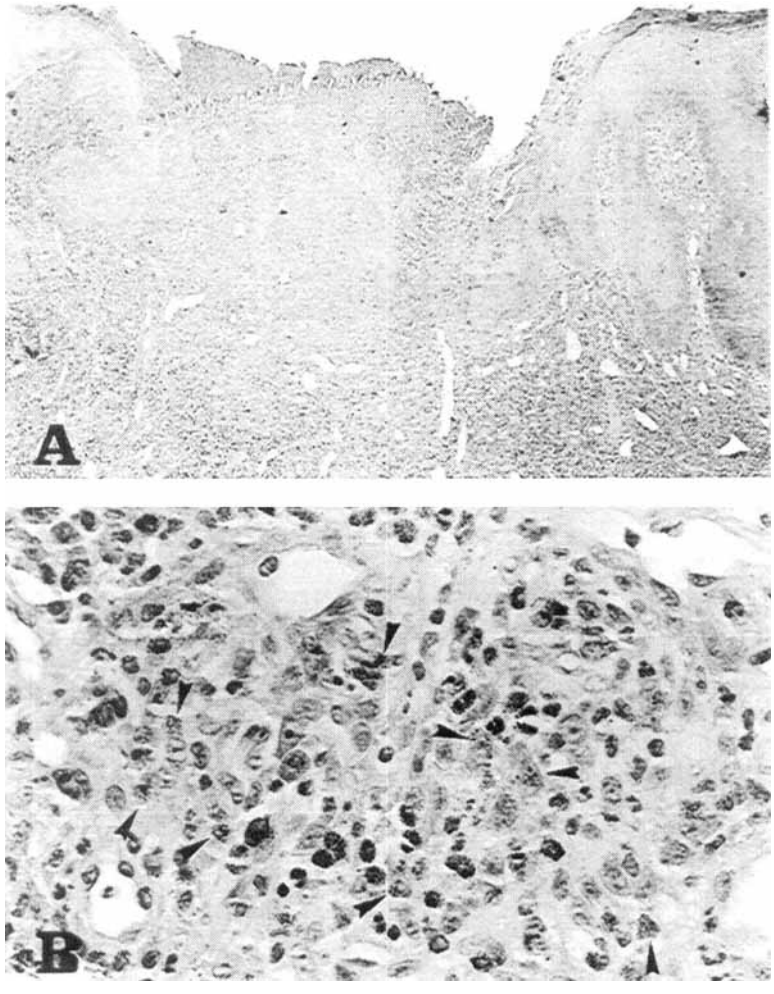
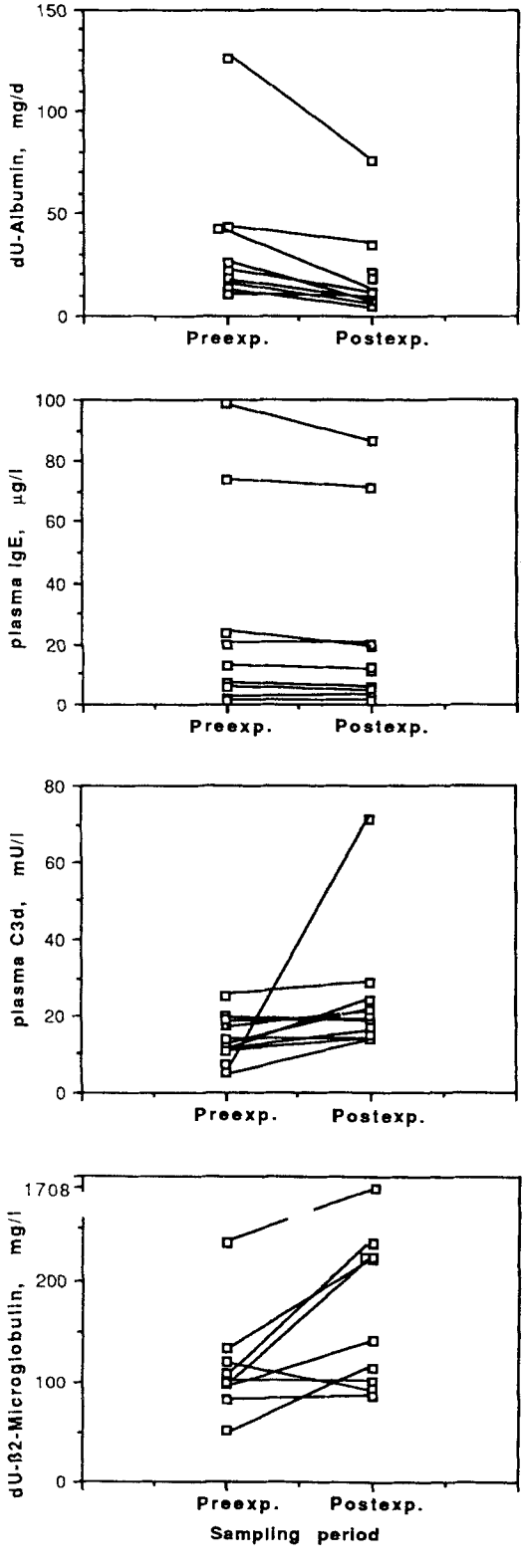


Fig. 1. Haemotoxylin–eosin-stained section from the oral mucosa (lateral tongue border) of a woman (patient I) with mercury allergy. 1A. An ulceration covered by a fibrin layer. The epithelium adjacent to the ulceration shows hyperparakeratinization and acanthosis. In the underlying vascular connective tissue a moderate chronic inflammatory reaction is seen. 1B. A higher magnification of the connective tissue. Note the abundant occurrence of eosinophilic granulocytes (arrowheads).

Discussion

Owing to difficulties in defining a proper control group it was decided that the patients should be compared pre- and post-experimentally. The patients differed considerably from the general population in that they were great consumers of medical care. All had visited several physicians, and many of them had long periods of absence from work owing to their symptoms, which could not be expressed in a common or evident diagnosis. They did not differ from the general population with regard to, for example, salivary gland function, urinary mercury excretion, dietary habits, the use of drugs, or most regular blood analyses. The patients complained of increasing adverse effects after amalgam removal, which raised the question of whether mercury might have contributed to their symptoms. The exposure to amalgam dust during removal of the restoration may have varied. The patients were also exposed to normal clinical routines during dental treatment, including local anesthesia, water spray under high pressure, and freshly prepared composite fillings. Eight patients also anticipated adverse reactions after the removal of the amalgam restoration. The



adverse effects occurring in the course of the experimental procedure in four of the patients were of such severity that they required acute medical attention.

From animal studies it is known that the administration of mercury chloride can cause immunologic activation, such as antinuclear antibodies, antibodies against glomerular basement membranes, immune complexes, and activation of the coagulation system (14–24). Eight of the patients in the present study had preexperimentally impaired values in one or more of such immune variables, indicating an altered immunologic activity.

Side effects to various heavy metals (22, 27–29, 48–51) can often be detected in the kidneys. There was evidence preexperimentally of interstitial nephritic changes in 8 of 10 patients in this study, as indicated by the decrease in kidney ability to concentrate urine on desmopressin stimulation. Although there was no further decrease in urinary concentration ability 2–3 days after the experimental procedure, further evidence of impairment of the renal tubular system was the significant increase in the urinary output of β_2-microglobulin.

An increase in the permeability of the basement membrane of the vessels within the glomeruli may be caused by local inflammation or deposits of various materials, including immune complexes. Such a reaction may result in albuminuria (22, 49, 50, 52) and has been reported to be more prevalent in dentists and dental assistants, although not correlated with their U-Hg (47). Similar findings were also made in this study, and microalbuminuria was present in six of the patients preexperimentally. Notable was the improvement of urinary albumin after the removal of one large amalgam restoration, while there was no significant increase of mercury in urine. The microalbuminuria in most of our patients was possibly the expression of an inflammatory process involving the basement membrane of the glomeruli and may also reflect an

Fig. 2. Comparison of preexperimental and postexperimental values of the four variables that were changed significantly.

involvement of the permeability of vessels other than glomerular—for example, in patients with diabetic nephropathy combined with generalized angiopathy (52).

Animal studies indicate that T cells are necessary for mercury-induced autoimmunity (16). In a previous study (53) an increase in T lymphocytes was noted after removal of amalgam restoration in three patients. An increase in T lymphocytes was not found in the present study after removal of one amalgam filling. However, three of the patients had a subnormal T4/T8 lymphocyte ratio, similar to what is found in immunosuppressed patients.

The high prevalence of positive reactions to metal salts in the patch test indicates that there might exist a predisposition for allergic reactions in at least five of the patients. However, it must be remembered that patch tests only reflect contact allergies. Thirty per cent of the patients in the present study reacted to mercury ammonium chloride. This exceeds the present overall prevalence, estimated to be below 10% (9, 54). The prevalence of allergic reactions to mercury is higher (16–62%) in individuals with mucosal lichenoid reactions (9, 54–56). In the present study, all three patients with skin reactions to mercury ammonium chloride showed localized lichenoid alterations of the oral mucosa. One of them also reacted with enanthema after exposure to amalgam powder during the experimental procedure.

After removal of the amalgam restoration, eight of the patients complained of an increased number of side effects. The onset of the symptoms varied from a few hours to several days. The duration varied between 3 days and 6 months. These reactions resembled findings noted in patients who have been sensitized. The presence of a hypersensitivity reaction to mercury is possible, as has been suggested by others (57–59). This may include neurologic disturbances, since inorganic mercury may interfere with the nerve conduction velocity (60) of myelin (61). A possible mechanism may be a mercury-induced alteration of the lipid part of plasma membranes of cell systems (30, 31, 49).

The reduction in serum IgE in this study may be explained by a decrease in exposure to a possible antigen (mercury), or it may be an expression of a non-compensated consumption of IgE occurring during the post-experimental period. The latter hypothesis is supported by the parallel increased activity of the complement system. The possible impact of an activated or overreacting immune system is demonstrated by the fact that administration of immunosuppressive drugs reduced or normalized immunologic manifestations caused by mercury exposure in rats (62). This hypothesis is supported by the improvement of side effects in our Patient I by short-term administration of steroids after the removal of one amalgam restoration.

In conclusion, the data indicate that the patients already preexperimentally displayed abnormalities. Some variables were affected by the experiment. Two of the variables with significant changes were improved (p-IgE and dU-albumin). These improvements should be referred to as decreased exposure to dental amalgam but hardly an added exposure (composite resin). It cannot be excluded that the changes of the two remaining variables (dU-β_2-microglobulin and C3d) are associated with the experimental procedure per se. It is notable that an increase in urinary β_2-microglobulin is used as a marker for impairment of tubular reabsorption (63), which is known to occur in heavy metal exposure (64, 65). Except for the change in urinary β_2-microglobulin there was no other laboratory evidence of a direct toxic side effect by mercury exposure. Instead, there are indications of allergic and/or immunologic reactions.

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