Review

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Amalgam Affects the Immune System

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Multiple sclerosis
Chronic fatigue syndrome
Multiple chemical sensitivities
Mercury

Abstract
Although in use for more than 150 years, dental amalgam has been questioned more or less vigorously as a dental restoration material due to its alleged health hazard. Humans are exposed to mercury and the other main dental amalgam metals (Ag, Sn, Cu, Zn) via vapour, corrosion products in swallowed saliva, and direct absorption into the blood from the oral cavity. Dental amalgam fillings are the most important source of mercury exposure in the general population. Local, and in some instances, systemic hypersensitivity reactions to dental amalgam metals, especially mercury, occur at a low frequency among amalgam bearers. Experimental and clinical data strongly indicate that these and other subclinical systemic adverse immunological reactions to dental amalgam metals in humans will be linked to certain MHC genotypes, and affect only a small number of the exposed individuals. These individuals will be very difficult to detect in a mixed population of susceptible and resistant individuals, including persons with alleged symptoms due to dental amalgam fillings, where many of the individuals are likely to suffer from conditions with no proven immunological background such as multiple chemical sensitivity syndrome. Intensified studies should be performed to identify such susceptible MHC genotypes, taking advantage of the reported cases of more heavily metal-exposed humans with systemic autoimmune reactions. Further studies will also be needed to ascertain whether the combined exposure to the metals in dental amalgam may lower the threshold for adverse immunological reactions, since recent studies have shown that the metals in alloy, especially silver, may induce autoimmunity in genetically susceptible mice.

A Short History of Dental Amalgam

Different kinds of dental amalgams have been used since the middle of the seventh century, as described in the Chinese medical literature [1]. Originally, they consisted of 100 parts mercury, 45 parts silver, and 900 parts tin. When first introduced in the West, more than 150 years ago, they comprised a mixture of silver grains and mercury, but only in the mid-1890s did dental amalgam become what is today known as 'conventional amalgam', when components such as copper, tin, and small amounts of zinc were added [2]. Thus, amalgam has been extensively used as a restorative material for many years, and it is estimated that about 100 million amalgam fillings are inserted annually in the United States [3].

It is not surprising that the common implantation in the human body of a material containing the well-known toxic element mercury has generated much debate over the years [4]. The first 'Amalgam War' took place in the United States in 1830–1850 and was due to serious side-effects of the primitive type of silver amalgam. This led to a statement of its toxicity by the American Association of Dental Surgeons in 1843. The second 'Amalgam War' began in 1928, and was caused by an article published by the German chemist Alfred Stock. Stock claimed that this own symptoms of bad memory, fatigue, and malaise were due to mercury intoxication [5], and a special clinic was opened at the Charité Hospital in Berlin to study the safety of amalgam. The third 'Amalgam War' started in the 1980s with reports of cardiovascular, psychiatric, and neurologic discorders linked to mercury release from dental amalgam [4]. This third war is still going on, waxing and waning with time and among countries. The debate has been particularly intense in Sweden, and dental amalgam is advised by the National Board of Health and Welfare not to be used in children and, starting in 1997, will not be advised for use in adults either. This ceasing of using amalgam has officially been declared on the grounds of environment safety, but the anti-amalgamist movement, claiming a multitude of adverse health effects due to mercury, has clearly played an important role in this decision, despite recommendations from the majority of the scientific community [6]. This is an example of one of the many unique effects of mercury, namely its ability to polarize opinions [7].

All these 'wars' have centred upon effects of the mercury component in dental fillings. The World Health Organization (WHO) committee on inorganic mercury in 1991 [8] recommended that further research should be performed, and directed at two main areas: neurobehavioural effects in occupationally exposed persons and in the fetus, and the importance of mercury in adverse immunological effects.

The Composition of Dental Amalgam

Dental amalgam is a multiphasic compound material composed primarily of three phases, γ (Ag_3Sn) γ_1 (Ag_2Hg_3) and γ_2 (Sn_8Hg), and is obtained by reacting a finely powdered alloy with metallic mercury [9, 10]. Freshly triturated conventional dental amalgam is composed of approximately 50% mercury, 35% silver, 13% tin, 1.4% copper, and 1% zinc. Amalgams can also contain traces of cadmium, palladium, platinum and indium. Copper-rich amalgams include an η phase (Cu_6Sn_5): e.g. Sybraloy with 28 wt% copper in the alloy [9]. Indium-containing non-γ_2 amalgam has an alloy copper concentration of 13% [11].

The grinding surface of a molar tooth filled with amalgam contains between 750 and 1,000 mg Hg and has an average serviceable life span in the human mouth of 7–9 years [12].

Release of Dental Amalgam Components

Mercury (fig. 1, 2)

It has been clearly shown that metallic mercury is continuously released from dental amalgams due to *corrosion*, especially of the γ_2 phase [9, 13, 14]. Saliva and dentinal fluid are the main liquids in the mouth which are in direct contact with dental fillings [15]. The moist environment of the mouth predisposes to corrosion. The corrosion rate of amalgam depends on many factors, such as the galvanic currents for transportation of corrosion products, mechanical factors and microbiological activity in the mouth, as well as the composition and pH of the saliva [15]. Proteins in the saliva may increase the release rate of various ions [16]. It has been postulated that the higher corrosion potential of high-copper amalgams would result in a higher rate of dissolution of mercury from these than from silver amalgams [17]. Mackert [18] has assessed the daily release of mercury from conventional amalgam as 1.2–1.8 µg for an individual with an average of 8–12 occlusal amalgam restorations.

Uptake of mercury of different oxidation states from amalgam fillings can occur via three routes: (1) inhalation of mercury vapour; (2) gastrointestinal absorption of mercury via saliva, and (3) direct absorption into the blood from oral mucous membrane, teeth roots, and surrounding bone.

Mercury Vapour

Dental amalgam fillings release mercury vapour into the mouth and it is almost completely (≈80%) absorbed from the lung alveoli [19, 20] and readily crosses cell membranes.

Mercury vapour release has been assessed by different authors as 0.1–0.6 µg/day/cm^2 from old restoration surfaces and about 4.5 µg/day/cm^2 from newly placed amalgam surfaces [21]. The mercury vapour concentration correlates statistically with the number of amalgam restorations [22–24]. It has also been shown that chewing gum for 5 min can cause the mercury vapour concentration to exceed the threshold limit value (TLV; the maximum amount of mercury vapour to which a worker may be exposed during an 8-hour day, over a 40-hour work week) of 50 µg/m^3 [23]. In a study by Utt [25], the mercury vapour level in the mouth increased to 50–150 µg/m^3 in 50 individuals after chewing gum. The insertion or removal of amalgam restorations, as well as condensation and carving of amalgam, results in an even higher mercury vapour content [26, 27]. The increase of mercury vapour after these conditions is, however, transient. But an individual with more than 6 amalgam restorations who is a heavy gum chewer may experience mercury concentration exceeding the TLV over hours [23]. A more

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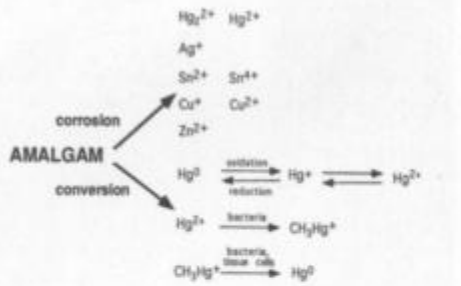

Fig. 1. Release of dental amalgam components by corrosion and the ways of biotransformation of various mercury forms [according to ref. 37, 256].

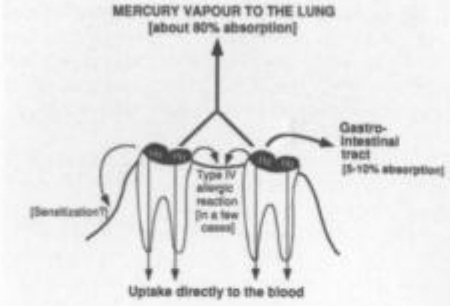

Fig. 2. Routes of uptake of dental amalgam components, mercury in particular. Note the low risk of sensitization via the oral mucous membrane and the possibility for contact allergic reaction in sensitized individuals.

severe loading of restorations occurs in the case of bruxism, but we have found no information regarding the amount of mercury released in this condition.

Data from four laboratories, reported by Clarkson et al. [28], have shown that the daily uptake of mercury vapour from dental amalgam fillings is between 2.9 and 17.5 µg. The disparity is suggested to reflect differences in measurement techniques and assumptions used in making the estimations. Clarkson [29] considered 8.0 µg to be a reasonable estimation.

Metallic mercury is rapidly oxidized by catalase and hydrogen peroxide to ionic mercury ($Hg^0 \rightarrow Hg_2^{2+} \rightarrow Hg^{2+}$) in red blood cells and tissues. This reaction can vary due to genetic and environmental factors [30]. The reaction requires several minutes, which means that elemental mercury exists in the blood long enough to reach the brain and placenta. After exposure to mercury vapour, the maximum concentration in red blood cells is found after about 1 h and in the plasma after about 10 h [19, 31, 32]. The plasma-to-cell ratio will initially be about 1:1, but gradually decreases due to uptake of mercury into tissues and excretion.

Mercury Release to Saliva

This release is proportional to the amalgam surface area [27]. Dissolved mercury vapour, oxidized corrosion products and amalgam microparticles are found in the saliva, especially after brushing the teeth. However, the intestinal absorption of inorganic mercury is not high and is estimated by the WHO [33] to be 7%, and by Elinder et al. [34] to be 5–10%. More recent toxicokinetic studies suggest an estimated 'true absorption' of about 20%, when the excretion of recently absorbed mercury is taken into consideration [35].

Uptake Directly to the Blood

Uptake can take place from the base of the tooth cavity, from surrounding bone, through the buccal mucous membrane, and from gingival and buccal mucosa connective tissue, as well as from corroded subgingival restorations [27, 36].

Biotransformation of Mercury

Many studies have demonstrated the formation of methyl mercury from conventional and admixed amalgam by both enzymatic and non-enzymatic reactions [37]. Methyl mercury can thus be produced in the mouth by the action of bacteria, as has been demonstrated in vitro [38]. Methyl mercury is readily and almost completely absorbed in the gastrointestinal tract. The biotransformation of inorganic mercury to methylmercury by bacteria in the gut will thus enhance the absorption of swallowed inorganic mercury. Inorganic mercury can be released from methyl mercury, and this biotransformation has been suggested to contribute to the toxicity of methyl mercury [39].

Are Blood and Urine Valid and Reliable Index Media for Estimation of Exposure?

Even a few days after exposure, the blood concentration will generally not be reliable for estimating the amount of inorganic mercury incorporated in the body [40]. Although Langworth et al. [41] and Schweinsberg [42] found a good correlation between the creatinine-adjusted urinary concentration of mercury and the number of dental amalgam surfaces, the mercury content in urine is considered to yield little information about the body burden from chronic exposure to mercury [40]. In the study by Schweinsberg [42], a considerable number of subjects with more than 25 amalgam fillings had an uncorrected urinary mercury concentration below 1 µg/l.

Silver

Dental amalgam alloy contains about 70 wt% silver which may be released in small amounts [43]. Absorption by inhalation of silver vapour has been reported but no estimates have been made [44]. The daily intake of silver from dietary sources, about 60–80 µg [45, 46], is suggested to be much higher than the amount released from dental amalgam [47]. The intestinal absorption of silver in saliva and food is less than 10% [48], and about 90% of the absorbed silver is faecally excreted, mainly by biliary excretion [44]. The regression coefficient between the silver content in faeces and the number of amalgam restorations has been calculated as 0.74 [49]. These authors also found a high correlation between the amount of mercury and silver excreted in the faeces. Human and animal experimental data indicate that 1–5% of all the silver absorbed may be retained in the body [50]. The concentration of silver in the blood is not a reliable index of exposure since silver is rapidly cleared from the blood via biliary excretion [50].

Tin

Commercial alloy contains about 27 wt% tin. The rate of release of tin from amalgam restorations in humans is not known but will certainly occur since metal alloys degrade in the electrolytic medium of the saliva [51]. The daily average intake has been estimated at 3–4 mg, with only low gastrointestinal absorption. The excretion is mainly faecal and less than 5% of the daily intake is found in the urine. There seems to be an efficient homeostatic mechanism for tin [52].

Copper

The copper content in conventional amalgam alloy is about 3 wt% and in non-γ_2 amalgam alloy up to 50 wt%. The rate of release from amalgam fillings is unknown, but implantation tests using alloys with more than 30 wt% copper have shown a high copper content in the surrounding tissues, especially in macrophages [51, 53]. Brune et al. [11] showed a high degree of copper release from copper amalgam with accumulation in the liver and kidney. Ionic copper is absorbed in the gastrointestinal tract, initially up to 30%, but the excretion of copper into the bile, bound to a non-absorbed protein complex, reduces the net absorption to 5% [52].

Zinc

This metal exists at only about 1 wt% in commercial amalgam alloy and can be presumed to leak out in small amounts into the saliva. It is also an ingredient in dental cement [$Zn_3(PO_4)_2$]. Sixty days after the implantation of alloy containing less than 1 wt% zinc, zinc was detectable in the surrounding tissues [51]. A recent study by Hanawa et al. [54] confirms that zinc is the most reactive element in amalgam and dissolves at a high rate from amalgam in aqueous medium. The gastrointestinal absorption of zinc averages about 50% but can be reduced by calcium, phosphorus, and copper [52].

Cadmium, Indium, Palladium, and Platinum

A high release of cadmium has been demonstrated from high-copper amalgam using artificial saliva [11]. Gastrointestinal administration of copper amalgam revealed cadmium in the liver and kidney, and indium was found in these organs after administration of non-γ_2 amalgam [11]. Recently, a dental amalgam called Indisperse has been introduced which contains indium in the alloy [55]. That this amalgam should release less mercury vapour during the setting reaction, as reported by Powell et al. [56], could not be confirmed in a later study [55]. High-copper amalgams may also contain palladium. No release of palladium could be detected after free corrosion in Ringer's solution but the presence of palladium was found to decrease the release of mercury and copper [57]. Platinum metal is biologically inert and reveals practically no tissue reaction or absorption [52].

Non-Dental Exposure to Mercury

Industrial Manufacture

Exposure to mercury may be high in many working places, such as the chloralkali industry, where chlorine and caustic soda are produced using brine electrolysis in mercury cells [58]. Workers in mercury mines and in plants for the production of fluorescent tubes, thermometers, and alkaline batteries are also exposed [58]. Biological monitoring of mercury in blood, urine, and hair has shown, not unexpectedly, high levels in these index media when such workers are compared to unexposed individuals [41].

Dental Practice

Many older studies demonstrated high mercury levels in dental offices, 10–20% above the hygiene standard (TLV; time-weighted average) safety limit of 50 μg/m^3 in the air [37]. The mercury concentration was also found to be increased in blood [59, 60] and urine [61]. Modern handling of mercury has considerably reduced the occupational exposure, and a more recent study demonstrated low levels of mercury in blood, plasma, and urine of dental personnel [62]. However, mercury levels were higher than in a reference group, and more so for nurses than for dentists. For this group of dental personnel, the effect of their own amalgam fillings was found to be as important as their occupational exposure. A recent study by Powell et al. [55] showed that inhalation of particulate amalgam may contribute even more to the mercury concentration in the ambient air than vapourized mercury.

Laboratories

Mercury and its compounds are an occupational hazard for laboratory technicians due to, for instance, spillage of corrosive sublimate solution in fixatives, as discussed by Stewart et al. [63].

Air, Water and Food

Mercury is ubiquitous, and intake occurs via air as well as water (a combined intake of about 0.1 μg), and food. The average daily intake via food (about 2–10 μg) stems mainly from fish and is present in the form of methylmercury. Most non-fish foods contain less than 20 μg/kg [41]. Methylmercury in food and seafood is derived from the biotransformation of elemental and ionic mercury in terrestrial and aquatic environments [34]. The proportion of methylmercury to inorganic mercury in fish varies between 4–9:1 in different geographic regions [28]. Accumulation of methylmercury in seafood from mercury-polluted water can exceed 10 mg/kg [34]. The acidification of lakes by acid rain enhances the level of methylmercury in fish [64]. High fish consumption can increase the ordinary intake of mercury 10-fold, with a corresponding increase in the blood concentration due to the almost complete gastrointestinal absorption. Only recent intake of organic mercury can, however, be monitored by determination of mercury in whole blood. A high-fish diet is associated with increased levels of mercury in hair, and makes it possible to control this intake by hair analysis [42]. The urinary mercury concentration does not reflect the methylmercury level because of the dominant faecal excretion [20].

Preservatives and Antiseptics

Many commercial γ-globulin preparations contain sodium ethylmercurithiosalicylate (Merthiolate, thiomersalate) as a bacteriostatic agent. Many other medical products such as vaccines and topical drugs for the eye and ear also contain thiomersalate, and it is also used as a preservative for contact lenses.

Daily Retention of Mercury in Non-Occupationally Exposed Individuals

The mean daily mercury retention is about 6–23 μg for non-occupationally mercury-exposed amalgam bearers (table 1).

Table 1. Estimated daily retention of mercury in non-occupationally exposed human amalgam bearers

	Respiration via mouth, μg Hg	Respiration via nose, μg Hg
Dental amalgam fillings		
Inhalation (vapour)	3.1–17	0
Saliva-gastrointestinal tract (vapour, microparticles)	~3.5	3.9–5.6
Non-dental sources (air, water, food)	~2.3 (99% methyl-Hg)	~2.3 (99% methyl-Hg)
Total retention	8.9–22.8	6.2–7.9

Excretion

Mercury is mainly excreted via faeces (F-Hg) and Skare and Engqvist [49] found a regression coefficient of 0.7 between the number of amalgam fillings and F-Hg/day. They concluded that the excretion of mercury in faeces is 20-fold higher than via urine and that the highest contribution of mercury comes from amalgams, less so from food and via the enterohepatic circulation. Mercury excreted in urine could possibly be derived mainly from the mercury absorbed in the gastrointestinal tract.

Distribution and Biological Half-Time of Amalgam Components

Distribution

Mercury

The kidney is the main depository of mercury and, on the basis of animal data, 50–90% of the body burden is found in this organ [8]. Inorganic mercury also accumulates in the thyroid, pituitary, brain, liver, pancreas, prostate, testicles, and ovaries. The distribution is not uniform between the different organs or within the same organ. Clarkson et al. [28] estimated a steady-state contribution to kidney and brain due to the release from amalgam fillings in humans. With a probable daily uptake of 2.9–17.5 μg Hg, the corresponding accumulation was calculated as 259–1,563 ng Hg/g for the kidney and 4.3–29 ng Hg/g for the brain. This conforms with the distribution of ^{203}Hg from 12 tooth dental fillings in sheep 29 days after placement [36]. The two main organs of accumulation for mercury were found to be the kidney and liver, but mercury was also found in the brain and endocrine organs. In a later study, the same group described the distribution of ^{203}Hg released from 16 dental amalgam tooth fillings in monkeys [12]. The highest mercury concentration was found in tooth alveolar bone and gingivae 28 days after placement. Lower mercury concentrations were present in the kidney and intestinal tract. This contrasted with the higher level of mercury concentration in monkey kidneys after placement of only 8 amalgam fillings, as described by Danscher et al. [65]. The difference could, however, be due to the longer exposure time in the latter study, thus favouring a higher accumulation of mercury in the kidneys. Hahn et al. [12] suggested that the mercury accumulations they described could impair kidney function and affect the intestinal and gingival bacterial floras. The effect on the intestinal flora led to antibiotic resistance [summarized in a recent paper by Summers et al., ref. 66]. Vimy et al. [67] also showed a fetal distribution of mercury from maternal dental amalgam fillings in sheep. The highest concentration in the fetus was found in the liver and pituitary gland, and post-partum in milk, thus providing a potential source of mercury to the newborn.

Silver appears to accumulate in humans with age, mainly in the kidney, spleen, and liver, but contribution from amalgam fillings release is unknown.

Copper is retained in muscle, which contains about 35% of the total body copper, and accumulation is also seen in the liver, brain, and kidney [52]. There is no information about the retention of copper derived from dental amalgam, which has to be added to the daily dietary intake of about 4–5 mg.

Half-Time Studies

The kinetics of inorganic mercury follow a complicated pattern during the first months after exposure, with biological half-times that vary for different tissues and for different times after exposure [19, 31, 32, 68–70). These authors showed that the first elimination phase from blood had a half-time of about 2–4 days, accounting for about 90% of the mercury, and was followed by a second phase with a half-time of 15–40 days. However, in some organs such as kidney, brain, thyroid, and pituitary, part of the mercury has a long biological half-time extending over a period of years [8]. Nylander et al. [71] analysed mercury concentrations in the occipital cortex and kidneys from autopsy cases and found a statistically significant correlation between the number of amalgam surfaces and the mercury level in these organs. High concentrations of mercury in thyroid glands and pituitaries could be shown in deceased dental personnel many years after termination of the exposure [72].

No data have been found on the biological half-times for the other components in dental amalgam.

Biocompatibility of Amalgam Components

Mercury and Derivatives

At the Cellular Level

The Hg^{2+} ion passes easily through cell membranes. It has no metabolic function in humans but mimics the behaviour of protons and binds with low specificity to many cell components via -SH, -OH, -NH_2, and -Cl groups. The bindings to thiols is especially strong and can attack the antioxidant glutathione. The toxicity of mercury has been suggested to be inversely related to the presence of intracellular glutathione [73].

Toxic Effects

Chronic mercury intoxication by ingestion of high doses or inhalation gives symptoms which are complex and slow to appear. The more commonly seen symptoms are ataxia, dysarthria, dysphagia, uncoordinated movements of arms and legs, impaired hearing, taste, and smell [52]. Low-level mercury intoxication, as reported after occupational exposure, is often focused on effects from the central nervous system. Memory disturbances, fatigue, irritability and tremor could be demonstrated in exposed workers by Roels et al. [74] and Fawer et al. [75]. A neuromyasthenia syndrome (headache, weakness, unsteady gait, tremor, and depression) has been described in mercury-exposed workers [76].

Silver, Tin, Copper, and Zinc

At the Cellular Level

Silver. A silver ion concentration of 5–10 μM activates polymorphonuclear neutrophils with production of superoxide anions, probably due to an augmentation of the respiratory burst initiated by a chemotactic peptide [77]. Silver is cytotoxic for cultured human epithelial cells [43], and also affects macrophage functions [78]. Subcutaneous implantation of alloy containing about 70 wt% silver can initiate a strong inflammatory response after 7 days with foreign body giant cells containing silver and other ions [51]. Silver accumulation was found in periapical granulomas which were removed from endodontically treated teeth in which the root canal had been filled with silver cones [79].

Tin. Subcutaneous implantation of alloys containing 26 wt% tin showed that tin could be detected around the implants in foreign-body-type giant cells over an observation time of 60 days [51]. Tin-containing alloy particles were also well phagocytized by macrophages in vitro [51]. Metallic tin and its inorganic salts are generally considered of low oral toxicity due to their poor alimentary absorption.

Copper. Copper ions damage lysosomes with release of acid hydrolases, resulting in cell degeneration [51, 52]. Copper, together with zinc, showed the highest cytotoxity among metals tested in culture [80]. Copper influences many physiological functions in humans, including the immune response, which is depressed after prolonged intake of even low doses [81]. This effect can, however, be due to its antagonistic interaction with zinc [81, 82].

Zinc. A considerable amount of zinc can be released from low-copper amalgam into culture medium and the concentration is sufficient to kill almost all cells in the culture [43]. Zinc released from amalgam is suggested to be very toxic in vitro, but the effect diminishes with time [80]. Increased concentrations of zinc have been shown to produce a T and B cell mitogen effect [83].

Amalgam Metals and the Immune System: In vitro Studies

Mercury

Human [84, 85] and animal [86] lymphocytes may react in vitro to mercury treatment with blastogenesis, increased DNA synthesis [85–91], and an increase in mitotic activity [84, 86, 91]. The proliferating lymphocytes have the characteristics of peripheral T cells in both humans [92] and rodents [91, 93], with an increase of CD8+ and especially CD4+ cells [94]. B cells are inhibited, both in mice [94, 95] and in humans [96]. Mercury also potentiates the lymphocyte proliferation induced by mitogens in both rodent [97, 98] and human [88] lymphocytes. However, some studies using murine [99–101] and human [96, 102] lymphocytes have only shown an inhibited lymphocyte proliferation after in vitro treatment with mercury. There are many possible explanations for these discrepancies.

First, multiple factors in the in vitro technique for cultivation of lymphocytes may affect the outcome: mercury is notorious for its handling difficulties [103]; serum in the medium modulates the proliferative response to mercury [104]; the commonly used 2-mercaptoethanol is highly inhibitory for mercury-induced lymphocyte proliferation [91], and cultivation time is critical, since the response to mercury occurs much later than for other mitogens [85, 91, 105]. The composition of the cell culture may be decisive for the reaction. Shenker et al. [102] found that the presence of monocytes in the mercury containing culture decreased mitogen-induced T cell proliferation, whereas depletion of monocytes caused an exaggerated proliferation. The concentration of mercury is also critical, since mercury is cytotoxic for lymphocytes at a concentration higher than 10^{-5} M, whereas the stimulation occurs at a slightly lower and narrowly restricted concentration range ($1–10\times10^{-6}$). Monocytes are more sensitive than lymphocytes [102], and these cells may be of special importance since adherent mouse cells respond to mercury with interleukin(IL)-1 secretion [106]. The cell concentration is also important since the mitogenic effect of mercury is prominent only at high cell concentrations (5×10^{6} cells/ml) [94], which has also been observed for other cations [107]. Finally, cell function may be affected at a dose which is much lower than the cytotoxic dose [96, 100–102, 108].

Secondly, species factors may play a decisive role in the effect of mercury. Human [92], but not murine [91], thymocytes respond with DNA synthesis to mercury. Human and guinea pig peripheral lymphocytes [85, 92, 104], but not mouse lymphocytes [91], were able to proliferate in response to mercury even after removal of adherent cells. The importance of adherent cells in murine cultures seems to be due to an early release of IL-1, which can be inhibited by anti-IL-1α antibodies, thus eliminating the mitogenic effect of mercury [94].

Thirdly, genetic factors may be important. Hultman and Johansson [97] described different effects of mercury on mitogen-induced lymphocyte proliferation in two inbred mouse strains, and Jiang and Möller [109] recently found that inbred mouse strains differ in their susceptibility to proliferate in response to mercury. Pollard et al. [94] found no correlation between the susceptibility to autoimmunity and the mitogenic effect.

In conclusion, mercury directly stimulates lymphocyte proliferation and also enhances proliferation induced by mitogens. However, species and genetic differences as well as an array of factors in the culture may profoundly influence the outcome of in vitro studies.

Amalgam Metals and the Immune System: Experimental in vivo Studies

At the in vivo level, mercury was for many years considered to act solely as an immunosuppressive agent which rendered experimental animals more susceptible to infectious agents [110, 111], possibly by inhibiting both the humoral [100, 110, 112–114] and the cellular [100, 114, 115] immune systems. However, in 1971, Bariety et al. [116] showed that 30% of outbred Wistar rats exposed to inorganic mercury developed an immune complex glomerulonephritis (GN). In 1977, Sapin et al. [117] further confirmed this new paradigm of an immunostimulatory effect of mercury, showing that a specific inbred rat strain, the Brown Norway (BN) rat, develops a systemic autoimmune disease in response to mercury [117]. A similar disease was then observed in mercury-treated New Zealand rabbits [118], followed by observation of antinuclear antibodies (ANA) and/or immune complex disease in the mouse [119, 120]. The rodent models have since then been extensively explored in order to better understand the mechanisms in autoimmune conditions. Since these models have recently been reviewed by a number of authors [121–123], the following discussion will be restricted to a brief overview and results of immediate importance for the discussion of amalgam.

Manifestations of Mercury-Induced Autoimmunity in Rodents

In genetically susceptible rats, mercury induces a massive polyclonal activation of B cells [124] and a biphasic, systemic immune-mediated disease beginning with anti-basement membrane antibodies mainly directed against laminin [125], followed by systemic immune complex deposits which may also involve anti-laminin antibodies [126, 127].

Mercury-treated, genetically susceptible mice of the H-2^s haplotype exhibit a general activation of the immune system with splenic cell hyperplasia, strong B cell activation, an increased number of immunoglobulin-secreting cells [128], and hyperimmunoglobulinaemia [129, 130]. The dominant autoantibodies in mercury-treated H-2^s mice are high-titreed anti-nucleolar IgG antibodies (ANoA) directed against the 34-kD nucleolar protein, fibrillarin [131, 132], which is part of the U3, U8, and U13 small nuclear ribonucleoprotein particles [133]. Interestingly, anti-fibrillarin antibodies (AFA) occur in [134], and are diagnostic for [135] human systemic scleroderma. Recent studies [136] have shown that the autoantibodies in human idiopathic systemic scleroderma and murine mercury-induced autoantibodies share common antigenic regions on fibrillarin, further linking the ANoA/AFA responses seen in mice and humans. In H-2^s mice, the development of AFA is followed by systemic immune complex deposits and a mild endocapillary proliferative GN [137]. The elution of ANoA from, kidneys of mercury-treated mice with immune complex deposits suggests that they are of pathogenetic importance for the GN [138].

Genetic Factors in Rodent Metal-Induced Autoimmunity

The seemingly contradictory views of mercury as an immunostimulating or immunosuppressive agent in vivo can be readily explained by genetic factors. In the rat, the genetic susceptibility to autoimmune GN is inherited as an autosomal dominant trait, and three genes, one of them localized in the rat MHC (RT1-B locus, class II region), are involved in both the development of anti-basement membrane antibodies as well as in immunostimulation determined as an increase in IgE [139, 140]. Examining a number of rat strains, Aten et al. [141] found that the susceptibility to induction of autoimmunity or immunosuppression by mercury were both dependent on the MHC class II haplotype. Moreover, in strains developing an autoimmune reaction, the type of glomerulopathy was influenced by non-MHC

genes. In mice, the susceptibility to development of ANoA/AFA resides in the mouse MHC (H-2) [142], more specifically in the Ir region and the H-2A locus [143, 144], and is codominantly inherited in a cross-breeding with resistant mice [145].

Pathogenetic Mechanisms in Rodent Metal-Induced Autoimmunity

For a long time, it was accepted that the autoantibodies found in mercury-treated rodents were the results of polyclonal B cell activation [121, 124], showing strong similarities with chronic graft-versus-host disease [146]. However, a number of observations indicate a T-cell-dependent reaction which could be driven by the autoantigen. These include a strong linkage of the susceptibility to the immune response region of H-2 [142–144], the need for T cells in the induction of autoimmunity [147, 148], the dominance of the IgG isotype [129, 130], and the strong skewing of the autoantibody production in mice towards the nucleolar protein fibrillarin [131, 132]. Recent studies by Aten et al. [149] strongly support an antigen-driven reaction to laminin as crucial in the autoimmune disease induced by mercury in the BN rat.

Although B cells can be directly activated by mercury [150], T cells are of paramount importance for the systemic autoimmune reactions seen in genetically susceptible rodents (see above). The activation of CD4+ cells with strong IL-4 mRNA expression in H-2^z strains, which demonstrate a strong B cell activation, has been attributed to a preferential activation of T helper(Th)2 cells by mercury [151]. In contrast, the T cells activated in H-2^s mice, reported to show contact sensitivity to mercury [152] and a higher secretion of interferon-8 [153], have been assumed to be Th1 cells. On the basis of these studies and a number of studies in rats [154], it has been suggested that the reaction to mercury in rodents should be a prominent example of a genetically determined Th1-Th2 imbalance, exaggerated activation of Th2 cells leading to systemic B cell autoimmunity, and activation of Th1 leading to a delayed-type (contact) hypersensitivity (DTH) [155]. Although an interesting hypothesis, some reports are difficult to combine with such a view. First, Ishii et al. [156] found that mouse strains susceptible to B cell activation with systemic autoimmunity were also high responders for mercury-induced contact hypersensitivity. Secondly, in vivo treatment with anti-IL-4 monoclonal antibodies (MAbs) acheived only a partial reduction in the increase of total and autoantigen-specific IgG1 [157], suggesting that the role of IL-4 is limited. Further understanding of these mechanisms is hampered by the lack of a complete cytokine profile in mercury-treated rodents.

An even more profound question, namely the nature of the stimulus which activates the T cell, is still unanswered. At the heart of this question lies the uncertainty whether mercury (and other metals) give rise to specific Th cells directed against the metal ions. This is difficult to link with the current paradigm of CD4+ cells reacting with small peptides presented by class II molecules. However, Wylie et al. [158] have recently reported development of a MAb reacting with high affinity to mercury. It can therefore not be excluded that mercuric ions contain Th cell epitopes.

A very specific role for T cells in the rodent models of mercury-induced autoimmunity was suggested by Druet et al. [121]. These authors showed mercury-activated autoreactive CD4+ cells which proliferated in the presence of syngeneic MHC-class-II-expressing cells in BN rats [159]; however, the exact stimulus could not be elucidated. A role for these autoreactive cells in the pathogenesis of the autoimmune disease was suggested by their ability to transfer the disease to syngeneic BN rats not treated with mercury [93]. However, this conclusion has recently been challenged [153] because of the presence of 8–10% of cells with unknown specificity in the transferred cell population. These cells could have been targeting mercury or self-proteins altered by mercury, a hypothesis supported by recent findings made by Kubicka-Muranyi et al. [153] in mice. By using the adoptive transfer popliteal lymph node test, these authors showed that mercury treatment in genetically susceptible mice generated T cells which were able to respond anamnestically to mercury and induce B cell activation not only after treatment with mercury but also after injection of peritoneal cells from mercury-treated animals. This was interpreted as either a reaction directed to the mercuric ion per se, or to self-proteins (for example class II molecules) altered by mercury. Again, the exact nature of the stimulus, as well as the importance of these cells for the autoimmune condition, remains unknown. The recent findings of silver as an efficient induce of autoantibodies targeting the same nucleolar protein as mercury in genetically susceptible mice [160] could be interpreted as an identical alteration in the nucleolar protein or in the nucleolar metabolism caused by mercury and silver.

Experimental Studies on DTH Reactions

Although the first descriptions of a contact-like reaction to mercury in humans were published at the end of the 18th century [161], similar reactions to chemical compounds were not reported in experimental animals until the 1930s by Bloch and Steiner-Wourlisch [see ref. 161]. Since then, a large number of modifications of these in vivo tests have been described, serving to 'maximize' the sensitivity mainly for drug testing. Although the sensitivity and predictive value of these test have been substantially increased since they were first described, they are now challenged for ethical reasons and because they ignored the role of genetic factors [162]. Polak et al. [163], described, as far back as 1968, the decisive role played by genetic factors in regulating the susceptibility to sensitization by chemicals. However, this factor has largely been ignored in the use of these tests, probably due to difficulties in obtaining and examining a large number of genetically defined strains instead of relying on a single (outbred) strain. In recent studies, contact sensitivity for mercury [156], nickel [164], and chromium [165, 166] have been studied in genetically defined strains of mice. These studies have strongly reinforced the above-metioned findings of a genetically regulated susceptibility for sensitization to metals, and have mapped the genetic susceptibility for these metals to the I-A region. Interestingly, the high- and low-responder haplotypes are not the same for the different metals, indicating that the antigenic epitope is not the same for these metals. Transfer experiments in these studies showed that the reaction was due to CD4+ cells, with no role being found for CD8+ cells in the low-responder strains.

Amalgam Components Other than Mercury

Recently, Hultman et al. [160] reported that silver, a dominant component of amalgam, is a highly efficient inducer of autoimmunity in genetically susceptible mice, causing ANoA which target the same nucleolar protein, fibrillarin, as mercury-induced ANoA. In contrast to mercury, silver only slightly stimulates the immune system, and also fails to provoke the systemic immune complex deposits and the anti-histone antibodies observed after mercury treatment. Genetic studies [Hultman et al., in prep.] show that the genetic susceptibility to silver is linked to the H-2A locus, although modifying non-H-2 factors have more influence than in mercury treatment.

In rodents, metallic tin induces a plasma cell response in the regional draining lymph nodes [167] and spleen [168], which is attributable to a polyclonal B cell activation that greatly increases the B cell response to injected antigens [169]. We did not find any studies on autoimmune effects or the influence of genetic factors. Copper administered orally in mice [81] caused a decrease in concanavalin-A-induced lymphocyte proliferation, whereas the lipopolysaccharide induced proliferation was increased after certain doses and decreased after others. In addition, the mice showed an increased production of autoantibodies against bromelain-treated erythrocytes. However, another study [170] found a consistent inhibition of B cell but not of T cell function. Thus, copper seems to interact with the immune system, but the findings are contradictory, possibly due to the different mouse strains used.

Amalgam and Alloy

Hultman et al. [171] have shown that genetically susceptible mice in which amalgam or alloy are implanted in the physiological milieu of the peritoneal cavity develop chronic hyperimmunoglobulinaemia, ANoA/AFA, and systemic immune complex deposits. There was also an increased expression of class II molecules on splenocytes, as well as a dose- and time-dependent change in mitogen-induced lymphocyte proliferation. Interestingly, implantation of alloy (not containing mercury) also caused AFA, which was attributed to the 70% silver component in the alloy, given the effects described in the same strain using pure silver [160]. Very recent results [Hultman et al., in prep.] in genetically susceptible rats implanted with conventional dental amalgam fillings in 4 teeth revealed a stimulation of B cells, measured as a significant increase in serum IgE, followed by systemic immune complex deposits.

Dose-Response Studies in Experimental Animals

Dose-response studies have been performed in mice and rats genetically susceptible to mercury-induced autoimmunity using peroral (p.o.), subcutaneous (s.o.), or intratracheal mercuric chloride treatment as well as exposure to mercury vapour. Three immune parameters have been evaluated: (1) development of autoantibodies; (2) B cell stimulation, and (3) systemic immune deposits. Autoantibodies, such as ANoA/AFA or anti-basement membrane antibodies, were first observed at a dose of 100–170 µg Hg/week/kg body weight (b.w.) in mice [172, 257] and rats [139, 173, 174], after exposure to $HgCl_2$ either p.o. or s.c., or Hg^0 via vapour. In contrast, no autoantibodies were found in these studies at a dose of 60–70 µg Hg/week/kg b.w. B cell stimulation was detected in mercury-vapour-treated mice at a dose of 360 µg Hg/week/kg b.w., and in rats at a dose of 340 µg Hg/week/kg b.w. However, since lower doses were not tested in the rats, B cell stimulation may occur at lower

doses. Systemic immune deposits were first observed in the rat at a dose of 110 µg Hg/week/kg b.w. [173], and in mice at a dose of 360 µg Hg/week/kg b.w. [257]. The corresponding body burden, measured as the renal mercury concentration, was 4.02±0.76 µg Hg/g kidney wet weight in mice at the lowest dose where autoantibodies were observed.

Immune Responses to Amalgam Components in Humans

Allergic Type I Reactions Induced by Mercury

Only a small number of cases of immediate, anaphylactic responses have been reported after placement of amalgam restorations and these were interpreted as being caused by mercury [175–177]. Localized or general symptoms, such as skin erythema, tachycardia, respiratory difficulty, and oedema have been described. Anaphylactic reactions to vaccines containing mercury as a preservative have also been described [178].

Allergic Type IV Reactions Induced by Mercury

How is the Patient Sensitized?

There is a lower tendency for sensitization through mucous membranes than through skin and it is not known if sensitization is possible via mercury released from dental amalgam. In 1957, Frykholm [179] noted that only one of seven individuals with mercury allergy showed oral lesions whereas all the others had a cutaneous reaction after newly placed amalgam fillings. Elicitation of a hypersensitivity reaction in the oral mucosa requires 5 to 12-fold higher mercury concentrations than elicitation through the skin [180]. The reason for this is not known, but protection by saliva and a more resistant epithelium in the oral cavity could be contributing factors. This would also mean that an epimucosal test is less sensitive than an epicutaneous one. Many sources for mercury sensitization exist, such as mercury-containing desinfectants, cosmetics, dyes, food, and preservatives (thiomersalate in particular) in vaccines and topical drugs. The frequency of a positive patch test to thiomersalate has been estimated at about 1–10% in various countries [89, 181]. This contact allergic reaction is suggested to result from a cross-reaction between different mercury compounds and metallic mercury [182].

Despite the widespread exposure to mercury, only a minority of individuals is sensitized, which indicates that both the induction and elicitation phases are subject to immunoregulatory mechanisms [183]. The involvement of genetic factors is supported by the recent findings by Stejskal et al. [184], who showed that genetically identical twins exhibited an identical reactivity pattern to metals.

Pathogenetic Mechanisms

The basic reaction is a DTH which implicates metal-specific CD4+ T cells recognizing metal-modified MHC-bound peptide molecules, as has been described for responses to nickel [185]. The same mechanism has been suggested for mercury with specific Th cells eliciting an immune reaction to mercury-peptide complexes bound to MHC molecules [153]. Another possibility is a direct interaction of mercury with MHC class II molecules, thus eliciting metal-specific T cells, which has been hypothesized for responses to gold [185]. The cytokine profile of the T cell clones resembles that of Th1 cells after nickel-induced DTH in the skin [186]. The extent to which these specific T cells expand may influence the degree of sensitization to the metal and the severity of the elicitation reaction [183]. It is also important to mention that there is no absolute prerequisite for the presence of Langerhans cells in the epidermis to initiate skin sensitization [183].

Signs and Symptoms of Systemic Type IV Reactions

A rash may appear on the face, neck, and the flexural areas of the limbs a few hours after placement of a new amalgam restoration in an individual harbouring old amalgam fillings [37]. This reaction will typically resolve after 10–14 days [37]. The frequency of allergic responses from dental amalgam is difficult to estimate but is considered to be less than 1% [37]. Véron et al. [176] stated, however, that amalgam is responsible for 16% of the allergic responses. Systemically induced allergic contact dermatitis (baboon syndrome) [187] can be induced not only by inhalation of mercury vapour during dental treatment but also after breaking of a clinical thermometer [188, 189]. In the latter cases, the erythema is characteristically seen over the buttocks, upper inner surface of the thighs and axillae [187]. Patch testing is positive for a number of mercury species in these cases. However, generalized symptoms caused by type IV mercury hypersensitivity are very rare and only a few cases have been reported [190]. The generalized symptoms can coincide with oral symptoms [190].

Allergic Oral Lesions – Localized Type IV Reaction

Lichen planus is a subacute or chronic skin disease characterized by small, flat-topped, shiny, polygonal, violaceous papules that may coalesce into plaques and are most often seen over the forearms and legs as well as on the oral mucosa [191]. The oral lesions consist of white papules, often in a reticular pattern, or composed of vesicles, erosions and ulcers in the buccal region, occasionally occurring as the only manifestation of the disease [191]. The aetiology is still unknown, but the disease has been seen in association with the ingestion of drugs, such as non-steroid anti-inflammatory drugs, β-blockers and gold salts [192]. An immune process directed against basal cells has also been suggested as a dominant pathogenic event [193].

James et al. [192] have proposed that oral erosive lichen planus could be associated with hypersensitivity to mercury. A positive epicutaneous test for mercury has been found among 16–62% of patients with this lesion, whereas mercury hypersensitivity is reported in only 1–4% of the general population [182, 190]. However, lesions of oral lichen planus are much more discrete than cutaneous lichen planus and this makes it uncertain if the oral lichen planus lesion should be classified without reservation as a type IV reaction [182]. The antigen effect is probably only weak in the oral mucosa due to the low release of mercury and dilution in saliva. James et al. [192] suggested that a persistent oral erosive lichen planus could be the result of allergy to mercury but in combination with cytotoxicity and trauma due to roughened amalgam. The uncertainty of the type of lesion described by the authors as oral lichen depends on the difficulty in distinguishing between contact type IV reactions and lichen planus. Bolewska et al. [194] suggested that only lesions of the oral mucosa in contact with dental amalgam fillings should be accepted as contact allergy, whereas lesions exceeding the contact area may have other causes such as oral lichen planus. This view is supported by the resolution of lesions in contact with amalgam fillings after extraction of the fillings [194]. In accordance with this opinion, Hietanen et al. [195] did not find any association between hypersensitivity to dental restorative metals and oral lichen planus in their study of 12 patients.

Recent studies by Warfvinge et al. [196] would seem to support such a view. These authors performed mercury provocation tests on normal oral mucosa in amalgam-bearing patients with clinically and histologically confirmed oral lichenoid lesions. No difference could be found in the phenotype of infiltrating lymphocytes in the mucosa between these patients and the amalgam bearers without oral lichenoid lesions. However, since only five patients with oral lichenoid lesions were tested, this does not exlude a genetically dependent susceptibility to react. Such an interpretation seems likely based on experimental findings in BN rats which are generally susceptible to mercury. These rats showed mononuclear cell infiltrates, rich in activated T cells, in the oral mucosa after repeated s.c. injections of mercuric chloride [197]. Ulceration of the oral mucosa occurred only at the site of local challenge with mercuric chloride, supporting the idea that direct contact is crucial for eliciting a type IV reaction to mercury in the oral cavity.

It is difficult to link allergic reactions with the local oral complaints (such as metallic taste, dryness, battery sensation, burning, smarting sensations) described in amalgam bearers [190, 198].

A cutaneous lichenoid drug reaction, histologically consistent with a type IV reaction, occurring together with ANA, was seen in a patient who had been occupationally exposed to mercury [199]. The important comment was made, that the patient must have shown an unusual susceptibility, since other more heavily exposed workers did not demonstrate any symptoms.

Type IV Reactions Induced by Non-Mercurial Amalgam Components

Silver

Allergic reactions are rare. One case was described by Catsakis and Sulica [200] and others by Véron et al. [176]. However, Bolewska et al. [194] did not find any positive cutaneous patch test to silver nitrate in patients with oral mucosal lesions. The same result was reported by Hietanen et al. [195].

Tin

Tin does not seem to give rise to allergic reactions, as far as can be seen from the literature.

Copper

In 1969, Frykholm et al. [201] described a case of a lichenoid reaction in the mouth due to copper, and James et al. [192] reported a positive allergic reaction to cupric nitrate in 8 of 29 patients with oral lichenoid lesions. Bolewska et al. [194] did not find signs of allergy to copper sulphate in 49 patients with oral mucosal lesions, nor did Hietanen et al. [195] in 12 patients with such lesions.

Zinc

Very few reports of positive allergic reactions have been reported [176, 192]. It is noteworthy that zinc is not included in standard series for epicutaneous tests to elucidate contact hypersensitivity to amalgam, and this might explain the lack of reports of allergic reactions.

Diagnosis of Allergic Metal Reactions

Two criteria are usually used to diagnose an allergic response to dental amalgam: a positive epicutaneous test (patch test) to the dental allergen, and recovery after elimination of the amalgam.

Patch Test

This test evolved from the observation of a type IV reaction in the area of a tattoo, usually elicited by mercuric sulphide [202]. It is routinely used for clinical diagnosis of occupational and environmental contact allergy, frequently with a battery of allergens. The patch test may be difficult to interpret, and especially to differentiate between irritant and allergic reactions. Classification of the phenotype of the infiltrating cells may help in this differentiation, since a simultaneous increase of Langerhans and T cells indicates a contact allergic reaction [203]. There is a risk for sensitization of previously non-allergic patients using the patch test [183, 204]. In addition, a boosting effect due to the application of a battery of test substances may aggravate somatic as well as central nervous system symptoms in certain individuals [205].

In vitro Tests

An in vitro correlate of DTH is the T cell proliferation assay. This so-called lymphocyte transformation test (LTT) is theoretically sensitive and specific, measuring proliferation of in-vivo-sensitized T cells when exposed to antigen in vitro. The proliferation is assessed by measuring the amount of ^{3}H-thymidine incorporated during DNA synthesis. A high count compared with the controls means that the lymphocytes have transformed due to their reactivity with the antigen. LTT is a test for T cell memory, but can not test the strength of the immune response against the antigen. A modification of this test, the so-called memory lymphocyte immunostimulation assay (MELISA), has been elaborated by Stejskal et al. [206, 207] and applied to drug-related allergies, but it is presently being evaluated as a tool for the assessment of metal allergy [184]. MELISA also takes advantage of the morphological assessment of blastogenesis, thus improving its accuracy. It is therefore a method that, when compared to the patch test, may better discriminate between toxic and allergic reactions to metals [184].

Type II Hypersensitivity (Antibody Mediated)

To our knowledge, there are no reports of an amalgam-induced antibody-dependent reaction against self-antigens, with the exception of a case of granulocytopenia due to a mercury-containing diuretic [208].

Type III Hypersensitivity (Immune Complex Mediated)

Nephrotic Syndrome – GN

A potential effect of amalgam components has been suggested from case reports regarding adverse effects due to occupational and therapeutic metal exposure, to mercury in particular.

An increased prevalence of proteinuria or nephrotic syndrome as well as GN after chronic mercury exposure was first reported in 1861 by Kussmaul [209]. Kazantzis et al. [210] described 4 occupationally mercury-exposed workers with nephrotic syndrome and noted that there were other workers excreting equally or even larger amounts of mercury who were unaffected. Tubbs et al. [211] reported two patients with industrial exposure to mercury who developed nephrotic syndrome due to immune-mediated membranous GN. Clinical remission was seen in one patient after elimination of the mercury exposure. Additional cases of occupationally mercury-induced glomerulopathies are referred to by Pairon et al. [212]. Stewart et al. [63] described proteinuria in 9 of 21 technicians occupationally exposed to mercury after spillage of corrosive sublimate and with a wide range in the excretion rate. This suggested a difference in susceptibility to the effect of mercury.

For centuries, mercury has played an important pharmacological role as an essential ingredient in antiluetics [209] and in diuretics, laxatives and some skin ointments. Many authors have demonstrated clinical signs of glomerular lesions after treatment with mercury-containing drugs and cosmetics. Pairon et al. [212] described 2 cases of glomerulopathy and referred to 5 other cases in the literature, most of them membranous GN caused by mercury-containing diuretics or skin-lightening creams. Additional cases of membranous GN due to mercury-containing cosmetics have been described by Lindqvist et al. [213], Kibukamusoke et al. [214], and Oliveira et al. [215]. It was later shown by Barr [216] that about 50% of patients in Kenya with nephrotic syndrome suffered from immune-mediated glomerulopathy. All of them had used skin-lightening cream containing mercury chloride, and an association to HLA phenotype was suggested. Many cases of membranous GN induced by calomel-containing diuretics can be found in the literature [217] and have been suggested to be immunologically provoked and probably T cell mediated [217].

Autoimmune Diseases

An interesting case of systemic autoimmune disease in an occupationally mercury-exposed worker has been described by Röger et al. [218]. This patient developed many symptoms, such as Raynaud's syndrome, polyarthralgia, polymyositis and sclerosis of the forearms and thighs. High titres of ANA in the serum with a speckled pattern were also demonstrated. The disorder was suggested to belong to the connective tissue overlap syndrome and to have probably been induced by mercury. The authors proposed that their case may represent a human analogue to the animal models of mercury-induced autoimmune reactions. They also recommended that exposure to mercury and other chemicals should be considered when taking the history of patients with systemic autoimmune disease.

Pathogenetic Mechanisms

Extrapolating from experimental findings in animals, an autoimmune reaction seems probable. The metal-specific T cells, as described by Sinigaglia [185], and the finding of the mercury ion as potentially antigenic [158] support the possibility of a T cell reactivity to mercury. It is most probably dependent on genetic factors, thus explaining the relatively few cases reported. Therefore, occupationally mercury-exposed individuals constitute an important cohort for immunotoxicological studies. However, it is necessary to evaluate the findings carefully and with the reservation that this cohort can be selected and not fully comparable with the general population due to, for example, the 'healthy worker effect' [219].

Immunological Effects of Amalgam Components

Acrodynia

This well-known disease caused by mercury – also called 'pink disease' – was first recognized in France in 1828 and shown to be linked to chronic mercury exposure from calomel in teething powders [220, 221]. The disease is restricted to children and has been reported after exposure to mercury vapour, phenylmercuric compounds, and both mercuric and mercurous salts [64]. In more recent case reports of acrodynia in children, the mercury exposure was from fluorescent light bulbs [222], but other sources, such as mercury-containing latex paint [223, 224] and ointments [222] have been described. Moreover, acrodynia appeared in a child after long-term injection of thiomersalate-containing γ-globulin [225]. The onset of this disease is often insidious and produces symptoms such as anorexia, irritability, photophobia and painful hands and feet. The puzzling finding that only a small number of the exposed children became sick [222] points to genetic factors. In 1980, Orlowski and Mercer [226] emphasized the marked individual susceptibility to mercury and proposed that acrodynia could represent an allergic reaction or idiosyncrasy to mercury. These authors also compared the clinical findings in acrodynia and mucocutaneous lymph node syndrome (Kawasaki's disease) and concluded that both could represent diseases caused by mercury. A link was also established early on between acrodynia and nephrotic syndrome [220, 227] (probably minimal-change disease). Only a small minority developed nephrotic syndrome and without relation to the urinary level of mercury, which again points to a genetically regulated immune reaction.

Effects on T Cells by Dental Amalgam

Egglestone [228] reported a decrease in the concentration of T cells in three patients after placing amalgam restoration; this was followed by an increase when the restorations were replaced by ceramics. These results could not be fully confirmed by Giuliani et al. [229] who analysed the total number of T cells and the subpopulation of CD4+ and CD8+ cells in 8 patients before and after placement of amalgam restorations. These authors found that half of the patients demonstrated a decrease and the other half an increase of T cells 15 days after amalgam fillings, with a return to pretreatment values after 2 months.

A small number of patients were reported by Wilhelm et al. [230], one group (n = 9) examined before and after placement of amalgam restorations for the first time and the other (n = 16) after removal of previous amalgam fillings. The authors were unable to find any significant differences between the two groups either in the number of T, B or other leukocytes, or in mitogen-induced T cell reactivity. It is, however, evident from this paper that 4 of the patients in the first group had an increase and 5 patients a decrease in T cells 3–6 weeks after the amalgam insertion. This is in accordance with the findings made by Giuliani [229] and illustrates the importance of considering individual reactions in these studies.

Hickel et al. [231] found that out of 50 amalgam bearers with symptoms alleged to be due to their fillings, 2 had distinctly abnormal CD4/CD8 values.

Recently, Herrström et al. [232] examined cellular and humoral immune factors in 41 healthy 15-year-old schoolchildren, and the relationship between the number of amalgam surfaces and the mercury concentration in plasma. No

significant correlation was found between the number of amalgam fillings or mercury concentration in plasma and the number of T, B or other blood cells, the immunoglobulin titres, and ANAs. Similar results have been published by Langworth et al. [233] who examined immunologic parameters in 16 women and 5 men with health complaints alleged to be secondary to mercury from amalgam fillings. Five of 9 patients in the allergy group and 1 out of 10 subjects in the amalgam group showed a positive reaction to mercury in the lymphocyte transformation test. No statistically significant group difference was seen in spontaneous DNA synthesis but the DNA synthesis was extremely high in a single individual in the amalgam group. The authors concluded that, despite the negative results, not all individuals are insensitive to low-dose mercury exposure. This supports the opinion that adverse effects from amalgam fillings could be expected in not more than about 1% of the population [234], possibly reflecting the relation to a certain genetic constitution. Animal studies have, therefore, been of great importance in exploring if and how immunotoxicological reactions are genetically linked.

Effects on T Cells after Occupational Exposure to Amalgam

Eedy et al. [235] compared the number of lymphocytes and their subclasses in the blood between groups of occupationally mercury-exposed dental and nonexposed medical students. The total lymphocyte count, total T cell numbers, CD4+ and CD8+ cell numbers were found to be significantly elevated in the mercury-exposed group. Interestingly, patch testing to mercury did not show evidence of cutaneous hypersensitivity in either group. This finding confirms the fact that dental personnel often have an only low cutaneous hypersensitivity to mercury [235].

T Cell Changes in Multiple Sclerosis (MS): Amalgam Effect?

The aetiology of MS is unknown but autoimmune responses against myelin basic protein (MBP) and proteolipid proteins (PLPs) have been suggested even if these proteins cannot be implicated directly as the target antigens. An increased number of MBP-specific T cells with encephalitogenic potential were, however, demonstrated in MS patients by Vandenbaark et al. [236]. Siblerud and Kienholz [237] compared blood findings between MS patients who had their amalgam removed and those with the amalgam left in place. They reported a higher percentage of CD8+ T cells and a lower CD4+/CD8+ ratio in the female amalgam removal group while the corresponding male group had a significantly higher total number of T cells compared to patients with amalgam left in place. Based on these and other findings, the authors hypothesized a relationship between mercury from dental amalgam and MS. It is, however, hard to evaluate their results without more knowledge about the (myelin) reactivity of the T cells. An association between dental amalgam and MS is, thus, unsolved, and in the USA, the Medical Advisory Board of the National Multiple Sclerosis Society has argued that there is no sound epidemiologic evidence that relates dental amalgam restorations to MS [238].

Effects of Mercury on B-Cell-Mediated Immunity

Data published by Stonard et al. [239] and Lauwerys et al. [240] indicate a possible autoimmune reaction in some workers exposed to mercury. An increased incidence of circulating immune complexes was found in a mercury-exposed group by Stonard et al. [239] and high levels of circulating ant-laminin antibodies were found in 8/62 workers in a chloralkali plant but in none of the controls [240]. These authors concluded that occupational exposure to mercury vapour can lead to immune dysfunction in a certain percentage of exposed individuals. However, in a later investigation by Bernard et al. [241], no increased prevalence of circulating anti-laminin antibodies was found in the serum of 58 workers exposed to mercury vapour for a mean of 8 years (a mean urinary excretion of Hg of 72 µg/g creatinine). Six years later, the same group of authors presented a different result after examining 50 male Belgian workers exposed to mercury vapour for an average of 11 years and with a mean mercury level in the urine of 22 µg/g creatinine [242]. They demonstrated that anti-DNA antibodies and total IgE in serum were positively associated with the levels of mercury in urine and blood. No signs of glomerular dysfunction could, however, be demonstrated and the authors speculated that the workers may have lacked the necessary genetic predisposition to develop immune-mediated GN.

A significant increase in serum concentrations of IgA, IgG, and IgM was noted by Queiroz et al. [243] in 40 of 44 workers exposed to mercury for 3–46 months and with a urinary mercury concentration below 50 µg/g creatinine. The increased immunoglobulin level persisted 6 months later despite significant reduction in the urinary concentration of mercury. The authors suggested that mercury levels, also withing the accepted threshold (< 50 µg/g creatinine), could lead to humoral immune stimulation. Apparently, the immunoglobulin concentrations varied at both examinations, as seen from the authors' figures, emphasizing the individual reactivity. Conflicting results have, again, been reported by Ellingsen et al. [244], who failed to reveal any increased prevalence of autoantibodies or changes in immunoglobulins or complement components among mercury-vapour-exposed chloralkali workers.

In conclusion, contradictory results like these correlate with the fact that there are relatively few reported cases of nephrotic syndrome in humans exposed to mercury and, as emphasized more than 30 years ago by Kazantzis et al. [210], this strongly suggests a genetic influence.

Systemic Effects Attributed to Dental Amalgam

Chronic Fatigue Syndrome (CFS)

This entity has been known as the chronic Epstein-Barr virus syndrome, later changed to CFS due to doubts regarding the causal relationship with Epstein-Barr virus infection [245]. A relation to immunological as well as neurological disturbances has been suggested, and CFS is therefore also known as the chronic fatigue immune dysfunction syndrome [246]. Recently, Straus et al. [247] reported a reduced level of CD4 T cells and CD4,CD45RA (naive T cells) in 18 patients with this condition. Their lymphocytes also demonstrated a low proliferative activity when treated with mitogens, but this finding was also seen with lymphocytes from fatigue patients who did not fulfill the criteria for CFS. The authors concluded that there is an increased state of differentiation of peripheral T cells in CFS which could be due to neuropsychiatric and/or neuroendocrine disturbances.

Fatigue, alleged to be an effect of mercury toxicity from amalgam fillings, was discussed by Michel et al. [248]. A significant positive correlation was found between symptoms of fatigue and psychosocial factors, but mercury release from dental amalgam did not seem to explain the fatigue symptoms. However, Stejskal et al. [184] recently described strong lymphocyte reactions to mercury compounds by the MELISA test in a few patients fulfilling the criteria of CFS according to Holmes et al. [245]. No immunovirologic data were, however, presented. Association of CFS to metal allergy is not addressed in the 600 articles about CFS appearing in the literature during the last 3½ years and the association must at present be regarded as an open question.

Multiple Chemical Sensitivities (MCS; Environmental Illness)

This syndrome, first introduced by Cullen [249] refers to somatic and psychiatric symptoms not consistent with the acceptable toxicological properties of the chemicals [250]. According to Cullen [249], the initial symptoms should be acquired in relation to low-level environmental exposure to a wide range of chemicals and present with pansystemic manifestations. Clinically, MCS is characterized by neurological, endocrine/metabolic, and immunological symptoms [251]. Symptoms include headaches, eye irritation, emotional irritability, short-term memory loss, and emotional depression [251]. According to Meggs [252], MCS is clearly distinct from IgE-mediated allergy but cellular immunity probably plays a role. Aberration of cellular immune profiles was suggested by Levin and Byers [251], who found that MCS patients often had a forme fruste of systemic lupus erythematosus, rheumatoid arthritis, autoimmune thyroiditis, or scleroderma. Many clinical studies have emphasized the polysymptomatic character of the complaints in patients who attribute their symptoms to amalgam intoxication [198]. The latter authors remarked on the finding that more than 1 of 4 patients examined for orofacial discomfort of dental restorations were under psychiatric treatment. This is in agreement with the high prevalence of mental disorders among patients with suspected mercury intoxication from amalgam fillings [253]. A large population study of 1,462 women showed an inverse correlation between the number of amalgam fillings and various symptoms and complaints as well as different biochemical tests [254, 255]. The authors did not, however, exclude a possible causal relationship in single individuals.

How many of the symptoms in 'amalgam disease' are caused by MCS? This question, which has not been asked before, seems relevant and important in order to have the amalgam controversy assessed in a broad manner. As noted by Levin and Byers [251], the genetic pool of the population has changed, now diluted with individuals with less durable and flexible adaptive mechanisms. At the same time, there is an increased exposure to toxic chemicals. Evidently, MCS is a very special disorder and, according to Megg [252], 'this illness is an exercise in walking through a mine field without getting blown up'. Elimination treatment protocols of patients attributing their complaints to amalgam, now in progress at different centres, is in line with how MCS is handled and with, obviously, good results [251].

Does Amalgam Affect the Immune System?

Is it possible to answer this question with the background that we have presented in this review?

There is sufficient evidence for stating that rare instances of type I hypersensitivity (immediate, anaphylactic reactions) to mercury do occur, as well as a low frequency of oral and systemic type IV hypersensitivity to mercury and, occasionally, to the other dental metals.

At present there is no evidence that immunological reactions due to amalgam metals are responsible for the local oral complaints reported by certain amalgam bearers, nor is there any convincing evidence that dental metals are the cause of CFS or MS. A number of patients with generalized symptoms alleged to be due to amalgam can probably be characterized as suffering from MCS, but the relation to immunological aberrations has not been proven. The situation is less clear for autoimmune and type III (immune complex mediated) reactions, and for the possible influence of dental amalgam components on markers for T and B cell immune function. The complexity of the situation is illustrated in figure 3, which addresses our view on how a combination of different factors may cooperate to cause adverse reactions to amalgam components.

Exposure stands for exposure to metals in the amalgam, not only to mercury, not only through inhalation of (mercury) vapour, and not only via dental fillings. As shown in recent animal studies, other components besides mercury, such as silver, may cause autoimmunity, and additive effects due to exposure to the multitude of metals in dental amalgam need to be considered. The main exposure route hitherto considered has been inhalation of vapour, but studies in primates given amalgam fillings and of the fecal content of mercury and silver in human amalgam bearers has shown that a substantial amount of the metals released from dental amalgam fillings are swallowed and pass through the gastrointestinal tract. Although the absorption rate is limited, this route will contribute to the body burden of these metals. Finally, one should realize that humans are also exposed to the metals in dental amalgam fillings from other sources such as food and water, and the amount leaking from the fillings should be considered as an extra burden.

It is thus evident that mercury, and to an unknown extent also the other elements in amalgam, accumulate in the body, but the crucial question is whether this accumulation is sufficient to adversely affect the immune system. Studies in rodents clearly show that *genetic factors*, especially MHC genes, play a decisive role regarding this question. Since it is very difficult to study dose-response relationship in a mixed population of susceptible and resistant individuals

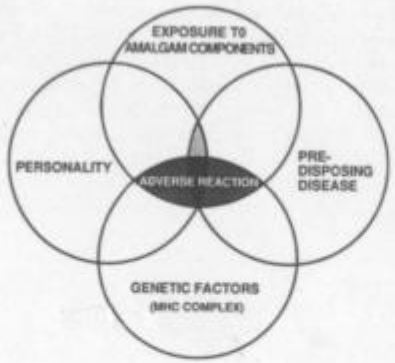

Fig. 3. Illustration of concurrent factors that could provoke adverse reactions to dental amalgam components. Note the small, central (black) 'high-risk' area depicting amalgam exposure in an individual with a hereditary susceptibility for immunological side-effects and prone to developing neuropsychiatric and/or neuroendocrine ailments. This zone, and the surrounding areas as well, include confounding elements which still make the 'amalgam disease' concept controversial and in need of further studies.

(for example, a population of humans), it is at present necessary to rely upon studies in susceptible and genetically homogeneous rodents. It can be calculated from such studies [8, 139, 257], that the lowest observed adverse effect level (LOAEL) for autoimmune manifestations is 16–24 µg Hg/day/kg body weight, equivalent to 1,100–1,680 µg Hg/day/70 kg. If a safety factor of 10 is applied for transforming data from animals to humans, and another factor of 10 is used for transforming the data from the LOAEL to no observed adverse effect level (NOAEL) [29], the allowable daily intake of mercury from deliberate mercury exposure would be 11–17 µg Hg/day for an ordinary adult. This is close to estimations of the amount of mercury taken up due to dental amalgam, i.e. 2.5–17 µg Hg/day [29]. However, under certain conditions (a large number of fillings, intensive gum chewing, bruxism) the uptake from dental fillings will be substantially higher. In addition, special circumstances (occupational exposure, high intake of fish foods) may lead to a much higher total exposure in the individual amalgam bearer. Genetic factors could play a role not only by determining the susceptibility to adverse reactions, but also by influencing the type of the immunological reaction. Based on rodent studies, it has been suggested [154, 155] that MHC genes may direct whether exposure to mercury leads to expansion of Th2 cells (mediating B cell activation and autoimmunity), or Th1 cells (mediating contact-sensitivity-like reactions). The existence of systemic autoimmune conditions due to mercury exposure in the occupational setting is an indication of the existence of Th2-mediated reactions after mercury exposure. It can be hypothesized that the contact sensitivity reactions, which have been clearly associated with dental amalgam fillings, may have an as yet unidentified counterpart in Th2-mediated, autoimmune reactions in amalgam bearers.

Very little is known about the importance of *predisposing illness*. Since the mechanisms which govern the development of autoimmune conditions are largely unknown, it is difficult to assess whether the extra body burden of metals resulting from dental amalgam fillings may trigger or accelerate the development of such diseases in genetically (metal)-susceptible individuals.

Personality factors such as an endocrine/metabolic or neuropsychiatric diathesis may be important for the polysymptomatic expression of metal-induced adverse effects. The relation of these symptoms to immunologic dysregulation and/or infectious causes is undefined. It is, however, reasonable to propose that organic as well as psychological contributions will be part of, and conceivably aggravate, the manifestations of a metal-induced reaction.

A need exists for further research into the effects of dental (amalgam) components (metals) on the immune system. Existing rodent models should be of value in examining the effect of simultaneous low-dose exposure to combinations of metals, and in further evaluating the mechanisms operating in metal-induced autoimmune conditions. Animal models should also be of importance for examining what effect metal exposure may have on spontaneously developing autoimmune conditions. Further studies on human metal-induced immunological adverse reactions are also warranted. Although such conditions are unusual, a substantial number of cases have been reported in the literature. It would be of great value to examine such cases to reveal whether there are one or more susceptible human genotypes, and to use this information when evaluating patients with symptoms alleged to amalgam fillings. Since many of the patients with such symptoms would seem to be suffering from other conditions, non-immunologically related, such a population will be of little use in studying conditions with genetically determined susceptibility. For the same reason, studies for immunological aberrations in occupationally metal-exposed individuals would profit substantially from the use of genotypic determinations.

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