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Impact of heavy metals on hormonal and immunological factors in women with repeated miscarriages

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TABLE OF CONTENTS

In 111 women with repeated miscarriages, the urinary excretion of heavy metals was determined in a challenge test with the chelating agent 2,3-dimercaptopropane-1-sulphonic acid in addition to hormonal, chromosomal, immunological and uterine investigations. The heavy metal excretion was correlated to different immunological (natural killer cells, T cell subpopulations) and hormonal (progesterone, oestradiol, prolactin, thyroid stimulating hormone) parameters. We conclude that heavy metals seem to have a negative impact on ovarian as well as on pituitary function. The heavy metal-induced immunological changes may interfere with the physiological adaptation of the immune system to the state of pregnancy with the result of a miscarriage. The observed heavy metal-induced hormonal and immunological changes may be important factors in the pathogenesis of repeated miscarriages.

Key words: DMPS testing/heavy metals/immunology/infertility/repeated miscarriages

Introduction

Hormonal and immunological disorders have been identified as the main causes of repeated miscarriages (Gerhard *et al*., 1981, 1996; Unander *et al*., 1985, 1987; Gerhard and Runnebaum 1986; Makino *et al*., 1992; Clifford *et al*., 1994). The diagnosis of immunologically caused recurrent miscarriages remains difficult and is made on the basis of excluding other common reasons. In recent years, we have been investigating the impact of harmful substances on the endocrine system and fertility in humans. It was observed in animal studies and accidental poisoning cases in humans that the increased uptake of lead, cadmium or mercury interfered with the normal pregnancy course and resulted in miscarriages, fetal malformation and stillbirth (Amin-Zaki *et al*., 1974; Flora and Tandon 1987; McMichael *et al*., 1986; Rowland *et al*., 1994; Colborn *et al*., 1996). In a previous study of infertile women, we found a significantly increased prevalence of hormonal disorders among subjects with high mercury body load. Furthermore, women with a history of miscarriages presented with a significantly greater cadmium body load than the other subjects (Gerhard, 1995a).

This study was designed to investigate possible connections between the heavy metal body load and hormonal or immunological changes in women with repeated miscarriages.

Observational patient group

The heavy metal excretion was investigated in 111 women with a history of at least two miscarriages, median age 31 years (range 21–39 years), in addition to the usual diagnostic procedures in our department between 1989 and 1993 (Table I). Forty-three women had a history of two, 35 subjects of three and 33 women of four or more miscarriages. Eighty women complained of primary miscarriages (patient has never been pregnant before), 31 women of secondary miscarriages (delivery of a least one baby with the same partner). Seventy-nine women complained of early miscarriages (≤12th week of gestation), six women of late miscarriages (13th–28th weeks of gestation). Twenty-six

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women had a history of both types. The mean body weight was 62.6 kg (range 45–98 kg), the median body mass index (BMI, kg/m^2) 22.4 (range 17.4–37.4). Fifteen per cent of the women were housewives, 72% office-workers and 13% industrial workers. Thirty-three women (29.8%) were smokers. Twenty women smoked <10, nine women 11–20 and four women >20 cigarettes per day. The menstrual cycles were found to be eumenorrhoeic (interval 25–30 days) in 70%, polymenorrhoeic (interval <25days) in 3%, oligomenorrhoeic (interval 31 days to 6 weeks) in 24% and amenorrhoeic (interval >6 months) in 3%. All subjects were medically fit and on no medication except for nine women on thyroxine for hypothyroidism.

General investigations

To identify the cause of repeated miscarriages hormonal, uterine, chromosomal and immunological investigations were performed (Table I). In women with luteal insufficiency (two different determinations of luteal progesterone <10 ng/ml) and/or hyperprolactinaemia (two different determinations of early follicular prolactin >450 mE/l) and/or hyperandrogenaemia [early follicular testosterone >500 pg/ml and/or dehydroepiandrosterone sulphate (DHEAS) >4500 ng/ml], the hormonal disorders were considered to be the reason for repeated miscarriages. As long-term treatment is required for thyroid disorders, these were not considered as hormonal disorders as cause of repeated miscarriages in the context of this study. The antiphospholipid syndrome (APS) was suspected in patients with a partial thromboplastin time (PTT) >30 s and/or anticardiolipin-antibodies >6 U/ml, though none of the patients fulfilled the clinical criteria, e.g. previous thrombosis, as defined by Harris (1990). Patients with various positive antibodies with uncertain pathogenetic relevance for repeated miscarriages included women who were positive for antinuclear antibodies (ANA), anti-DNA antibodies, human leukocyte antigen (HLA) antibodies or for blocking factors in cross-match. The details of the hormonal and immunological analyses have been described recently, as were the methods, sensitivity, the coefficients of intra- and interassay variance (Gerhard *et al*., 1991, 1993, 1997a; Daniel *et al*., 1996).

Table I. Investigations of the causes of repeated miscarriages and abnormal results in the total group of women with repeated miscarriages (*n* = 111)

Investigation	*n*	Abnormal results	*n*
Hormonal tests:	111		
Follicular phase: gonadotrophins (FSH, LH), prolactin, testosterone, oestradiol, DHEAS, T3, T4, TSH (basal, 30 min after TRH)		Hyperprolactinaemia	10
		Hyperandrogenaemia	8
		Mixed hormonal disorder	1
Luteal phase: progesterone		Luteal insufficiency	16
Uterine and tubal investigation:	73		
Hysterosalpingography, chromolaparoscopy		Uterus septus, bicornis	5
		Uterine fibroids	1
		Partial or complete blockage of one tube	11
Chromosomal analysis:	79		
Karyogram		Mosaic 46XX (92%), 47XXX (8%)	1
Immunology:			
Anticardiolipin antibodies	93	Positive >6 GPL/ml	6
Anti-DNA	85	Positive	2
Antinuclear antibodies (ANA)	85	Positive	2
HLA antibodies	111	Positive	1
HLA typing of the couple	101	Matching antigens: 1 – *n* = 39, 2 – *n* = 26, 3 – *n* = 12, 4 – *n* = 6	
Lymphocyte subpopulations	102		
Cross-match	85	Positive blocking factors	4
Interleukin-2 receptor	22	Increased (>5000 pg/ml)	7
Immunoelectrophoresis	86	Increased IgM (>2.8 g/l)	11
Various:			
Partial thromboplastin time	90	Prolonged (>30 s)	6
Full blood count and differential, biochemical profile	111		

FSH = follicle stimulating hormone; LH = luteinizing hormone; DHEAS = dehydroepiandrosterone sulphate; TSH = thyroid stimulating hormone; TRH = thyrotrophin releasing hormone; HLA = human leukocyte antigen.

Investigation of heavy metal body load

To estimate the heavy metal body load, a challenge test with the chelating agent 2,3-dimercaptopropane-1-sulphonic acid (DMPS) was performed as follows: After a 12 h fast, a 10 ml urine sample was collected at 08.00 h. DMPS capsules (Dimaval® Heyl Co., Berlin) were given orally (10 mg/kg). A further 10 ml of urine was collected after 2 and 3 h when the maximum excretion takes place (Gerhard *et al*., 1992b). The concentrations of mercury (Hg), lead (Pb), cadmium (Cd) and arsenic (As) were determined in each sample. Zinc (Zn) and selenium (Sn) concentrations were measured only in the basal sample. The concentrations were set in relation to the corresponding creatinine content of the sample. The following analytical methods were used.

Lead and cadmium

Atomic absorption spectrometry (AAS) with graphite tube (Perkin-Elmer 1100 B; Überlingen, Germany). For extraction, sodium diethyldithiocarbamate (NaDDTC) and methylisobutylketone (MIBK) were used. Sensitivity: lead 2 μg/l, cadmium 0.1 μg/l. Linear area: lead up to 100 μg/l, cadmium up to 6 μg/l. Coefficient of variation: lead 50 μg/l up to 5%, cadmium 13 μg/l up to 3%.

Mercury

AAS cold steam technique (Perkin-Elmer 2100) in combination with flow injection system. 100 μl nitric acid was added to the urine. Reduction was performed with sodium boron hydride solution 0.02%. Sensitivity: 1 μg/l. Linear area: up to 200 μg/l. Coefficient of variation: 7 μg/l up to 8%.

Arsenic

AAS hydride technique (Perkin-Elmer 3030) in combination with MHS-20-hydride unit. Direct analysis was performed in comparison to aqueous arsenic (III) standard solution. Sensitivity: 1 μg/l. Linear area: up to 40 μg/l. Coefficient of variation: 10 μg/l up to 7%.

Zinc

Flame technique AAS (Perkin-Elmer 2100). Direct analysis was performed in comparison to aqueous standard solution. Sensitivity: 20 mg/l. Linear area: up to 1000 mg/l. Coefficient of variation: 650 mg/l up to 6.5%.

Selenium

AAS hydride technique (Perkin-Elmer 3030) in combination with MHS-20-hydride unit. Breaking down of the samples was performed with nitric acid and hydrochloric acid. Reduction was performed with sodium boron hydride solution 15%. Sensitivity: 2 μg/l. Linear area: up to 100 μg/l. Coefficient of variation: 70 μg/l up to 9%.

To estimate a simultaneous body load of lead, cadmium and mercury, we used the metal score. In the total group, the values of maximum stimulated urinary excretion were divided into percentiles. Each subject scored 1 to 4 points for the urinary excretion of lead, cadmium and mercury (1 point: excretion <25th percentile; 2 points: excretion 25th–50th percentile; 3 points: 50th–75th percentile; 4 points: 75th–100th percentile). Therefore, the minimum score was 3, the maximum score 12.

Statistical analysis

Statistical analysis was performed using the Statistical Analysis System program package (SAS Institute; Sachs, 1997). For discrete variables, χ^2-test or (for smaller numbers) Fisher's exact test was applied. As the continuous variables were usually not normally distributed, the Spearman correlation coefficient was used. To compare discrete and continuous variables, for two variables the *U*-test according to Wilcox, Mann and Whitney and for more than two independent variables the *H*-test according to Kruskal–Wallis were applied. Differences were considered significant at $P < 0.05$.

Results of general investigations

The results of hormonal, immunological, chromosomal and uterine investigations are summarized in Table I. The following causes of repeated miscarriages were found: hormonal disorders in 35 women (31.5%), uterine abnormalities in six women, chromosomal abnormality in one subject, the antiphospholipid syndrome in 12 women and various positive antibodies with uncertain pathogenetic relevance (ANA, anti-DNA, HLA antibodies or cross-match positive) in nine women. The cause remained unknown in 48 patients.

The differential blood count revealed a reduced lymphocyte count (<1.5/nl) in 20% of the women. The monocytes were increased in 20% (>6%), the platelets in 12% (>440/nl). Women with primary versus secondary miscarriages had a significantly higher platelet (303 versus 250/nl, $P = 0.0004$), total T cell (1.454, 1107–2050 versus 1.238, 788–1665/μl, $P = 0.0406$) and T helper cell count (1002, 670–1320 versus 753, 498–1133/μl, $P = 0.0434$). Subjects with late (2.09, 1.46–2.37 versus 1.59, 1.18–2.23 g/l, $P = 0.06$) or secondary (2.09, 1.55–2.61 versus 1.57, 1.27–2.23 g/l, $P = 0.0414$) miscarriages had significantly higher IgM concentrations than women with

early or primary miscarriages. Serum selenium concentrations (normal range 73–165 µg/l) were reduced in 38.5% and serum zinc (normal range 70–150 µg/l) concentrations in 18.5%.

Results of heavy metal and laboratory findings

The urinary heavy metal excretion is shown in Table II. Basal cadmium excretion was significantly greater in women with primary versus secondary miscarriages (0.33 versus 0.2 µg/g creatinine, $P = 0.0136$). No differences in heavy metal excretion were noted in women with early versus late miscarriages, smoker versus non-smoker or in association with general parameters, e.g. BMI. A significant direct correlation between lead and age was found (Spearman correlation coefficient $r = 0.22973$, $P = 0.02$). The urinary mercury excretion was significantly associated with the number of amalgam tooth fillings (Figure 1). The number of fillings correlated more strongly with the DMPS-stimulated excretion ($r = 0.68$, $P = 0.0001$) than with the basal mercury excretion ($r = 0.35$, $P = 0.001$).

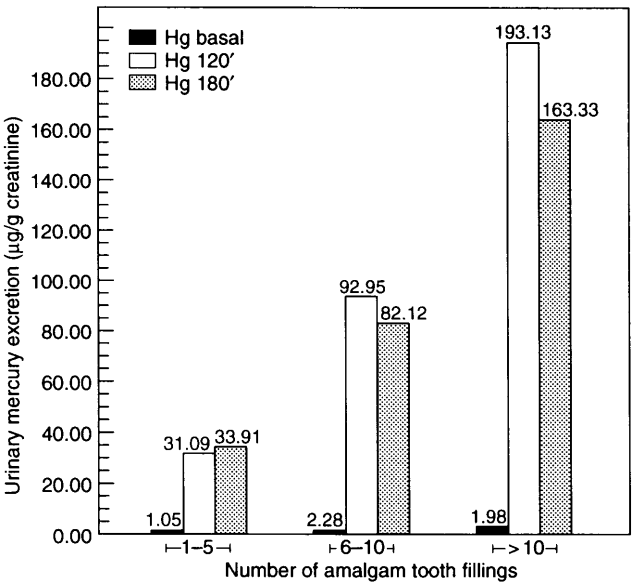

Figure 1. Mean urinary mercury (Hg) excretion and the number of amalgam tooth fillings. Basal and 2,3-dimercaptopropane-1-sulphonic acid-stimulated (120 and 180 min) urinary excretion are shown.

Correlation between heavy metals and laboratory findings

Mercury (Table III)

In women with secondary miscarriages, basal mercury excretion correlated inversely with the percentage of T suppressor cells. The lymphocyte count was directly associated with the stimulated mercury excretion. In women with primary miscarriages, decreasing progesterone concentrations were noted the greater the stimulated urinary mercury excretion. Decreasing stimulated thyroid stimulating hormone (TSH) concentrations were found with increasing basal or stimulated mercury excretion in the total group and in women with secondary miscarriages. Oestradiol concentrations were directly correlated to basal mercury excretion in women with early miscarriages.

Table II. Urinary heavy metal excretion (µg/g creatinine) in the group of women with repeated miscarriages. Excretion is shown at baseline and 120 and 180 min after oral 2,3-dimercaptopropane-1-sulphonic acid stimulation (10 mg/kg body weight)

Excreted substance (µg/g creatinine)	Reference[a]	*n*	Mean	SD	Min. value	25th percentile	Median	75th percentile	Max. value
Arsenic basal	<25	84	4.80	4.58	1	2	3	5	11.11
Arsenic 120 min		78	14.86	18.35	1	4	8	15.5	62.5
Arsenic 180 min		77	15.31	19.63	1.5	5	7	14	44.0
Cadmium basal	<3.0	111	0.52	0.63	0.05	0.15	0.3	0.55	2.5
Cadmium 120 min	<5.0	101	0.86	0.99	0.05	0.3	0.55	1.05	4.35
Cadmium 180 min	<5.0	97	0.84	1.16	0.1	0.3	0.5	0.8	2.8
Mercury basal	<4.0	111	5.4	19.7	0.4	1	1.1	2.55	64.28
Mercury 120 min	<50	102	94.25	105.79	2.54	28.5	60.75	132.7	579.71
Mercury 180 min	<50	99	97.14	104.87	3.5	29.2	66	127.6	410.0
Lead basal	<50	100	3.9	5.21	1	1.5	2	4	32.14
Lead 120 min	<150	102	37.32	40.74	5	15	26	36	188.24
Lead 180 min	<150	98	41.73	33.28	5	20	28	41	122.0

[a]Normal reference values (µg/g creatinine).
n = number of subjects; Min. value = minimum value; Max. value = maximum value.

Table III. Correlation between the basal or maximum stimulated urinary mercury excretion (μg/g creatinine) with different hormonal and immunological parameters

Parameter	*n*	*r*	*P*	Excretion	Groups
Stimulated TSH	85	–0.240	0.0269	Basal	Total group (*n* = 111)
Stimulated TSH	20	–0.630	0.0029	Stimulated	Secondary miscarriages (*n* = 31)
Progesterone	71	–0.2560	0.031	Stimulated	Primary miscarriages (*n* = 80)
Oestradiol	65	0.2757	0.0262	Basal	Early miscarriages (*n* = 79)
OKIa1 positive %	24	–0.608	0.0016	Basal	Secondary miscarriages
CD8 %	20	–0.582	0.0071	Basal	Secondary miscarriages
Lymphocytes	24	0.406	0.0490	Stimulated	Secondary miscarriages
IgA	54	–0.2849	0.0376	Stimulated	Early miscarriages
IgG	18	–0.594	0.0092	Basal	Secondary miscarriages
Platelets	25	0.410	0.0413	Stimulated	Late miscarriages (*n* = 26)

[a]Spearman correlation coefficient.
TSH = thyroid stimulating hormone.

Table IV. Correlation between the basal or maximum stimulated urinary cadmium excretion (μg/g creatinine) with different immunological and other laboratory parameters

Parameter	n	r[a]	P	Excretion	Groups
Oestradiol	65	–0.2701	0.0284	Basal	Early miscarriages
Prolactin	24	–0.4684	0.0210	Stimulated	Late miscarriages
Prolactin	21	–0.442	0.044	Stimulated	Secondary miscarriages (*n* = 31)
Natural killer (NK) cells	85	0.2352	0.030	Basal	Total group (*n* = 111)
NK cells % (cluster CD16)	25	0.506	0.009	Basal	Late miscarriages (*n* = 26)
Lymphocytes/μl blood	59	0.310	0.015	Stimulated	Early miscarriages (*n* = 79)
Zinc (serum)	66	–0.254	0.0393	Basal	Early miscarriages (*n* = 79)
PTT	57	0.2735	0.0395	Basal	Primary miscarriages (*n* = 80)

[a]Spearman correlation coefficient.
PTT = partial thromboplastin time.

Cadmium (Table IV)

The greater the basal excretion, the higher was the natural killer (NK) cell count, especially in women with late miscarriages. The lymphocyte count correlated directly with the stimulated cadmium excretion in women with early miscarriages. Prolactin and oestradiol concentrations were inversely correlated to the basal and stimulated cadmium excretion respectively. Zinc concentrations correlated inversely with basal cadmium excretion.

Lead (Table V)

Basal lead excretion was directly associated with the percentage of NK cells and indirectly with interleukin-2 receptor (IL-2R)-positive cells. In women with late miscarriages, the basal excretion was directly correlated to the T suppressor cell count. Oestradiol concentrations correlated directly with the basal lead excretion, prolactin concentrations inversely with the stimulated lead excretion.

Arsenic (Table VI)

In women with early miscarriages, the stimulated As excretion was inversely associated with the percentage of NK cells. Basal As excretion was inversely correlated to progesterone concentrations.

Metal score

No significant differences in metal score values were found between women with early versus late miscarriages (7.5, 5–9 versus 8.5, 6.5–10, $P = 0.1643$) or primary versus secondary miscarriages (8, 5–9 versus 7, 6–9, $P = 0.7591$). On dividing the total group of patients ($n = 92$) with calculated metal score, in women with a high (>6, $n = 35$) or with a lower (≤6, $n = 57$) score, IgA concentrations (1.83, 1.49–2.34 versus 2.55, 1.9–3.28 g/l, $P = 0.0015$) were lower in the former group. The NK cell (161, 74–248 versus 132, 93–170/μl, $P = 0.05$) and T helper (1045, 701–1372 versus 861, 670–1047/μl, $P = 0.05$) cell count was higher in the group of women with high score.

Table V. Correlation between the basal or maximum stimulated urinary lead excretion (µg/g creatinine) with different immunological and other laboratory parameters

Parameter	n	r[a]	P	Excretion	Groups
Oestradiol	66	0.2935	0.0168	Basal	Total group (*n* = 111)
Oestradiol	68	0.309	0.011	Basal	Primary miscarriages (*n* = 80)
Prolactin	71	–0.3009	0.0034	Stimulated	Total group (*n* = 111)
Prolactin	61	–0.315	0.0074	Stimulated	Primary miscarriages (*n* = 80)
NK cells % (cluster CD16)	86	0.222	0.0398	Basal	Total group (*n* = 111)
Interleukin 2-receptor	20	-0.456	0.0430	Basal	Total group (*n* = 111)
T suppressor cells (cluster CD8)	23	0.525	0.010	Basal	Late miscarriages (*n* = 26)
PTT	21	0.512	0.017	Basal	Late miscarriages (*n* = 26)
Platelets	20	0.3892	0.032	Basal	Late miscarriages (*n* = 26)

[a]Spearman correlation coefficient.
NK cells = natural killer cells; PTT = partial thromboplastin time.

Table VI. Correlation between the basal or maximum stimulated urinary arsenic excretion (µg/g creatinine) with different immunological and other laboratory parameters

Parameter	*n*	*r*[a]	*P*	Excretion	Groups
Progesterone	75	–0.3921	0.0173	Basal	Total group (*n* =111)
NK cells % (cluster CD16)	45	–0.353	0.0172	Stimulated	Early miscarriages (*n* = 79)
IgA	18	–0.566	0.0142	Basal	Late miscarriages (*n* = 26)
Platelets	19	–0.4581	0.0486	Basal	Secondary miscarriages (*n* = 21)
PTT	14	0.633	0.0150	Basal	Late miscarriages (*n* = 26)

[a]Spearman correlation coefficient.
NK cells = natural killer cells; PTT = partial thromboplastin time.

Table VII. Progesterone concentrations, maximum urinary cadmium and mercury excretion and metal score in women with different causes of repeated miscarriages

Parameter	Unit	Group 1[a]	Group 2[b]	Group 3[c]	*P*
Progesterone[d]	ng/ml	4 (2.5–5.5)	12 (9–15)	11 (7.5–15.5)	0.05
Cd max[e]	µg/g creatinine	0.7 (0.46–1.39)	0.3 (0.22–0.56)	0.6 (0.4–1.3)	0.06
Hg max[f]	µg/g creatinine	113 (62–207)	62 (37–152)	57 (34–113)	0.05
Metal score		8.5 (6.5–10)	6.5 (4–8.5)	8 (5–9)	0.16

[a]Group 1: women with hormonal disorders (*n* = 35).
[b]Group 2: women with antiphospholipid syndrome (*n* = 12).
[c]Group 3: women with idiopathic recurrent miscarriages (*n* = 48).
[d]Progesterone in luteal phase of menstrual cycle.
[e]Maximum stimulated urinary cadmium excretion.
[f]Maximum stimulated urinary mercury excretion.

Causes of miscarriages and heavy metals

To evaluate connections between causes of repeated miscarriages and heavy metal excretion, women with hormonal disorders (*n* = 35), antiphospholipid syndrome (*n* = 12) and with unknown reason for repeated miscarriages (*n* = 48) were compared (Table VII). In women with hormonal disorders, lower luteal progesterone concentrations and the greatest stimulated mercury excretion were found.

Discussion

Negative effects of heavy metals on fertility, normal menstrual cycles and the course of pregnancy have been previously noted in human and animal studies. Increased rates of menstrual irregularities and miscarriages were observed among the female staff of dental surgeries dependent on the extent of the mercury exposure (Rowland *et al*., 1994). Chronic lead exposure caused amenorrhoea and anovulation in animals (Vermande-van Eck and Meigs 1960). In rats, intrauterine lead exposure resulted in menstrual irregularity in later life (Wiebke *et al*., 1988; McGivern *et al*., 1991). Increased rates of miscarriages, stillbirths and fetal growth retardation were noted in a lead-contaminated region of South Australia (McMichael *et al*., 1986). The prevalence of miscarriages or infertility was increased among female workers in the ceramics industry with exposure to lead (Rom, 1976). In a previous study of 400 infertile women, the cadmium excretion was positively associated with a history of miscarriages (Gerhard *et al*., 1997b).

The urinary mercury excretion was strongly linked to the number of amalgam tooth fillings present, as published by the World Health Organization in 1991 and previously observed (Vimy and Lorscheider, 1985; Molin *et al*., 1990; Aposhian *et al*., 1992; Gerhard and Runnebaum, 1992, 1997; Gerhard, 1993a; Skare and Engquist, 1994; Lorscheider *et al*., 1995). We found decreasing progesterone concentrations the greater the stimulated urinary mercury excretion. The highest excretion was noted in women with hormonal disorders. The release of mercury vapour from amalgam tooth fillings may interfere with the normal corpus luteum function. In cultures of human granulosa cells, increasing mercury concentrations led to a reduced progesterone production (Vallon *et al*., 1995). In a study of infertile women, the prevalence of luteal insufficiency was significantly increased in women with 10 or more amalgam tooth fillings (Gerhard, 1995b; Gerhard *et al*., 1997a). We also observed a direct correlation between follicular oestradiol concentrations and the mercury or lead excretion. A reduced ovarian function causes an increase of early follicular oestradiol concentrations and results in luteal insufficiency (Gerhard *et al*., 1992a). Arsenic also seems to affect the luteal function negatively, as indicated by the inverse correlation between progesterone concentrations and arsenic excretion. Cadmium, however, correlated inversely with follicular oestradiol concentrations. The cadmium and lead excretion was negatively associated with prolactin, the mercury excretion with stimulated TSH concentrations. Thus to a certain extent, heavy metals seem to have a negative impact on ovarian, especially luteal, as well as on pituitary function, which may be important in the pathogenesis of menstrual irregularity, infertility and repeated miscarriages.

As previously observed, urinary lead excretion increased with age (Schweinsberg and von Karsa, 1990). This association has also been shown previously for cadmium, but was not evident in this study (Schweinsberg and von Karsa 1990; Gerhard and Runnebaum, 1992).
Immunological changes in women, which may be induced by environmental factors, e.g. harmful substances such as heavy metals, may be an important factor causing repeated miscarriages (Gleichmann *et al*., 1987; Colborn *et al*., 1996). The immune system is quite sensitive to the effects of heavy metals (Koller, 1980; Gleichmann *et al*., 1987; Luster *et al*., 1987). Cadmium has been found to act on the cytomembranes and to modulate B lymphocytic function directly (Fujimaki, 1985). It affects IgG synthesis indirectly through interaction with T cells and modulates the interleukin production (Sowa and Steibert, 1985; Schenker *et al*., 1992). Mercury interferes with the monocytic function which also affects the production of T cells and interleukin (Schenker *et al*., 1992). In healthy subjects, the maternal immune system is able to recognize foreign, paternal antigens and to sustain the pregnancy with help of local, immunosuppressive reactions. In the normal pregnancy, the maternal NK cell count decreases. Mallmann *et al*. (1991) found reduced interleukin-2 concentrations in women with recurrent miscarriages which is a key factor in the production of T and B lymphocytes, and thus affects the synthesis of immunoglobulins. Heavy metals have a stimulating effect on the NK cell count and induce changes in the T helper and T suppressor cell populations. We found a direct correlation between the NK cell count on the one hand and a high heavy metal score, cadmium or lead excretion on the other hand. The arsenic excretion was inversely correlated to the percentage of NK cells, however. In women with secondary miscarriages, the mercury excretion was inversely correlated to the percentage of T suppressor cells. Lead was directly associated with the T suppressor cell count and indirectly with the count of IL-2R-positive cells. Cadmium and mercury correlated directly with the total lymphocyte count. These immunological changes may interfere with the physiological adaptation of the immune system to the state of pregnancy with the result of a miscarriage. IL-2 and -12 are known to be produced by interacting lymphocytes and macrophages in the deciduum (Marzusch *et al*., 1997), and are thought to regulate trophoblast invasion, but several other immunological models have been suggested (Christiansen, 1996; Loke and King, 1996). Recent experiments of our group have demonstrated that methods which induce an increase

in T helper cells and a decrease in the total lymphocyte count may prove to be potential treatment in women with repeated early miscarriages (Pfeifer *et al*., 1997).

Up to now significantly different causes of early versus late or primary versus secondary miscarriages could not be proven, which was confirmed in our study. Women with primary miscarriages had a higher count of platelets, total T cells and T helper cells and lower IgM concentrations than subjects with secondary miscarriages. The basal cadmium excretion was significantly greater in women with primary versus secondary miscarriages. We found variable changes of lymphocyte subpopulations in association with heavy metals for women with different types of repeated miscarriages. Thus, though not completely clarified, early versus late and primary versus secondary miscarriages may be caused by different mechanisms altogether.

It was previously noted that the toxic effects of heavy metals can be reduced by the administration of zinc, calcium or vitamin C (Perger, 1989; Gerhard, 1993b). Heavy metals may, therefore, also act via certain trace elements. In our study, zinc and selenium concentrations were decreased in a significant percentage of women, which may potentiate the toxic effects of heavy metals. Cadmium replaces zinc in its cellular linkages (Hoadley and Cousins, 1985). Zinc or iron deficiency results in a 4-fold increase of the intestinal cadmium absorption from food. Furthermore, zinc promotes the renal elimination of cadmium (Sowa and Steibert, 1985). Correspondingly, we found an inverse correlation between cadmium and zinc in women with early miscarriages. In subjects with increased cadmium load, zinc administration improved placental blood supply. In pregnant animals, the administration of cadmium-containing drinking water had fetotoxic effects which were reduced by zinc administration (Sorell and Gaziano, 1990). Selenium is an essential factor in the elimination of mercury (Köstler, 1990; Schrauzer, 1990; Wu *et al*., 1990). In in-vitro and in-vivo studies, selenium completely inhibited the cytotoxic effects of mercury (Wu *et al*., 1990).

In summary, associations between the heavy metal excretion and different hormonal and immunological factors were shown. One has to emphasize that this is an observational study conducted to detect possible associations between different heavy metals and hormonal or immunological changes and to create new working hypotheses for controlled studies. The statistically significant findings (~30) could well have arisen by chance due to the large number of parameters tested. As the results partly show similar changes, however, these findings may indicate real pathogenic and aetiological associations. Heavy metals seem to have a negative impact on ovarian as well as on pituitary function, as seen by inverse associations with progesterone, prolactin and TSH and direct associations with oestradiol. Different heavy metal-induced immunological changes, such as an increase of NK cells and changes of T suppressor and T helper cell counts, may interfere with the physiological adaptation of the immune system to the state of pregnancy with the result of a miscarriage. The observed hormonal and immunological changes due to heavy metals may be important factors in the pathogenesis of repeated miscarriages.

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