HEAVY METALS AND FERTILITY

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Heavy metals have been identified as factors affecting human fertility. This study was designed to investigate whether the urinary heavy metal excretion is associated with different factors of infertility. The urinary heavy metal excretion was determined in 501 infertile women after oral administration of the chelating agent 2,3-dimercaptopropane-1-sulfonic acid (DMPS). Furthermore, the influence of trace element and vitamin administration on metal excretion was investigated. Significant correlations were found between different heavy metals and clinical parameters (age, body mass index, nationality) as well as gynecological conditions (uterine fibroids, miscarriages, hormonal disorders). Diagnosis and reduction of an increased heavy metal body load improved the spontaneous conception chances of infertile women. The DMPS test was a useful and complementary diagnostic method. Adequate treatment provides successful alternatives to conventional hormonal therapy.

The prevalence of infertility has increased from 8 to 15% over the past 2 decades in industrialized countries (Templeton et al., 1990; Dondero et al., 1991; Runnebaum et al., 1997). Hormonal disorders, such as hypo- or hyperthyroidism, hyperprolactinemia, or hyperandrogenemia, are the main causes of female infertility in Western populations (Runnebaum et al., 1997).

Certain environmental factors may influence the female endocrine system, and thus may play a role in the increasing infertility problem. Heavy metals, for example, induce modifications of neurotransmitters in the central nervous system and impair the pulsatile, hypothalamic release of gonadotropin-releasing hormone (GnRH) (Klages et al., 1987; Cagiano et al., 1990; Duhr et al., 1991; Lindstrom et al., 1991; McGivern et al., 1991; Foster et al., 1993; Lakshmana et al., 1993). A number of harmful substances, such as mercury, are stored in the pituitary gland and affect the production of gonadotropins (Danscher et al., 1990). In the adrenal gland, heavy metals are deposited in the lipid-rich cortex and block various enzymatic pathways, causing hyperandrogenemia or partial hypo-adrenalism (Gerhard et al., 1991; Mgbonyebi et al., 1994). Hypo- and hyperthyroidism can result from lead or cadmium exposure (Klages et al., 1987). Thus, the hypothalamic-pituitary-ovarian axis can be affected by heavy metals either directly or indirectly through modifications of the secretion of prolactin, adrenocortical steroids, or thyroid hormones. In the

ovary itself, accumulation of heavy metals impairs the production of estradiol and progesterone (Watanabe et al., 1982; Wiebe et al., 1988; Pisa et al., 1990; Pasky et al., 1992a, 1992b; Piasek & Laskey, 1994). This may interfere with the normal oocytic development and cause chromosomal damage (Al-Hakkak et al., 1986; Johansson & Pelliciari, 1988). Pregnancies that occur despite an elevated heavy metal body load are at a greater risk of miscarriage, fetal malformation, placental insufficiency, and premature birth (McMichael et al., 1986; Laudanski et al., 1991; Fagher et al., 1993; Farris et al., 1993; Pinon-Lataillade et al., 1993).

Heavy metals are incorporated through food ingestion and inhalation. Lead is commonly used in different industrial areas, for example, paint, print, galvanization, and the fuel industry, and may dissolve from leaded pipes for drinking water and from pottery. Insecticides (e.g., for winegrowing) contain arsenic, which is also used in the metal-processing industry (Daunderer, 1990). Cadmium is ingested with food, such as fish and rice from areas with contaminated groundwater. It is also obtained in different industries and phosphate containing fertilizers (Daunderer, 1990). There are three species of mercury: (1) organic mercury, derived from fish, seafood, fungicides, herbicides, and wood preservatives; (2) inorganic mercury, from antiseptic and dermatological preparations; and (3) elemental mercury, used in the production of batteries, thermometers, and fluorescent tubes (Daunderer, 1990). Dental amalgam fillings contain elemental mercury in concentrations up to 50%. According to the World Health Organization (WHO) and other studies, amalgam tooth fillings were identified as the major source of mercury contributing to the mercury body burden in humans (Snapp et al., 1989; World Health Organization, 1991; Drasch et al., 1997). The determination of blood or urinary heavy metal concentrations is only useful in cases of acute intoxication. As heavy metals are quickly deposited in body tissues, metal level determinations are poor indicators of the body burden in cases of chronic low-level exposure. The extent of chronic exposure can only be estimated by stimulation tests. The sodium salt of 2,3-dimercaptopropane-1-sulfonic acid (DMPS) has been used efficiently and safely in humans intoxicated with heavy metals, such as mercury, arsenic or lead. Since Cherian et al. (1988) have shown that the body content of a heavy metal can be estimated from its urinary concentration after DMPS challenge, this test has been widely used in human diagnostics over the last 10 yr (Cherian et al., 1988; Aposhian et al., 1992; Gerhard et al., 1992; Gonzalez-Ramirez et al., 1995). This study was designed to investigate whether the body burden of heavy metals as estimated by the DMPS challenge test has an impact on the hormonal profile and fertility in women.

MATERIALS AND METHODS

Subjects

In 501 women, aged 30 ± 7 yr, the heavy metal body load was determined in addition to the usual endocrinological investigations in our de-

partment from 1991 to 1993. Ninety-one percent of the women were German. At 3%, Turkish women presented the largest group among the foreign nationalities. The occupations of the women were as follows: 20% housewives, 21% in commerce, 23% in services trade, 14% in health service, 11% teachers or social employees, 5% high-school or university students and 6% industrial workers.

Twenty-four percent of the women had a known allergy to metals, house dust or pollen; 17% suffered from thyroid dysfunction. All women were medically fit and symptom-free at the time of this study. The women were on no medication except for those on thyroxin for hypothyroidism.

Fifty-one percent were lifelong nonsmokers, 17% had given up smoking for more than 2 yr, and 32% smoked an average number of 5 cigarettes daily (range 2–30). Thirty-eight percent drank no alcohol, 14% drank on a regular basis, and 48% only occasionally. The dental status was known in 329 women: 32% had less than 4, 36% had 5–9, and 32% had 10 or more amalgam tooth fillings. Seventy-four percent of the women were referred because of infertility (failure to conceive after 2 yr of regular intercourse without contraception), which lasted 2 yr in 37%, 3–5 yr in 39%, 6–9 yr in 17%, and 10 yr or more in 7%. The other 26% of the women complained of similar gynecological conditions as the 74% with infertility but did not have a wish for a child. Of the women with infertility, 63% presented with primary sterility (patient has never been pregnant). Secondary sterility was found following normal delivery in 11%, miscarriages in 21%, and abortions in 5%.

One hundred and sixty-five women presented with symptoms of hyperandrogenism (discussed later): Hirsutism was diagnosed in 59 patients, alopecia in 51, acne in 29, PCO syndrome [ultrasound, histology, luteinizing hormone (LH)/follicle-stimulating hormone (FSH) ratio > 1.5] in 15, and multiple symptoms in 11 patients. One Hundred and seventeen women were obese (body mass index, BMI ≥ 25 kg/m^2). Thirty patients had endometriosis (laparoscopy, histology), and 18 women uterine fibroids.

Individual treatment was given as indicated by the underlying cause of infertility: hormonal therapy [Clomiphene, FSH, human menopausal gonadotropin (HMG)] to improve follicular maturation and luteal function, homologous insemination, corticosteroids in women with hyperandrogenemia, bromocriptine in patients with hyperprolactinemia, and surgical intervention in patients with uterine abnormality and endometriosis (if indicated).

Investigations and Hormonal Analysis

On d 2–5 of the menstrual cycle, a random blood sample was taken to determine the following hormones: follicle-stimulating hormone (FSH), luteinizing hormone (LH), prolactin, testosterone (T), dehydroepiandrosterone sulfate (DHEAS), and estradiol-17β. Thyroid-stimulating hormone (TSH) was measured basally and after stimulation with thyrotropin-releasing hormone (TRH). On three different days in the luteal phase of the menstrual cycle, estradiol and progesterone were determined. The hormonal analyses were performed with commercially available radio-

immunoassays (RIA); the intra- and interassay variance was less than 10% for all assays applied. Details have been described recently (Gerhard & Runnebaum, 1992c). The infertile women had the following routine investigations: check of tubal patency (pertubation, hysterosalpingography, and/or chromolaparoscopy), sperm analysis of the spouse, Sims–Huhner postcoital test and in vitro sperm-mucus penetration test according to Kremer, and determination of antisperm antibodies. Details about these additional tests have been described previously (Gerhard et al., 1992).

Heavy Metal Analysis

The DMPS test was performed as follows: After a 12-h fast, a 10-ml urine sample was obtained at 8.00 h. DMPS (Dimaval, Heyl Co., Berlin) was given orally (10 mg/kg body weight). A further 10 ml of urine was collected after 2 and 3 h. The concentrations of mercury (Hg), lead (Pb), copper (Cu), cadmium (Cd), and arsenic (As) were determined in each sample with the following analytical methods: As, Hg: hydride atomic absorption (AAS); Cd, Pb: graphite tube AAS; Cu: flame AAS. The concentrations were set in relation to the corresponding creatinine content of the sample. The sensitivity was 1 μg/L, and the intra- and interassay coefficients of variation were less than 15% for all methods applied.

In women with elevated Hg excretion and dental amalgam, a chewing test was performed additionally ($n = 255$): After an overnight fast, 5 ml saliva was collected before and during 10 min of chewing (sugar-free chewing gum) to determine Hg and tin (Sn) levels.

Statistical Analysis

Nonparametric tests were applied. The Spearman correlation coefficient was used to compare two continuous variables, and the Kruskal–Wallis test to compare continuous with discrete variables after constitution of percentiles to convert continuous into discrete variables. The chi-squared test or Fisher's exact test was applied for discrete variables. The level of significance was $\alpha = .05$.

Multivariate regression analyses were applied for the following target variables:

1. Endometriosis: $n = 30$
2. Uterine fibroids: $n = 18$
3. Miscarriage: $n = 87$
4. Primary infertility: ≥ 2 yr; $n = 234$
5. Hyperandrogenemia: early follicular phase testosterone > 500 pg/ml; DHEAS > 4500 ng/ml, $n = 76$
6. Hormonal disorders: hyperandrogenemia, $n = 76$; and/or hyperprolactinemia (prolactin > 700 mE/L, $n = 27$; and/or luteal insufficiency (luteal progesterone < 10 ng/ml), $n = 103$; and/or anovulation, $n = 113$; and/or amenorrhoea (no menstruation for ≥ 3 mo); $n = 18$

7. Pregnancy: within 1 yr after the DMPS test, irrespective of the treatment applied, 40% spontaneous conceptions, $n = 72$
8. Alopecia of unknown origin: $n = 48$

Multivariate regression analysis was applied to test the hypotheses that these target variables were associated with the heavy metal load (Hg, Pb, Cd, As) and general factors (age, BMI, smoking). Univariate logistic regression analysis was applied first to identify independent variables with possible significance in multivariate analysis. To ensure the identification of all important factors, every independent variable was included if the p value of the coefficient was ≤.25. Multivariate analysis was performed first as a complex model including all important factors as identified in the preliminary analysis. For the final analysis, variables were only included if the p values of the coefficients were ≤.05. Age as the most important biological factor was the only exception in this setting, as it was only excluded from multivariate analysis if the p value of the coefficient was clearly >.05.

RESULTS

The urinary heavy metal concentrations during DMPS challenge are shown in Table 1.

General Factors Influencing the Heavy Metal Excretion

The stimulated Hg excretion was significantly greater in younger (<30 yr) than in older (≥30 yr) women [62/80 (median/75th–25th percentile) vs. 31/50 µg/g creatinine], corresponding to the greater median number of amalgam tooth fillings in the former (12/6 vs. 8/5). By contrast, Cd excretion was higher in older women (Figure 1).

Underweight women excreted significantly more Hg after DMPS stimulation than normal-weight or overweight women (Figure 2). Cd excretion after DMPS was significantly lower in German women compared to foreign nationalities (0.44/0.5 vs. 0.71/0.6 µg/g creatinine). The mean duration of residence in Germany of the foreign women was 3 ± 5 yr. No significant associations were found between heavy metal excretion and occupation, smoking habits, or alcohol intake.

Heavy Metal Excretion, Dental Amalgam, and the Reproductive System

Cd excretion was significantly increased in women with a history of previous miscarriages ($n = 87$, Figure 3), uterine fibroids (n = 18, basal 0.42/0.41 vs. 0.25/0.31 µg/g creatinine), or hirsutism (basal 0.43/0.44 vs. 0.37/0.35 µg/g creatinine).

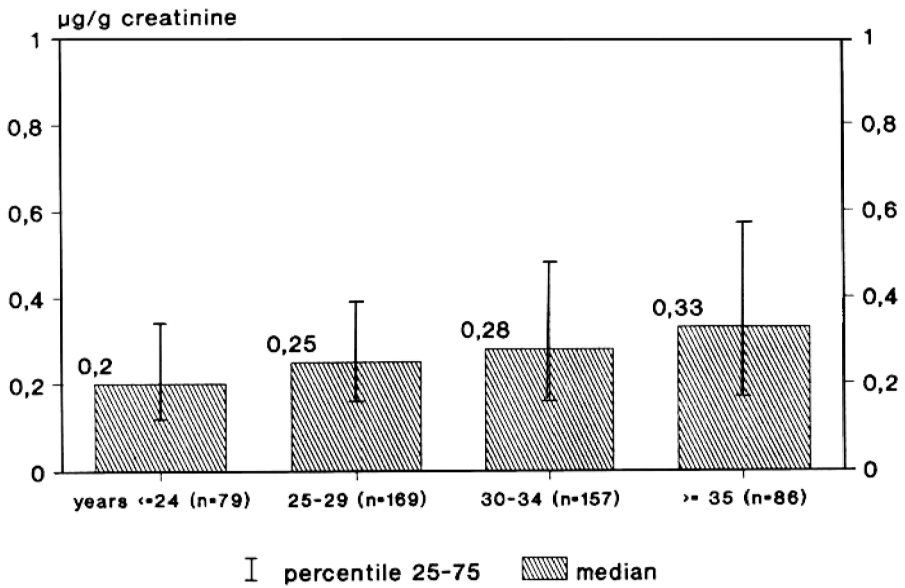If the basal (>4 µg/g creatinine) or stimulated (>150 µg/g creatinine) Hg excretion was elevated (n = 202), secondary infertility (30% vs. 16%), and luteal insufficiency or hyperandrogenemia (51% vs. 32%) were observed

TABLE 1. Urinary heavy metal excretion (in µg/g creatinine) after oral DMPS administration (10 mg/kg) in 501 women with hormonal disorders or fertility problems

Excreted substance (in µg/g creatinine)	Mean	Minimum	10th Percentile	50th Percentile	75th Percentile	90th Percentile	Maximum
Mercury							
basal	2.4	0.2	0.5	1.3	21.1	3.7	63
stimulated	109	0.3	10	46	101	221	11081
Lead							
basal	2.9	0.5	1.0	2.1	3.5	5.3	28.7
stimulated	32	1.0	12	28	41	55	195
Cadmium							
basal	0.4	0.02	0.1	0.3	0.4	0.8	3.5
stimulated	0.7	0.03	0.2	0.5	0.7	1.2	13.4
Copper							
basal	39	2	17	31	44	58	739
stimulated	1378	21	572	1307	1689	2110	16836
Arsenic							
basal	3.4	0.3	1.0	2.7	4.2	6.5	33
stimulated	14	0.6	4	10	17	27	148

Note. Basal and maximal stimulated excretion after 2 or 3 h is shown.

Basal cadmium excretion

µg/g creatinine

0,2   0,25   0,28   0,33

years <=24 (n=79)   25-29 (n=169)   30-34 (n=157)   >= 35 (n=86)

Ⅰ percentile 25-75   ▨ median

p=0,0067

Stimulated cadmium excretion

µg/g creatinine

0,34   0,42   0,49   0,59

years <=24 (n=79)   25-29 (n=169)   30-34 (n=161)   >= 35 (n=89)

Ⅰ percentile 25-75   ▨ median
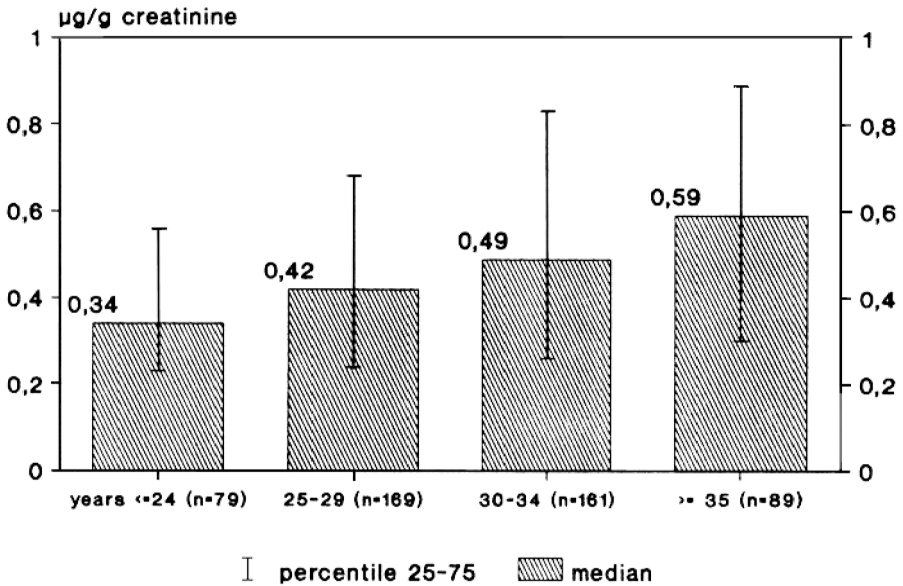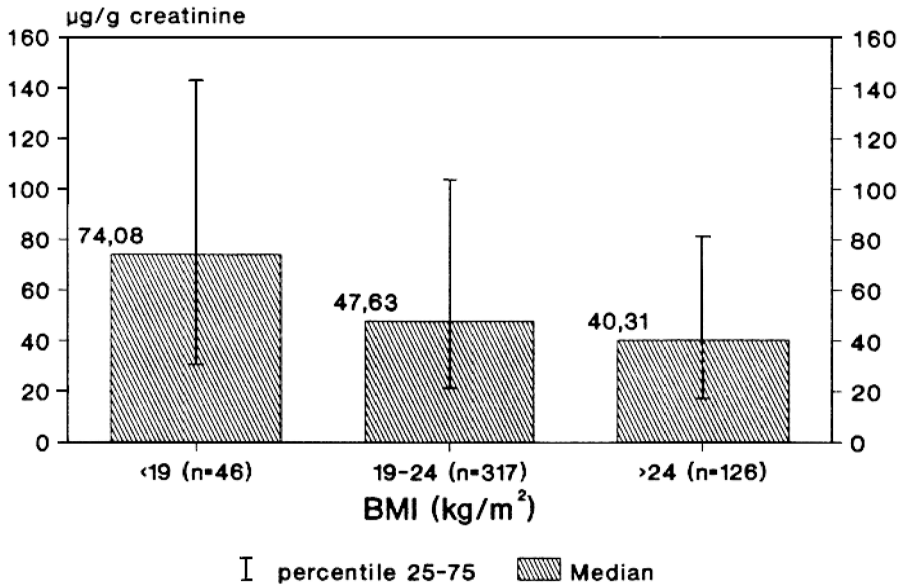
p=0,0017

FIGURE 1. Basal and stimulated urinary cadmium excretion in different age groups.

Stimulated mercury excretion

FIGURE 2. DMPS-stimulated urinary mercury excretion and body mass index (BMI).

more frequently. In the group of women with hormonal disorders, a higher median stimulated Hg excretion was noted (Figure 4). Significantly increased stimulated Hg levels were found in women with thyroid dysfunction ($n = 103$, 52.8/100 vs. 33.4/80 µg/g creatinine), PCO syndrome ($n = 13$, 99.6/110 vs. 44.3/70 µg/g creatinine), and metal allergies ($n = 123$, 67.8/60 vs. 43.5/60 µg/g creatinine).

The saliva concentrations of Hg and Sn rose significantly with increasing numbers of amalgam tooth fillings (Figure 5), as was the stimulated urinary Hg excretion (Figure 6). In contrast, the basal Hg excretion was not influenced by the number of amalgam fillings. Interestingly, in almost 50% of the women the highest salivary Hg level was found before chewing.

On dividing the women according to their stimulated urinary Hg excretion into a subgroup with high (>75th percentile = 230 µg/g creatinine) and lower excretion, the mean number of amalgam tooth fillings and the basal salivary Hg concentration were significantly greater in the former (Figure 7). The difference in salivary Hg levels was even more pronounced after chewing, with four times higher concentrations in women with high urinary Hg excretion. In women with more than 10 amalgam tooth fillings, luteal insufficiency was significantly more frequent than in

the other women (0–4 fillings, 20.3%; 5–9 fillings, 21.6%; ≥10 fillings, 34.7%). For arsenic and copper, no significant correlations were found.

Multivariate Regression: Heavy Metals, Gynecological Conditions, Fertility

The incidence of primary infertility was greater in younger women, whereas recurrent miscarriages were seen more often with increasing age and urinary Cd excretion (Table 2). With increasing urinary Cd excretion women were also more likely to suffer from uterine fibroids. No significant associations were found for women with endometriosis. With increasing age, hormonal disorders and hyperandrogenemia were seen less often. Pregnancy was more likely to occur with increasing urinary Pb excretion. The Pb excretion was 35/25 µg/g creatinine in women who conceived, versus 29.9/22 µg/g creatinine in women who did not.
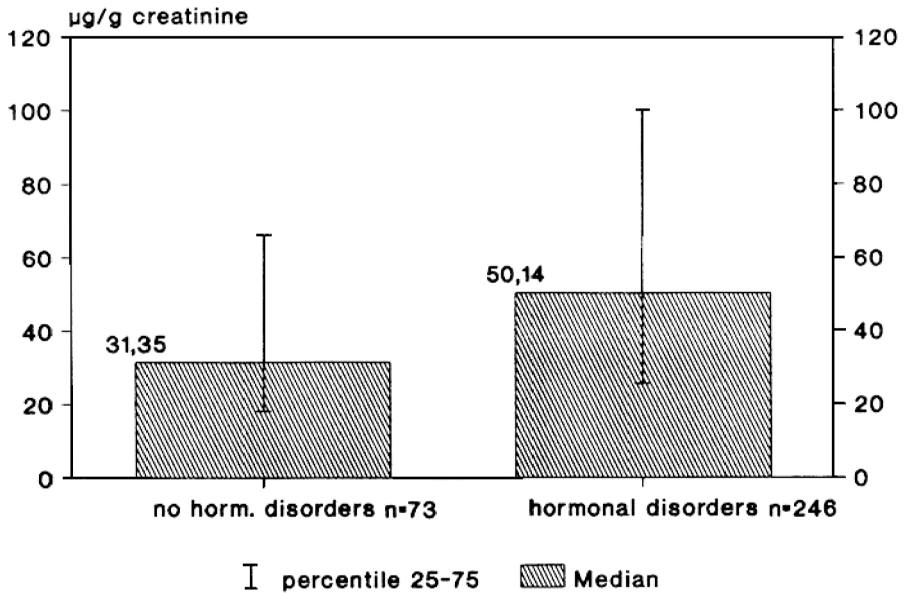
DISCUSSION

Despite general agreement that the basal urinary heavy metal excretion does not reflect conclusions about the body burden, there is contro-
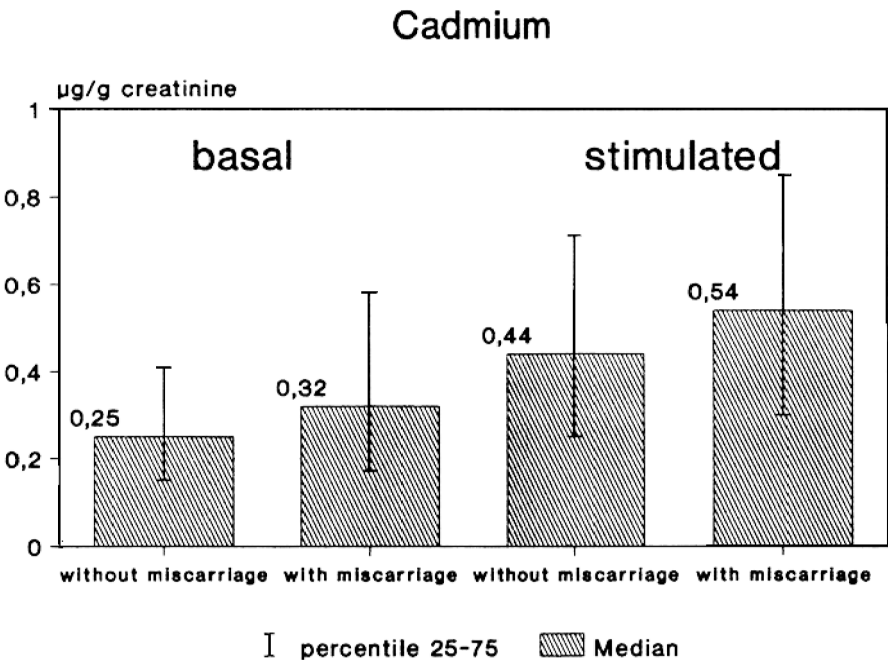
Cadmium

p(bas/stim)=0.034/0.007

FIGURE 3. Basal and stimulated urinary cadmium excretion in women with ($n = 87$) or without ($n = 229$) a history of previous miscarriages.

Stimulated mercury excretion

µg/g creatinine

FIGURE 4. Stimulated urinary mercury excretion in women with ($n = 246$) or without ($n = 73$) hormonal disorders (hyperandrogenemia, hyperprolactinemia, luteal insufficiency).

versy about the role and performance of the DMPS challenge test. As shown in earlier studies, the DMPS-stimulated excretion of all heavy metals reaches a maximum after 2–3 h and decreases thereafter, to return to baseline levels after 8 h (Aposhian et al., 1992; Gerhard & Runnebaum, 1992a, 1992c). Recently it was demonstrated that the most valuable information about the Hg body burden can be obtained from urine samples at maximal excretion 45 min after iv DMPS compared to urine collections over 10 h (Gerhard et al., 1997). This was confirmed by Drasch et al. (1997), who found that the renal mercury content showed the closest correlation to the DMPS stimulated maximal mercury excretion.

Mercury and cadmium concentrations were found to be age dependent (Gerhard et al., 1992). Hg excretion was greater in younger women, since they had more amalgam tooth fillings than older women, who had more gold alloys. In contrast, cadmium levels rose with increasing age. In a recent Chinese study, this association was confirmed for women, but not for men (Qu et al., 1993).

The incorporation of heavy metals into the human body depends on dietary and environmental factors. Foreign women with a mean duration of residence in Germany of 3 ± 5 yr excreted significantly more cadmium

than German women. Nationality-dependent differences in the heavy metal body burden may lead to different causes of infertility. This could explain the result of a previous study, where foreign women were found to respond significantly less successfully to conventional infertility treatment (Gerhard et al., 1990).

It was noted that underweight women excreted significantly more urinary mercury than normal-weight women. The increased mercury body load may be the primary event leading to alterations of metabolism or eating habits, as it is known from Hg intoxication (Daunderer, 1990; Lakshmana et al., 1993). The distribution of mercury and its excretion after DMPS may also be different in underweight subjects. In mice on a protein-reduced diet, feeding of methylmercury resulted in a significantly lower urinary methylmercury excretion than in mice on normal diet (Adachi et al., 1992). After injection of a specific inhibitor of the γ-glutamyl-transpeptidase, the mercury excretion was twice as high in the former as in the latter group. Thus, proteins seem to modulate the metabolism of Hg.

In contrast to other studies, associations between smoking habits and lead or cadmium levels were not found (Qu et al., 1993). This may be
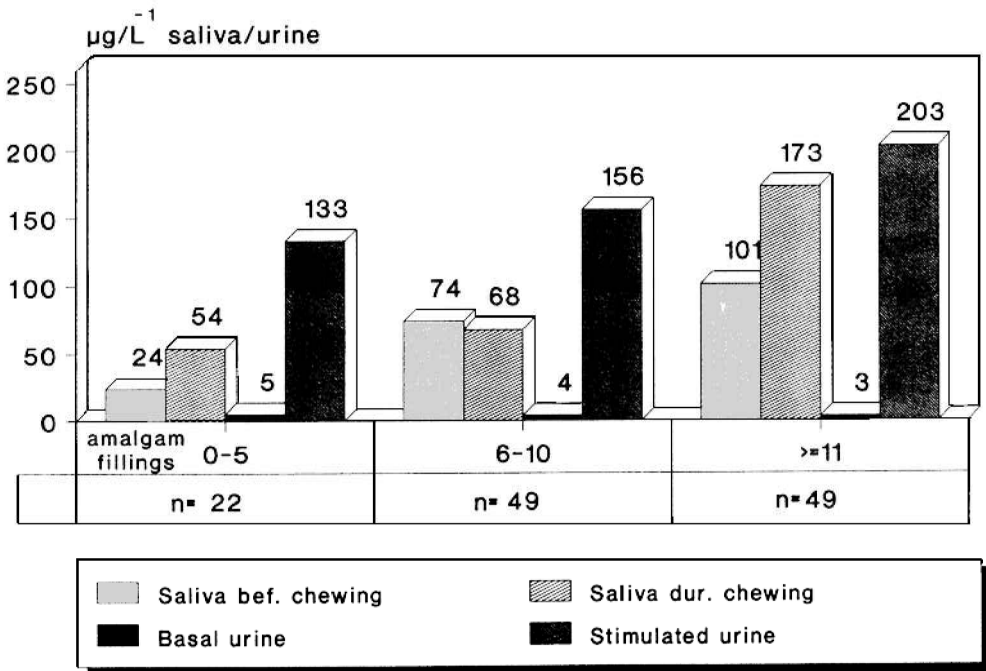
Mercury and tin in saliva
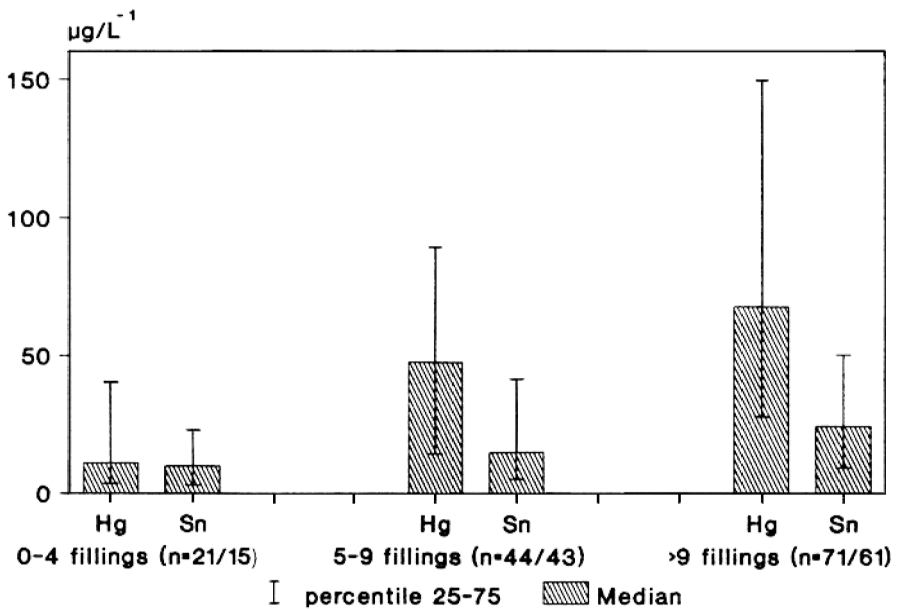
Hg: p=0.0001 Sn:p=0.0223

FIGURE 5. Maximal salivary mercury and tin concentrations during the chewing gum test in relation to the number of amalgam tooth fillings.
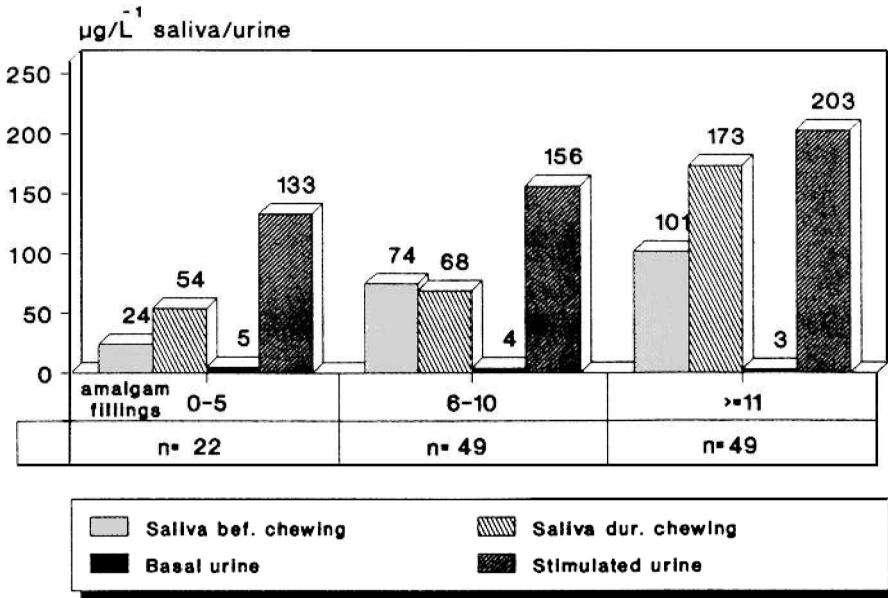
Mercury

μg/L^{-1} saliva/urine

FIGURE 6. Association between the number of amalgam tooth fillings and the mercury release into saliva (chewing gum test) and urine (DMPS test).

due to the low average number of cigarettes smoked by our group of smokers or due to the variable heavy metal concentrations in different brands of cigarettes in different countries (Preston, 1991).

Lead concentrations in blood, urine, and hair depend on the age of the subjects and on environmental factors, such as the place of residence. No age correlation was found in our patients. In recent years, lead pollution has decreased in Germany. However, a greater lead incorporation into the body must be expected in subjects in certain occupations as well as in their families (e.g., workers in car repair shops), resulting in subclinical intoxication (Nunez et al., 1993). An occupational lead exposure was found in only two women. Further tests are required to detect which reproductive parameters and hormones are affected by lead. The somehow puzzling fact of a higher lead excretion in women who conceived during the observation period compared to those with persistent infertility needs to be investigated further. Increased rates of miscarriages have been noted previously in areas with lead contamination (Graziano et al., 1990;

FIGURE 7. Number of amalgam tooth fillings and salivary mercury concentrations (chewing gum test) in women with a high (>75th percentile) or lower (≤75th percentile) DMPS-stimulated urinary mercury excretion.

TABLE 2. Correlations between different gynecological conditions and women's age, urinary lead, or cadmium excretion

Gynecological condition	Estimated		Chi-square	Level of significance
Pregnancy	Age:	−0.0282	2.1819	0.1396
	Pb:	0.00831	4.2667	0.0389
Uterine fibroids	Age:	0.053	3.27	0.070
	Cd:	0.273	7.49	0.006
Recurrent miscarriages	Age:	0.0569	11.6326	0.0006
	Cd:	0.2535	6.7188	0.0095
Endometriosis	—		—	—
Hormonal disorders	Age:	−0.0963	11.3762	0.0007
Primary infertility	Age:	−0.0662	26.7827	0.0001
Alopecia	Age:	0.0346	4.914	0.0266
Hyperandrogenemia	Age:	−0.1225	31.894	0.0001

Murphy et al., 1991; Baghurst et al., 1991; Factor-Litvak et al., 1991; Lindbohm et al., 1991). In animal studies, lead induced changes in pulsatile GnRH secretion as well as prolactin and progesterone concentrations (Klages et al., 1987; Foster et al., 1993). Interestingly, it was noted that intrauterine lead exposure in female animals resulted in disturbances of the menstrual cycle, infertility, and receptor defects at the time of puberty (Wiebe et al., 1988; McGivern et al., 1991). Thus, hormonal disorders in this reproducing generation may be due to lead exposure of their mothers, which might have been greater at the time. Behavioral disturbances and diminished cognitive skills in children that were associated with increased maternal blood lead levels may be indicative of this connection (Ernhart & Green, 1990; Koren et al., 1990; Bellinger et al., 1991, 1992; Conaway et al., 1992; Huel et al., 1992; Lewis et al., 1992; Dietrich et al., 1993; Colborn et al., 1996).

Though the lowest heavy metal concentrations were found for cadmium, it is more toxic than the other heavy metals. Cadmium in low concentrations can impair the synthesis of DNA, RNA, and ribosomes. In the itai-itai epidemic, the Cd urinary excretion remained elevated for years after exposure and was the best indicator for the total Cd body load (Nogawa & Kido, 1993). An association between increased Cd concentrations and uterine fibroids was found. Biopsy studies are required to determine whether Cd is deposited in the uterine wall and contributes to the degenerative changes. In our study, women with a history of previous miscarriages exhibited a higher cadmium excretion. In rats and mice, the time of Cd exposure during pregnancy determined whether miscarriage, growth retardation, or fetal malformation was induced (Padmanabhan & Hameed, 1990; Soukupova & Dostal, 1991; De et al., 1993; Pinon-Lataillade et al., 1993). With increasing Cd accumulation in oocytes, the number of oocytes reaching the second metaphase dropped significantly (Pisa et al., 1990). In mice, a single subcutaneous Cd injection on the first day of pregnancy lead to a time- and dose-dependent Cd accumulation in the tubes and a significant decrease of ovarian progesterone production resulting in infertility (Pasky et al., 1992a). Urinary Cd concentrations were also elevated in women with hirsutism. Cd is stored preferentially in renal and adrenal tissue. The latter may reduce the enzymatic activity of 21-hydroxylase and 3β-hydroxysteroid dehydrogenase, resulting in the synthesis of more potent androgens.

Over the past decade, the safety of amalgam tooth fillings has been under investigation (Lorscheider et al., 1995). The DMPS-stimulated urinary Hg excretion depends significantly on the number of amalgam tooth fillings. It is well known that the daily mercury incorporation is more a result of absorption from amalgam tooth fillings than from food, water, or air (World Health Organization, 1991). Hg accumulation occurs in endocrine organs, especially the pituitary and the adrenal glands (Nylander et al., 1989; Störtebecker, 1989; Danscher et al., 1990; Hahn et al., 1990;

Drasch et al., 1992). This may explain the increased rate of hormonal disorders observed among women with increased mercury load. Adrenal Hg deposition may cause enzymatic defects resulting in hyperandrogenemia and PCO syndrome (Gerhard & Runnebaum, 1997). Hg also induces neurotransmitter changes in certain cerebral areas, which may influence hormonal feedback mechanisms (Lakshmana et al., 1993; Siblerud et al., 1993). In peripheral rat nerves, a retrograde axonal transport of Hg to the anterior horn cells was demonstrated (Schionning, 1993). In dentists, who are exposed to increased Hg loads by inhalation, the highest Hg levels were found in the pituitary gland, so a retrograde transport may also take place in the olfactory nerve (Störtebecker, 1989). Dietary history did not reveal a high consumption of fish in our patients. Only two patients had been occupationally exposed to inorganic mercury. In a follow-up study of workers with exposure to mercury vapor, urinary Hg excretion was not increased in comparison to controls once the exposure had stopped. However, the Hg excretion was significantly correlated to the amount of dental amalgam present (Ellingsen et al., 1993).

In this study, luteal insufficiency was more frequent in women with a high urinary Hg excretion. In vitro, human granulosa cells produced less progesterone after incubation with low Hg concentrations (Vallon et al., 1995). In female guinea pigs, injections of mercuric chloride at different times of the menstrual cycle lead to anovulation. Menstrual irregularities seem to be more frequent among women with occupational low-dose exposure to Hg compared to controls (Rowlands et al., 1994). In a recent study, the probability to conceive was estimated at 50% for dental nurses compared to 95% for women without Hg exposure (Rowlands et al., 1994). Since mercury can trigger allergies and immunological disorders, embryotoxic antibodies, as detected in rats, may play a role (Gleichmann et al., 1987, 1989; Chambers & Klein, 1993; Hultman et al., 1994).

In our study, the chewing test revealed a significant association between salivary Hg concentrations and the urinary DMPS-stimulated mercury excretion. Concentrations up to 1500 µg Hg/L saliva were found. For comparison, the upper limit for Hg in drinking water set by the WHO is 1 µg/L (World Health Organization, 1991). It was previously shown that chewing results in a significant increase of Hg concentrations in saliva and exhaled air. A daily uptake of 8–30 µg Hg was calculated in subjects with 4–12 fillings (Vimy & Lorscheider, 1985). Arsenic and copper are essential trace elements. However, symptoms of poisoning occur at greatly elevated concentrations which was not observed in our patients. Certainly, a great number of additional factors do influence heavy metal concentrations in the blood and the DMPS challenge test, such as diet, exercise, working and living conditions, smoking, alcohol consumption, and the use of other drugs. In a previous study, significant hormonal changes were observed in men and women and a reduced pregnancy rate was shown due to the absorption of harmful substances from cigarette smok-

ing (Gerhard & Runnebaum, 1992b). Though it was previously shown that alcohol reduced Hg uptake, this could not be confirmed in our study, probably due to the low alcohol intake in our population (Hursh et al., 1980).

The results of our observational study do not permit causative, analytical conclusions. The data can be used, however, as a base to find possible connections and new hypotheses in the etiology of infertility. The DMPS test is a useful instrument to estimate the heavy metal body load. Further controlled studies are required to investigate the hypotheses discussed.

REFERENCES

Adachi, T., Yasutake, A., and Hirayama, K. 1992. Influence of dietary protein levels on the fate of methylmercury and glutathione metabolism in mice. *Toxicology* 72:17–26.

Al-Hakkak, Z. S., Hamamy, H. A., Murad, A. M., and Hussain, A. F. 1986. Chromosome aberrations in workers at a storage battery plant in Iraq. *Mutat. Res.* 171:53–60.

Aposhian, H. V., Bruce, D. C., Alter, W., Dart, R. C., Hurlbut, K. M., and Aposhian, M. M. 1992. Urinary mercury after administration of 2,3-dimercaptopropane-1-sulfonic acid: Correlation with dental amalgam score. *FASEB J.* 6:2472–2476.

Baghurst, P. A., Robertson, E. F., Oldfield, R. K., King, B. M., McMichael, A. J., and Wigg, N. R. 1991. Lead in the placenta, membranes, and umbilical cord in relation to pregnancy outcome in a lead-smelter community. *Environ. Health Perspect.* 90:315–320.

Bellinger, D., Sloman, J., Leviton, A., Rabinowitz, M., Needleman, H. L., and Waternaux, C. 1991. Low-level lead exposure and children's cognitive function in the preschool years. *Pediatrics* 87:219–227.

Bellinger, D., Stiles, K. M., and Needleman, H. L. 1992. Low-level lead exposure, intelligence and academic achievement: A long-term follow-up study. *Pediatrics* 90:855–861.

Cagiano, R., De Salvia, M. A., Renna, G., Tortella, E., Braghiroli, D., Parenti, C., Zanoli, P., Baraldi, M., Annau, Z., and Cuomo, V. 1990. Evidence that exposure to methyl mercury during gestation induces behavioral and neurochemical changes in offspring of rats. *Neurotoxicol. Teratol.* 12:23–28.

Chambers, B. J., and Klein, N. W. 1993. Role of laminin autoantibodies on the embryo toxicity of sera from mercuric chloride treated brown Norway rats. *Reprod. Toxicol.* 7:333–341.

Cherian, M. G., Miles, E. F., Clarkson, T. W., and Cox, C. 1988. Estimation of mercury burdens in rats by chelation with dimercaptopropane sulfonate. *J. Pharmacol. Exp. Ther.* 245:479–484.

Colborn, T., Dumanoski, D., and Myers, J. P. 1996. *Our stolen future.* New York: Dutton Press, Penguin Group.

Conaway, M. R., Waternaux, C., Allred, E., Bellinger, D., and Leviton, A. 1992. Pre-natal blood lead levels and learning difficulties in children: An analysis of non-randomly missing categorical data. *Stat. Med.* 11:799–811.

Danscher, G. P., Horstedt-Bindslev, P., and Rungby, J. 1990. Traces of mercury in organs from primates with amalgam fillings. *Exp. Mol. Pathol.* 52:291–299.

Daunderer, M. 1990. *Handbuch der Umweltgifte.* Landsberg/Lech: Ecomed-Verlag.

De, S. K., Paria, B. C., Dey, S. K., and Andrews, G. K. 1993. Stage-specific effects of cadmium on preimplantation embryo development and implantation in the mouse. *Toxicology* 80:13–25.

Dietrich, K. N., Berger, O. G., Succop, P. A., Hammond, P. B., and Bornschein, R. L. 1993. The developmental consequences of low to moderate prenatal and postnatal lead exposure: Intellectual attainment in the Cincinnati Lead Study Cohort following school entry. *Neurotoxicol. Teratol.* 15:37–44.

Dondero, F., Lenzi, A., Lombardo, F., and Gandini, L. 1991. Therapy of immunologic infertility. *Acta Eur. Fertil.* 22:139–145.

Drasch, G., Schupp, I., Riedel, G., and Günter, G. 1992. Einfluⱴ von Amalgamfüllungen auf die Quecksilberkonzentration in menschlichen Organen. *Dtsch. Zahnärztliche. Z.* 47:490–496.

Drasch, G., Scharl, K., Roider, G., Schiwara, H.-W., and Zilker, T. 1997. Aussagekraft des DMPS-Test auf Quecksilber. *Umweltmed. Forsch. Prax.* 2(1):2–10.

Duhr, E., Pendergrass, C., Kasarskis, E., Slevin, J., and Haley, B. 1991. Hg^{2+} induces GTP–tubulin interactions in rat brain similar to those observed in Alzheimer's disease. *FASEB J.* 5:456.

Ellingsen, D. G., Holland, R. I., Thomassen, Y., Landro-Olstad, M., Frech, W., and Kjuus, H. 1993. Mercury and selenium in workers previously exposed to mercury vapour at a chloralkali plant. *Br. J. Ind. Med.* 50:745–752.

Ernhart, C. B., and Greene, T. 1990. Low-level lead exposure in the prenatal and preschool periods: Language development. *Arch. Environ. Health* 45:342–354.

Factor-Litvak, P., Graziano, J. H., Kline, J. K., Popovac, D., Mehmeti, A., Ahmedi, G., Shrout, P., Murphy, M. J., Gashi, E., and Haxhiu, R. 1991. A prospective study of birthweight and length of gestation in a population surrounding a lead smelter in Kosovo, Yugoslavia. *Int. J. Epidemiol.* 20:722–728.

Fagher, U., Laudanski, T., Schutz, A., Sipowicz, M., and Akerlund, M. 1993. The relationship between cadmium and lead burdens and preterm labor. *Int. J. Gynaecol. Obstet.* 40:109–114.

Farris, F. F., Dedrick, R. L., Allen, P. V., and Smith, J. C. 1993. Physiological model for the pharma-cokinetics of methyl mercury on the growing rat. *Toxicol. Appl. Pharmacol.* 119:74–90.

Foster, W. G., McMahon, A., Young Lai, E. V., Hughes, E. G., and Rice, D. C. 1993. Reproductive endocrine effects of chronic lead exposure in the male cynomolgus monkey. *Reprod. Toxicol.* 7:203–209.

Gerhard, I., and Runnebaum, B. 1992a. Schadstoffe und Fertilitätsstörungen—Schwermetalle und Mineralstoffe. *Geburtshilfe Frauenheilkd.* 52:383–396.

Gerhard, I., and Runnebaum, B. 1992b. Schadstoffe und Fertilitätsstörungen—Genußgifte. *Geburtshilfe Frauenheilkd.* 52:509–515.

Gerhard, I., and Runnebaum, B. 1992c. Grenzen der Hormonsubstitution bei Schadstoffbelastung und Fertilitätsstörungen. *Zentralbl. Gynäkol.* 114:593–602.

Gerhard, I., and Runnebaum, B. 1997. Environmental contamination and infertility. In *Gynecological endocrinology and reproductive medicine,* eds. B. Runnebaum and T. Rabe, vol. 2, pp. 187–224. New York: Springer-Verlag.

Gerhard, I., Reischmann, T., Eggert-Kruse, W., and Runnebaum, B. 1990. Untersuchungen der Faktoren, die den Schwangerschaftseintritt und -ausgang durch medikamentöse Therapie bei sterilen Frauen beeinflussen. I. Anamnestische und klinische Parameter. *Fertilität* 6:123–125.

Gerhard, I., Becker, T., Eggert-Kruse, W., Klinga, K., and Runnebaum, B. 1991. Thyroid and ovarian function in infertile women. *Human. Reprod.* 6:338–345.

Gerhard, I., Waldbrenner, A., Thuro, H., and Runnebaum, B. 1992. Diagnostik von Schwermetallbelastungen mit dem peroralen DMPS-Test und dem Kaugummitest. *Klin. Lab.* 38:404–411.

Gerhard, I., Frick, A., and Monga, B. 1997. Diagnosis of mercury body burden. *Clin. Lab.* 43:637–647.

Gleichmann, E., Vohr, H. W., Mirtschewa, J., Stiller-Winkler, R., and Vogeler, S. 1987. Zur Induktion von Autoimmunkrankheiten und anderen stimulativen Immunopathien durch Quecksilber und andere Chemikalien. In *Umwelthygiene, Jahresbericht 1986/1987,* vol. 19, ed. K. Beyen, pp. 270–319. Düsseldorf: Gesellschaft zur Förderung der Lufthygiene und Silikoseforschung e.V.

Gleichmann, E., Kimber, I., and Purchase, F. H. 1989. Immunotoxicology: Suppressive and stimulatory effects of drugs and environmental chemicals on the immune system. *Arch. Toxicol.* 63:257–273.

Gonzalez-Ramirez, D., Maiorino, R. M., and Zuniga-Charles, M. 1995. Sodium 2,3-dimercapto-propane-1-sulfonate challenge test for mercury in humans: II. Urinary mercury, porphyrins and neurobehavioral changes of dental workers in Monterrey, Mexico. *J. Pharmacol. Exp. Ther.* 272:264–274.

Graziano, J. H., Popovac, D., Factor-Litvak, P., Shrout, P., Kline, J. K., Murphy, M. J., Zhao, Y. H., Mehmeti, A., Ahmedi, X., and Rajovic, B. 1990. Determinants of elevated blood lead during pregnancy in a population surrounding a lead smelter in Kosovo, Yugoslavia. *Environ. Health Perspect.* 89:95–100.

Hahn, L. J., Kloiber, R., Leininger, R. W., Vimy, M. J., and Lorscheider, F. L. 1990. Whole-body imaging of the distribution of mercury released from dental fillings into monkey tissues. *FASEB J.* 4: 3256–3260.

Huel, G., Tubert, P., Frery, N., Moreau, T., and Dreyfus, J. 1992. Joint effect of gestational age and maternal lead exposure on psychomotor development of the child at six years. *Neurotoxicology* 13:249–254.

Hultman, P., Johansson, U., Turley, S. J., Lindh, U., Eneström, S., and Pollard, K. M. 1994. Adverse immunological effects and autoimmunity induced by dental amalgam and alloy in mice. *FASEB J.* 8:1183–1190.

Hursh, J. B., Greenwood, M. R., Clarkson, T. W., Allen, J., and Demuth, S. 1980. The effect of ethanol on the fate of mercury vapor inhaled by man. *J. Pharmacol. Exp. Ther.* 214:520–527.

Johansson, L., and Pelliciari, C. E. 1988. Lead-induced changes in the stabilization of the mouse sperm chromatin. *Toxicology* 51:11–24.

Klages, K., Sourgens, H., Bertram, H. P., Müller, C., and Kemper, F. H. 1987. Lead exposure and endocrine functions: Impact on the hypophyseal-gonadal axis and the pituitary-thyroid system. *Acta Endocrinol. Suppl.* 283:17–18.

Koren, G., Chang, N., Gonen, R., Klein, J., Weiner, L., Demshar, H., Pizzolato, S., Radde, I., and Shime, J. 1990. Lead exposure among mothers and their newborns in Toronto. *Can. Med. Assoc. J.* 142:1241–1244.

Lakshmana, M. K., Desiraju, T., and Raju, T. R. 1993. Mercuric chloride-induced alterations of levels of noradrenaline, dopamine, serotonin and acetylcholine esterase activity in different regions of rat brain during postnatal development. *Arch. Toxicol.* 67:422–427.

Laudanski, T., Sipowicz, M., Modzelewski, P., Bolinski, J., Szamatowicz, J., Razniewska, G., and Akerlund, M. 1991. Influence of high lead and cadmium soil content on human reproductive outcome. *Int. J. Gynaecol. Obstet.* 36:309–315.

Lewis, M., Worobey, J., Ramsey, D. S., and McCormack, M. K. 1992. Prenatal exposure to heavy metals: Effect on childhood cognitive skills and health status. *Pediatrics* 9:1010–1015.

Lindbohm, M. L., Hemminki, K., Bonhomme, M. G., Anttila, A., Rantala, K., Heikkila, P., and Rosenberg, M. J. 1991. Effects of paternal occupational lead exposure on spontaneous abortions. *Am. J. Public Health* 81:1029–1033.

Lindstrom, H., Luthman, J., Oskarsson, A., Sundberg, J., and Olson, L. 1991. Effects of long-term treatment with methyl mercury on the developing rat brain. *Environ. Res.* 56:158–169.

Lorscheider, F. L., Vimy, M. J., and Summers, A. O. 1995. Mercury exposure from "silver" tooth fillings: Emerging evidence questions a traditional dental paradigm. *FASEB J.* 9:504–508.

McGivern, R. F., Sokol, R. Z., and Berman, N. G. 1991. Prenatal lead exposure in the rat during the third week of gestation: Long-term behavioral, physiological, and anatomical effects associated with reproduction. *Toxicol. Appl. Pharmacol.* 110:206–215.

McMichael, A. J., Vimpani, G. V., Robertson, E. F., Baghurst, P. A., and Clark, P. D. 1986. The Port Pirie cohort study: Maternal blood lead and pregnancy outcome. *J. Epidemiol. Commun. Health* 40:18–25.

Mgbonyebi, O. P., Smothers, C. T., and Mrotek, J. J. 1994. Modulation of adrenal function by cadmium salts: 2. Sites affected by $CdCl_2$ during unstimulated steroid synthesis. *Cell. Biol. Toxicol.* 10:23–33.

Murphy, M. J., Graziano, J. H., Popovac, D., Kline, J. K., Mehmeti, A., Factor-Litvak, P., Ahmedi, G., Shrout, P., Rajovic, B., and Nenezic, D. U. 1991. Past pregnancy outcomes among women living in the vicinity of a lead smelter in Kosovo, Yugoslavia. *Am. J. Public Health* 80:33–35.

Nogawa, K., and Kido, T. 1993. Biological monitoring of cadmium exposure in itai-itai disease epidemiology. *Int. Arch. Occup. Environ. Health* 65:43–46.

Nunez, C. M., Klitzman, S., and Goodman A. 1993. Lead exposure among automobile radiator repair workers and their children in New York City. *Am. J. Ind. Med.* 23:763–777.

Nylander, M., Friberg, L., Eggleston, D., and Björkman, L. 1989. Mercury accumulation in tissues from dental staff and controls in relation to exposure. *Swed. Dent. J.* 13:235–243.

Padmanabhan, R., and Hameed, M. S. 1990. Characteristics of the limb malformations induced by maternal exposure to cadmium in the mouse. *Reprod. Toxicol.* 4:291–304.

Pasky, K., Varga, B., Naray, M., Olajos, F., and Folly, G. 1992a. Altered ovarian progesterone secretion induced by cadmium fails to interfere with embryo transport in the oviduct of the rat. *Reprod. Toxicol.* 6:77–83.

Pasky, K., Varga, B., and Lazar, P. 1992b. Cadmium interferes with steroid biosynthesis in rat granulosa and luteal cells in vitro. *Biometals* 5:245–250.

Piasek, M., and Laskey, J. W. 1994. Acute cadmium exposure and ovarian steroidogenesis in cycling and pregnant rats. *Reprod. Toxicol.* 8:495–507.

Pinon-Lataillade, G., Thoreux-Manlay, A., Coffigny, H., Monchaux, G., Masse, R., and Soufir, J. C. 1993. Effects of ingestion and inhalation of lead on the reproductive system and fertility of adult male rats and their progeny. *Human. Exp. Toxicol.* 12:165–172.

Pisa, J., Cibulka, J., and Ptacek, M. 1990. Effect of subcutaneous application of a single cadmium dose on oocyte maturation in vitro. *Physiol. Bohemoslov.* 39:185–190.

Preston, A. M. 1991. Cigarette smoking—Nutritional implications. *Prog. Food Nutr. Sci.* 15:183–217.

Qu, J.-B., Xin, X.-F., Li, S.-X., and Ikeda, M. 1993. Blood lead and cadmium in a general population in Jinan City, China. *Int. Arch. Occup. Environ. Health* 65:201–204.

Rowlands, A. S., Baird, D. D., Weinberg, C. P., Shore, D. L., Shy, C. M., and Wilcox, A. J. 1994. The effect of occupational exposure to mercury vapour on the fertility of female dental assistants. *Occup. Environ. Med.* 51:28–34.

Runnebaum, B., Rabe, T., Sillem, M., and Eggert-Kruse, W. 1997. Infertility. In *Gynecological endocrinology and reproductive medicine,* eds. B. Runnebaum and T. Rabe, vol. 2, pp. 107–164. New York: Springer-Verlag.

Schionning, J. D. 1993. Retrograde axonal transport of mercury in rat sciatic nerve. *Toxicol. Appl. Pharmacol.* 121:43–49.

Siblerud, R. L., Kienholz, E., and Motl, J. 1993. Evidence that mercury from silver dental fillings may be an etiological factor in smoking. *Toxicol. Lett.* 68:307–310.

Snapp, K. R., Boyer, D. B., Peterson, L. C., and Svare, C. W. 1989. The contribution of amalgam to mercury in blood. *J. Dent. Res.* 68:780–785.

Soukupova, D., and Dostal, M. 1991. Developmental toxicity of cadmium in mice I. Embryotoxic effects. *Funct. Dev. Morphol.* 1:3–9.

Störtebecker, P. 1989. Mercury poisoning from dental amalgam. *Lancet* 3:9.

Templeton, A., Fraser, C., and Thompson, B. 1990. The epidemiology of infertility in Aberdeen. *Br. Med. J.* 301:148–152.

Vallon, V., Roller, E., and Clédon, P. 1995. Schwermetallionen beeinflussen die Progesteronsynthese von humanen Granulosazellen bei i.v.F.-Patientinnen: Anwendung eines alternativen in-vitro-Zytotoxizitätstests. *J. Fertil. Reprod.* 3:31.

Vimy, M. J., and Lorscheider, F. L. 1985. Serial measurement of intraoral air mercury: Estimation of daily dose from dental amalgam. *J. Dent. Res.* 64:1972–1975.

Watanabe, T., Shimada, T., and Endo, A. 1982. Effects of mercury compounds on ovulation and meiotic and mitotic chromosomes in female golden hamster. *Teratology* 25:381–384.

Wiebe, J. P., Barr, K. J., and Buckingham, K. D. 1988 Effects of prenatal and neonatal exposure to lead on gonadotropin receptors and steroidogenesis in rat ovaries. *J. Toxicol. Environ. Health* 24:461–476.

World Health Organization. 1991. Environmental Health Criteria 118, Inorganic Mercury. Geneva: WHO.