Markers of early renal changes induced by industrial pollutants. I Application to workers exposed to mercury vapour

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Abstract

Several markers of renal changes have been measured in a cohort of 50 workers exposed to elemental mercury (Hg) and in 50 control workers. After application of selection criteria 44 exposed and 49 control workers were retained for the final statistical analysis. Exposed workers excreted on average 22 μg Hg/g creatinine and their mean duration of exposure was 11 years. Three types of renal markers were studied—namely, functional markers (creatinine and β_2-microglobulin in serum, urinary proteins of low or high molecular weight); cytotoxicity markers (tubular antigens and enzymes in urine), and biochemical markers (eicosanoids, thromboxane, fibronectin, kallikrein, sialic acid, glycosaminoglycans in urine, red blood cell membrane negative charges). Several blood-borne indicators of polyclonal activation were also measured to test the hypothesis that an immune mechanism might be involved in the renal toxicity of elemental Hg. The main renal changes associated with exposure to Hg were indicative of tubular cytotoxicity (increased leakage of tubular antigens and enzymes in urine) and biochemical alterations (decreased urinary excretion of some eicosanoids and glycosaminoglycans and lowering of urinary pH). The concentrations of anti-DNA antibodies and total immunoglobulin E in serum were also positively associated with the concentration of Hg in urine and in blood respectively. The renal effects were mainly found in workers excreting more than 50 μg Hg/g creatinine, which corroborates our previous estimate of the biological threshold of Hg in urine. As these effects, however, were unrelated to the duration of exposure and not accompanied by functional changes (for example, microproteinuria), they may not necessarily represent clinically significant alterations of renal function.

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The prevention of renal diseases induced by exposure to industrial or environmental chemicals largely relies on the capability to detect nephrotoxic effects at a stage when they are still reversible or at least not yet compromising renal function.[1 2] During the past decade various tests have been proposed for the early detection of toxic effects at different sites on the nephron. Some of these tests have been validated in specific situations (for example, microproteinuria in workers exposed to cadmium),[1] but in most cases an epidemiological validation is still lacking. The need for such a validation has been recently stressed during a CEC-IPCS workshop.[3]

To assess various biological markers of nephrotoxicity, an international collaborative study with several cohorts of workers has been carried out within the European Community. In this study, three cohorts of Belgian workers exposed to lead, cadmium, or Hg vapour have been examined.

The markers of nephrotoxicity analysed fell into three categories arbitrarily defined as (1) functional markers (creatinine and β_2-microglobulin (β_2-m) in

serum, urinary proteins of low or high molecular weight), (2) cytotoxicity markers (tubular antigens and enzymes in urine), (3) biochemical markers (eicosanoids, fibronectin, kallikrein activity, sialic acid, and glycosaminoglycans in urine, red blood cell (RBC) membrane negative charges).

This paper reports the results obtained in the cohort of workers exposed to Hg vapour. The kidney is one of the main target organs during chronic exposure to Hg vapour.[4-6] Both glomerular and tubular effects have been reported.[7-20] The glomerular effects range from an increased prevalence of high molecular weight proteinuria to a nephrotic syndrome.[7-14] The reported tubular changes consist of an enhanced urinary excretion of enzymes (for example, N-acetylglucosaminidase, β-galactosidase)[15-20] or retinol binding protein (RBP).[17] Some studies, however, have failed to find any signs of renal tubular or glomerular impairment, presumably because of low exposure.[21-24]

The cited markers have been measured in a cohort of 50 workers exposed to elemental Hg and in 50 controls. Several bloodborne indicators of polyclonal activation were also examined to determine whether, as suggested by animal studies,[25,26] an immune mechanism might be involved in the renal toxicity of Hg vapour.

Subjects and methods

STUDY POPULATION

The study was carried out on 50 male Belgian workers exposed to Hg vapour in a chloralkali plant for at least one year. The control group consisted of 50 male workers from other departments of the same plant but with no exposure to Hg. From biological monitoring data and information collected through a questionnaire it was ascertained that controls and workers exposed to Hg (1) did not have kidney diseases or any disease likely to impair renal function (diabetes, gout); (2) did not regularly consume drugs with potential nephrotoxicity (analgesics, lithium); (3) had concentrations of lead in blood (Pb-B) lower than 350 μg/l, zinc protoporphyrin (ZPP) in blood lower than 2·5 μg/g Hb, cadmium in urine (Cd-U) below 2 μg/g creatinine and Hg in urine (Hg-U) below (controls) or higher (exposed) than 5 μg/g creatinine. Also, subjects with a urinary creatinine concentration (crt-U; g/l) lower than 0·3 or higher than 4 were excluded.

After the application of the aforementioned criteria the cohort of workers exposed to Hg and the matched control group comprised 44 and 49 subjects respectively.

BLOOD AND URINE SAMPLING

All syringes, tubes, and urine containers were previously checked for the absence of contamination with heavy metals. A sample of venous blood (25 ml) was taken from each participant. Ten ml were immediately transferred to two tubes of 5 ml each containing 50 μl of sodium EDTA (10%) and kept at 4°C. One tube was used for metal and ZPP determination and the other for the measurement of RBC surface negative charges and of the sialic acid concentration in plasma and RBC membranes. The remainder was used for the separation of serum, which was stored at −20°C until the analysis of creatinine, β_2-m, IgE, anti-DNA and antiglomerular basement membrane (GBM) antibodies, and rheumatoid factor.

A spot urine sample (100 ml) was also collected from each subject and immediately divided: 10 ml were kept at 4°C without additives for the analysis of creatinine, cadmium, and Hg; 20 ml were distributed in four tubes of 5 ml each containing 0·4 ml of a 0·2% NaN_3 solution in 1 M phosphate buffer pH 7·6 and kept at −20°C until analysis for albumin, transferrin, IgG, β_2-m, RBP, protein 1, sialic acid, and the brush border tubular antigens (BBA, BB50, and HF5); 10 ml were stored at −20°C with 10 μl of lysine acetylsalicylate (1%) for the analysis of the eicosanoids: 6-keto-prostaglandin $F_{1\alpha}$(6-keto-$PGF_{1\alpha}$), prostaglandin E_2 (PGE_2), prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$), and thromboxane B_2 (TXB_2); 5 ml were kept at 4°C, after the addition of 50 μl of NaN_3 (1%) as preservative, for the analysis of Tamm-Horsfall glycoprotein (THG), glycosaminoglycans (GAG), and N-acetyl-β-D-glucosaminidase (NAG) activity; 5 ml were kept at −20°C without additives for the measurement of pH, kallikrein activity, and sodium concentration; 5 ml were stored at 4°C with 250 μl of a stabilising buffer consisting of 1 M imidazole buffer (pH 7) supplemented with 2% triton X-100, 20 mM benzamidine, 2000 U/ml aprotinine, and NaN_3 (1%). These tubes served for the assays of intestinal alkaline phosphatase (IAP) and tissue non-specific alkaline phosphatase (TNAP) activities; 5 ml were stored at −20°C with 50 μl of phenylmethylsulphonyl fluoride (0·1 M in DMSO) for the analysis of fibronectin.

BIOLOGICAL ANALYSIS

The Hg-B and Hg-U were determined by a cold vapour atomic absorption technique with $SnCl_2$ as reducing agent.[27]

The Cd-U, Cd-B, and Pb-B were determined by electrothermal atomic absorption spectrometry using a Perkin-Elmer Zeeman 3030 spectrometer.[28] The concentration of ZPP in RBCs was measured with a haematofluorimeter (AVIV Associates, Lakewood, NJ).

Creatinine in serum (crt-S) and urine (crt-U) was measured by the methods of Heinegård and Tiderström,[29] and Jaffé[30] respectively, slightly modified for automation (Technicon RA1000; Tarrytown, USA).

Negative charges on RBCs were evaluated by the binding of the cationic dye alcian blue (AB) as described previously.[31] The concentrations of sialic acid in urine, plasma, and RBC membranes were determined by a fluorimetric technique.[31]

The concentrations of albumin, transferrin, IgG, β_2-m, RBP, protein 1, THG, rheumatoid factor, IgE complexes, and of total IgE were determined by an automated non-isotopic immunoassay based on latex particle agglutination.[32 33] The concentrations of anti-DNA antibodies in serum was measured by a radioimmunoassay method (Amersham kit).

The brush border tubular antigens BB50, BBA, and HF5 were analysed by a sandwich enzyme linked immunoassay using monoclonal antibodies.[34] Eicosanoids (6-keto-PGF_{1_2}, PGE_2, PGF_{2_2}, and TXB_2) were measured by a radioimmunoassay after extraction with Sep-Pak C18 cartridges and separation by high performance liquid chromatography.[35] Values of PGE_2 in urine that were abnormally increased (higher than 900 ng/g creatinine) were rejected because of possible seminal contamination.

Concentration of GAG in urine was determined by Whiteman's method.[36] The NAG activity in urine was measured fluorimetrically by the method of Tucker *et al.*[37] Values of urinary kallikrein activity obtained from urine with a sodium concentration lower than 35 mmol/g creatinine were discarded because an abnormally low sodium intake may cause a pronounced increase of this enzyme.[38] The sodium content in urine was analysed by flame atomic absorption.[39] Kallikrein activity was determined by a colorimetric method with the chromogenic tripeptide S-2266 (Kabi Vitrum; Stockholm, Sweden).[40]

Activities of IAP and TNAP were determined by an enzyme antigen immunoassay using monoclonal antibodies.[41] Urinary fibronectin concentration was measured by the enzyme linked immunosorbent sandwich antigen assay of Engval.[42] Polyclonal rabbit antihuman fibronectin immunoglobulin G (Dakopatts, Hamburg, Germany) was used as catching antibody. Enzyme labelling of this antibody with alkaline phosphatase (Sigma, Delsenhofen, Germany) was performed according to Avrameas.[43] The anti-GBM antibodies in serum were measured by a modification of the method of Fish *et al.*[44]

STATISTICAL ANALYSIS

All parameters measured in serum (except β_2-m and creatinine), whole blood, or urine were normalised by log transformation. To assess the influence of age, a stepwise regression analysis was first performed on the control group with crt-U, body mass index (BMI), smoking habit (yes or no), consumption of alcohol (yes or no), and age as independent variables. After standardisation for age, multivariate regression analysis was carried out in the whole population with crt-U, BMI, smoking habits, alcohol consumption, and Hg-B or Hg-U as possible determinants. The effect of duration of exposure was tested by adding this parameter as an independent variable in a multiple regression analysis conducted in the group exposed to Hg. To avoid collinearities, variables considered in the model were centred. In some cases, the association between variables was also evaluated by Pearson's correlation.

Student's *t* test for unpaired data and Duncan's test were used to compare group means. Prevalences of abnormal values were compared by the 2×2 Fisher's exact test. The cut off values were defined as the 95th or the 5th percentile of the values of the control group, depending on whether the abnormality corresponded to an increased or a decreased value. A logistic regression model was used to study the relations between the frequency of abnormal

Table 1 Characteristics of control and workers exposed to Hg

	Control workers (n = 49)		*Exposed workers (n = 44)*		
	Mean or No	*SD or (range)*	*Mean or No*	*SD or (range)*	*p Value**
Age (y)	29·8	9·5	38·6	9·8	<0·0001
Body mass index	24·2	2·6	25·5	3·0	<0·05
Smokers	17		10		NS
Duration (y)	13·1	11·0	19·7	11·2	NS
Cigarettes/day	13·0	9·3	16·3	6·8	NS
Alcohol drinkers	45		33		<0·05
Glasses/week	13·5	16·6	11·1	14·0	NS
Exposure (y)	—		11·2	6·8	—
Hg-U† (μg/l)	2·3	(0·5–10·8)	31·9	(3·7–169)	<0·001
Hg-U† (μg/g creatinine)	1·6	(0·6–4·7)	21·9	(5·1–92·0)	<0·001
Hg-B† (μg/l)	1·0	(0·5–5·4)	7·2	(1·1–30·3)	<0·001
Cd-U† (μg/l)	0·47	(0·12–2·77)	0·63	(0·06–3·2)	NS
Cd-U† (μg/g creatinine)	0·31	(0·10–1·5)	0·44	(0·20–1·4)	<0·05
Pb-B (μg/l)	72	28	71	26	NS
ZPP in blood (μg/g Hb)	0·97	0·24	0·99	0·20	NS
Crt-U (g/l)	1·66	0·77	1·62	0·66	NS

*Student's *t* test or χ^2 test.
†Geometric means.

Table 2 Significant associations found by multiple regression analysis

*Dependent variables**	*Independent variables**	*Partial regression coefficient*	*Partial r^2*	*p Value*
Urine:				
Albumin	Crt-U	0·618	0·220	0·0001
Transferrin	Crt-U	0·629	0·103	0·0017
IgG	Crt-U	0·465	0·129	0·0004
β_2-m	Crt-U	0·867	0·168	0·0001
	Hg-U	−0·173	0·083	0·0022
RBP	Crt-U	0·984	0·541	0·0001
Protein 1	Crt-U	0·756	0·222	0·0001
THG	Crt-U	0·660	0·308	0·0001
	Hg-U	0·164	0·106	0·0001
NAG	Crt-U	0·547	0·355	0·0001
	Hg-U	0·062	0·031	0·0355
BB50	Crt-U	0·718	0·255	0·0001
	Hg-U	0·098	0·032	0·0480
BBA	Crt-U	0·806	0·252	0·0001
HF5	Crt-U	0·527	0·203	0·0001
	Hg-U	0·112	0·054	0·0121
IAP	Crt-U	0·879	0·305	0·0001
	Hg-U	0·167	0·068	0·0025
TNAP	Crt-U	1·558	0·254	0·0001
Fibronectin	Crt-U	0·344	0·231	0·0001
6-keto-$PGF_{1\alpha}$	Crt-U	0·628	0·666	0·0001
	Smoker	0·061	0·026	0·0075
PGE_2	Crt-U	0·881	0·387	0·0001
	Hg-B	−0·210	0·133	0·0001
$PGF_{2\alpha}$	Crt-U	1·218	0·447	0·0001
	Hg-U	−0·167	0·091	0·0001
TXB_2	Crt-U	0·616	0·408	0·0001
	Smoker	0·178	0·124	0·0001
	Hg-B	−0·100	0·070	0·0001
Kallikrein	Crt-U	1·090	0·437	0·0001
GAG†	Crt-U	0·791	0·741	0·0001
	Hg-B	−0·080	0·060	0·0001
Sialic acid	Crt-U	1·049	0·688	0·0001
pH urine	Hg-U	−0·482	0·175	0·0001
	Crt-U	−1·178	0·068	0·0055
Blood:				
Anti-DNA in serum	Hg-U	0·107	0·118	0·0017
Total IgE in serum	Hg-B	0·113	0·057	0·0213

*Blood Hg and variables measured in serum or in urine were log transformed. All urinary parameters were expressed per litre.
†Standardised for age.
For abbreviations see subjects and methods section.

values of the renal markers and Hg-B or Hg-U. All statistical analyses were performed with SAS procedures.[45] In all cases, $p \leqslant 0{\cdot}05$ was considered as statistically significant.

Results

Table 1 presents the characteristics of the controls and workers exposed to Hg. The duration of exposure to Hg vapour ranged from 1·5 to 25 years (on average about 11 years). The cohorts did not differ in smoking habits. Workers exposed to Hg were on average older than their controls and also showed a slightly higher mean BMI and Cd-U. The number of alcohol drinkers was lower in the Hg group (75% *v* 92%); however, the consumption did not seem to be different between control and exposed subjects. The mean values of Pb-B, ZPP, and crt-U were similar in both groups. The Hg-U (μg/g creatinine) and Hg-B (μg/l) were closely related (log Hg-U = 0·292 + 1·09 log Hg-B; r = 0·91, p = 0·0001).

In the control group, only the urinary excretion of GAG was found to be positively associated with age. Table 2 summarises the results of the multivariate correlation analysis performed on the whole study population (n = 93). Only the determinants significantly correlated with the dependent variables, and their partial correlation (r^2) and regression coefficients are listed. As expected all urinary variables were positively correlated with crt-U. The urinary excretion of THG, NAG, BB50, HF5, and IAP, and the serum concentrations of anti-DNA antibodies were also positively associated with Hg-U. The concentration of total IgE in serum was correlated with Hg-B. The urinary output of β_2-m, PGE_2, $PGF_{2\alpha}$, TXB_2, and GAG and urinary pH showed a negative association with Hg-U or Hg-B. None of the renal effects seen in the Hg workers were significantly correlated with the duration of exposure.

After standardisation for the determinants unrelated to exposure to Hg (table 2), workers

Table 3 Urinary and bloodborne markers of nephrotoxicity in controls and workers exposed to Hg

Marker	*Control workers (n = 49)§* *Mean (SD or range)*	*Exposed workers (n = 44)¶* *Mean (SD or range)*	*p Value**
Urine:†			
Albumin (mg/l)	9·2 (2·8–48·3)	8·5 (3·7–64·3)	NS
Transferrin (μg/l)	333 (82–10224)	240 (12–1995)	NS
IgG (μg/l)	1622 (329–5680)	1498 (226–3850)	NS
β_2-m (μg/l)	77 (24–232)	48 (4–277)	<0·01
RBP (μg/l)	96 (34–226)	93 (26–311)	NS
Protein 1 (μg/l)	130 (20–588)	124 (15–605)	NS
THG (mg/l)	16·1 (2·9–65·3)	27·1 (11·4–72·5)	<0·001
NAG (U/l)	1·55 (0·83–4·66)	1·78 (0·38–7·23)	NS
BB50 (U/l)	9·2 (2·0–32·0)	12·5 (1·8–58·3)	<0·05
BBA (U/l)	5·7 (1·1–22·7)	7·9 (1·9–35·7)	<0·05
HF5 (U/l)	6·4 (1·4–37·8)	9·0 (2·4–38·5)	<0·01
IAP (U/l)	0·67(0·13–3·52)	1·02 (0·22–8·26)	<0·01
TNAP (U/l)	0·145 (0·002–1·43)	0·213 (0·005–1·28)	NS
Fibronectin (μg/l)	14·5 (8·7–59·0)	14·3 (74–26·9)	NS
6-keto-$PGF_{1\alpha}$ (ng/l)	292 (159–644)	308 (211–518)	NS
PGE_2 (ng/l)	244 (106–569)	159 (49–411)	<0·001
$PGF_{2\alpha}$ (ng/l)	531 (127–1685)	337 (19–1305)	<0·001
TXB_2 (ng/l)	98 (31–175)	66 (32–177)	<0·001
Kallikrein (U/l)	0·97 (0·29–3·03)	0·79 (0·11–2·58)	NS
GAG (mg/l)	55 (32–78)	46·5 (16·2–92)	<0·001
Sialic acid (mg/l)	304 (145–692)	325 (165–2711)	NS
Blood:‡			
Creatinine/serum (mg/l)	10·8 (1·1)	10·1 (1·1)	<0·01
β_2-m/serum (mg/l)	1·48 (0·31)	1·56 (0·41)	NS
Sialic acid/plasma (mg/l)	601 (67)	601 (75)	NS
Sialic acid/RBC (μg/mg protein)	25·8 (2·1)	25·5 (2·2)	NS
AB binding/RBC (ng/10^6 RBC)	196 (18)	197 (16)	NS

*Student's *t* test.
†Geometric means; ‡arithmetic means.
§For PGE_2 and kallikrein n = 46 and 44 respectively.
¶ For PGE_2 and kallikrein n = 43 and 41 respectively.
For abbreviations see subjects and methods section.
All markers of nephrotoxicity were standardised for the determinants unrelated to Hg exposure (see table 2). Standardisation was based on the mean of the total population.

exposed to the metal presented a significantly higher urinary excretion of THG and several tubular antigens (BB50, BBA, HF5, IAP) than the controls (table 3). Decreased urinary concentrations of β_2-m, GAG, PGE_2, $PGF_{2\alpha}$, and TXB_2 were also found (table 3) as well as a significant reduction in urinary pH (6·74 *v* 6·23, $p < 0·01$). The urinary concentration of β_2-m was correlated with urinary pH ($\log \beta_2\text{-m} = 0·52 + 0·194\,pH$, $r = 0·49$, $p = 0·0001$, Pearson's correlation). In fact, when it was included as an independent variable in the multivariate correlation analysis, urinary pH, and not Hg-U (table 2), was significantly associated with the concentration of β_2-m in urine (partial $r^2 = 0·20$, $p = 0·0001$; partial regression coefficient = 0·19).

No effect of Hg could be found on the concentrations of β_2-m in serum, sialic acid in plasma or in RBCs, or on AB binding to RBC membranes. By contrast, crt-S was on average lower in the group of workers exposed to Hg.

The comparison of prevalences of abnormal values between the two cohorts (table 4) showed almost the same pattern of changes in markers of nephrotoxicity as that seen with their mean values.

To study dose-effect and dose-response relations, workers exposed to Hg and their referents were combined and divided into three groups as a function of Hg-U. The values of 5 μg Hg/g creatinine (upper limit of normal in non-occupationally exposed populations) and 50 μg Hg/g creatinine (the previously proposed critical value for the risk of renal dysfunction)[12 17] were selected as landmarks (table 5 and figure). As well as the effects described, a significant increase in mean urinary NAG activity (table 5) and a decrease in prevalence of urinary kallikrein activity (figure) were found in the group with the highest exposure to the metal. The renal changes were generally dose related whether the comparison was made on the basis of prevalences (figure) or on the basis of mean values (table 5).

Most of these changes reached statistical significance at Hg-U higher than 50 μg/g creatinine. The urinary excretion of several prostaglandins, however, was already reduced at Hg-U below 50 μg/g creatinine. An attempt was made with a logistic regression model to assess further the dose-response relations between Hg-B or Hg-U and the renal effects. With the exception of a statistically significant association between the prevalence of reduced GAG values and Hg-U and between the

Table 4 Prevalences of abnormal values of urinary and bloodborne markers of nephrotoxicity in controls and workers exposed to Hg

Marker	Cut off value†	Control workers (n = 49)‡ No	%	Exposed workers (n = 44)§ No	%	p Value*
Urine:						
Albumin	> 24·5	2	4·1	4	9·1	NS
Transferrin	>1200	2	4·1	3	6·8	NS
IgG	>4222	2	4·1	0	—	NS
β_2-m	> 179	2	4·1	2	4·5	NS
RBP	> 196	2	4·1	1	2·3	NS
Protein 1	> 461	2	4·1	1	2·3	NS
THG	> 35·3	2	4·1	8	18·2	<0·05
NAG	> 3·31	2	4·1	2	4·5	NS
BB50	> 27·4	2	4·1	8	18·2	<0·05
BBA	> 19·0	2	4·1	7	15·9	NS
HF5	> 14·1	2	4·1	12	27·3	<0·01
IAP	> 3·00	2	4·1	4	9·1	NS
TNAP	> 1·04	2	4·1	1	2·3	NS
Fibronectin	> 26·1	2	4·1	1	2·3	NS
6-keto-$PGF_{1\alpha}$	< 221	2	4·1	3	6·8	NS
PGE_2	< 116	2	4·3	13	30·2	<0·01
$PGF_{2\alpha}$	< 297	2	4·1	15	34·1	<0·001
TXB_2	< 65	2	4·1	13	29·5	<0·001
Kallikrein	< 0·42	2	4·5	6	14·6	NS
GAG	< 42	2	4·1	12	27·3	<0·01
Sialic acid	> 455	2	4·1	5	11·4	NS
Blood:						
Creatinine/serum	> 12·8	2	4·1	1	2·3	NS
β_2-m/serum	> 2·23	2	4·1	5	11·4	NS
Sialic acid/plasma	> 685	2	4·1	3	6·8	NS
Sialic acid/RBC	< 22·6	2	4·1	3	6·8	NS
AB binding/RBC	< 172	2	4·1	1	2·3	NS

*Fisher's exact test.
†For the units see table 3 and for abbreviations see subjects and methods section.
‡For PGE_2 and kallikrein n = 46 and 44 respectively.
§For PGE_2 and kallikrein n = 43 and 41 respectively.
All markers of nephrotoxicity were standardised for the determinants unrelated to Hg exposure (see table 2). Standardisation was based on the mean of the total population.

prevalence of decreased PGE_2 values and Hg-B no other significant logistic regression was identified, probably because of the limited number of measurements. For GAG and PGE_2 the prevalences of abnormal results were significantly increased above background when Hg-U and Hg-B exceeded 35 μg/g creatinine and 4·3 μg/l respectively.

Of the immunological parameters measured to test the polyclonal activation hypothesis only anti-DNA antibodies showed a statistically significant increase in the exposed group, for means (table 6) and prevalences of raised values (table 7). Although the total IgE concentration in serum was positively associated with Hg-B its mean value and the prevalence of increased results were not significantly different between controls and workers exposed to Hg. Likewise the serum concentrations of anti-GBM antibodies, IgE complexes, and rheumatoid factors were not significantly affected by exposure to Hg (tables 6 and 7).

Discussion

The cohort of workers exposed to Hg had a mean Hg-U of 22 μg/g creatinine, which indicates a low exposure. In agreement with previous observations,[21 24] this exposure did not lead to an increased urinary excretion of proteins (tables 3 and 5). Exposed workers, however, showed several signs of renal cytotoxicity (increased leakage into urine of tubular antigens and enzymes: BBA, BB50, HF5, IAP, NAG, and THG) and biochemical alterations (decrease in the urinary output of some eicosanoids and GAG).

Together with the changes in urinary excretion of eicosanoids, the increased urinary excretion of THG appears in this study as an early renal effect induced by exposure to Hg vapour (figure and table 5). This increase could reflect an injury to the epithelial cells of the thick ascending limb of the loop of Henle and of the most proximal part of the distal convoluted tubule where this glycoprotein is localised.[46] The physiological function of THG is still obscure. It might have several important functions such as rendering the ascending limb of Henle's loop impermeable to water, transport of sodium (2Cl-K-Na cotransporter), defence against infection, or the immunoregulation of several cytokines.[46 47] Measurement of urinary THG concentration has found little application in the assessment of toxic

Table 5 Mean values of urinary and bloodborne markers of nephrotoxicity as a function of Hg-U (μg/g creatinine)

Marker	*Hg-U < 5 (n = 49)‡*		*5 ≤ Hg-U ≤ 50 (n = 35)§¶*		*Hg-U > 50 (n = 9)‖**	
Urine:*						
Albumin	9·2	(a)††	8·8	(a)	7·3	(a)
Transferrin	333	(a)	257	(a)	182	(a)
IgG	1852	(a)	1616	(a)	1426	(a)
β_2-m	77	(a)	49	(ab)	44	(b)
RBP	92	(a)	94	(a)	89	(a)
Protein 1	130	(a)	123	(a)	129	(a)
THG	16·1	(a)	26·9	(b)	27·9	(b)
NAG	1·55	(a)	1·68	(ab)	2·19	(b)
BB50	9·2	(a)	10·8	(a)	22·0	(b)
BBA	5·7	(a)	6·8	(a)	13·9	(b)
HF5	6·4	(a)	7·8	(a)	15·9	(b)
IAP	0·67	(a)	0·92	(a)	1·53	(b)
TNAP	0·15	(a)	0·17	(a)	0·54	(b)
Fibronectin	14·5	(a)	14·7	(a)	13·0	(a)
6-keto-$PGF_{1\alpha}$	292	(a)	316	(a)	279	(a)
PGE_2	244	(a)	161	(b)	150	(b)
$PGF_{2\alpha}$	531	(a)	335	(b)	344	(b)
TXB_2	98	(a)	79	(ab)	72	(b)
Kallikrein	0·97	(a)	0·82	(a)	0·63	(a)
GAG	55	(a)	48	(a)	41	(b)
Sialic acid	304	(a)	319	(a)	350	(a)
Blood:†						
Creatinine/serum	10·8	(a)	10·2	(ab)	10·0	(b)
β_2-m/serum	1·48	(a)	1·57	(a)	1·53	(a)
Sialic acid/plasma	601	(a)	596	(a)	623	(a)
Sialic acid/RBC	25·8	(a)	25·3	(a)	26·0	(a)
AB binding/RBC	196	(a)	197	(a)	196	(a)

*Geometric means. †Arithmetic means.
‡For PGE_2 and kallikrein n = 46 and 44 respectively.
§For PGE_2 and kallikrein n = 34.
‖For kallikrein n = 7.
¶**Average duration of exposure to Hg 11·8 and 8·5 years respectively.
††Means with the same letter do not differ significantly (Duncan's test).
For units see table 3 and for abbreviations see subjects and methods section.
All markers of nephrotoxicity were standardised for the determinants unrelated to Hg exposure (see table 2). Standardisation was based on the mean of the total population.

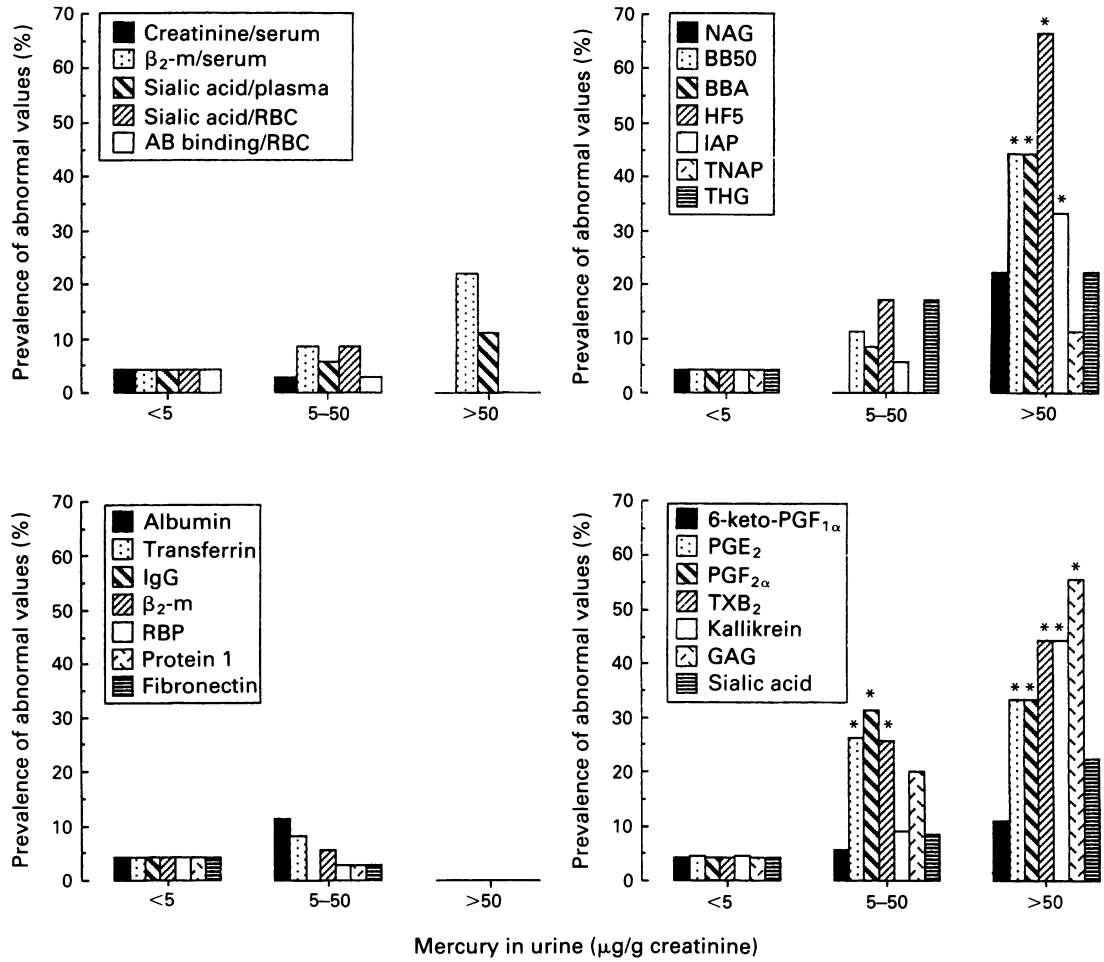
nephropathies and it is presently impossible to evaluate the clinical significance of its increase in the urine of workers exposed to Hg.

The increased urinary excretion of the brush border antigens BB50, BBA, HF5, and IAP (specific for S3 segment) is in all likelihood a reflection of damage to proximal tubular cells.[2 34 41 48] This increase is related to the urinary excretion of Hg (table 2) and appears mainly when Hg-U is above 50 μg/g creatinine (figure and table 5) as this has already been reported in a previous study on BB50 excretion.[48] No correlation was found between urinary excretion of the brush border tubular antigens and that of high (albumin, transferrin, IgG) or low (RBP, β_2-m, protein 1) molecular weight proteins, which is at variance with findings from previous investigations.[34 48 49]

Activity of NAG in urine was significantly increased in the group of workers with Hg-U higher than 50 μg/g creatinine (figure and table 5), which agrees with previously published data[16 18 20] and with the effects seen on excretion of brush border antigens.

Workers exposed to Hg showed a significant reduction of β_2-microglobulinuria that was certainly the consequence of the enhanced degradation of this protein due to lower urinary pH values. This interpretation is corroborated by the significant correlation between pH and β_2-m in the urine of these workers. This decreased excretion of β_2-m, which has been almost always found in studies on workers exposed to Hg vapour,[14 15 17 20 22] cannot be simply accidental and is likely the reflection of an effect of Hg on the urinary excretion of protons. Conceivably, the tubulotoxicity of Hg might be associated with an inhibition of Na^+ transport in the proximal tubules or in the loop of Henle, leading in the distal tubules to a compensatory increase in sodium reabsorption in exchange with H^+ excretion, a process known to occur with several diuretic drugs. This effect might be related to the ability of mercury chloride (like several diuretics) to bind to THG, which, as already mentioned, might be the 2Cl-K-Na cotransporter in the thick ascending limb of the loop of Henle.[47]

The interpretation of the results for urinary prostaglandins is difficult since these represent only a fraction of total renal synthesis. Substantial amounts of prostaglandins can indeed be degraded by or secreted in renal venous blood or can originate from



*Prevalences of abnormal values of markers of nephrotoxicity as a function of urinary Hg in workers exposed to Hg and their referents. Prevalences were established on the basis of the cut off values given in table 4. For group size and duration of exposure see table 5. *Significantly different from the group with Hg-U < 5 μg/g creatinine (2 × 2 Fisher's exact test).*

extrarenal sources.[50-52] It is generally accepted, however, that urinary 6-keto-$PGF_{1\alpha}$ and TXB_2 primarily reflect the glomerular synthesis of prostacyclin and of thromboxane A_2 whereas urinary PGE_2 and $PGF_{2\alpha}$ are largely contributed by the renal medulla.[50] The decrease in PGE_2, $PGF_{2\alpha}$, and TXB_2 concentration that could result from biochemical or cytotoxic effects in the medulla and glomeruli represents, together with the increase in THG excretion, the earliest renal changes associated with exposure to Hg in the present study (table 5 and figure). Inhibition of prostaglandin synthesis in healthy subjects does not induce a significant decline in renal function, presumably because it can be compensated for by other regulatory mechanisms.[53] Nevertheless, the integrity of renal prostaglandin synthesis seems necessary to maintain renal cortical and medullary function in patients with various types of diseases.[50] The decreased synthesis of some eicosanoids induced by exposure to Hg (table 3) might thus be regarded as an adverse effect able to precipitate the decline of renal function in some circumstances (for instance in hypertension, cirrhosis). Further studies are necessary to test this hypothesis.

The GAGs are polysaccharides composed of repetitive disaccharide units. They are found in glomeruli and tubules and their leakage into urine has been suggested to be a marker of injury to the nephron,[54,55] but the significance of GAG for the

Table 6 Immunological markers in control and workers exposed to Hg

Marker (serum)	*Control workers (n = 49) Mean (range)*	*Exposed workers (n = 44) Mean (range)*	*p Value**
Anti-GBM (U l)†	21·0 (10·1–54·4)	19·6 (2·3–46·8)	NS
Anti-DNA (kU l)†	2·2 (0·5–5·0)	3·1 (1·0–6·5)	<0·01
IgE complexes (arbitrary unit l)†	108 (26–799)	120 (28–1293)	NS
Total IgE (kU l)†	467 (124–2053)	557 (191–1504)	NS
Rheumatoid factor (U l)†	928 (441–28076)	902 (426–8998)	NS

*Student's *t* test.
†Geometric means.

detection of glomerular or tubular injury is unclear as no relation has been found with other indices of renal damage in humans.[56] An increased excretion of GAG has also been suggested to be an indicator of damage to the renal papilla,[57] which is rich in GAG.[58] Like other kidney derived components (for example, Tamm-Horsfall glycoprotein), the urinary excretion of GAG is also dependent on renal synthesis, a reduction of which might explain the slight decrease in urinary GAG found in the cohort exposed to Hg (table 3).

Urinary kallikrein is a serine protease (EC 4.4.21.35), synthesised by distal tubule cells, which might serve as an index of distal nephrotoxicity.[40 59 60] As most of the kallikrein is associated with the membranes that face the urinary compartment,[61] an increased urinary excretion of kallikrein could result from toxic damage in distal tubule cells, and this has been reported in experimental molybdenum nephropathy.[60] On the other hand, a decrease as seen in chronic exposure to cadmium[40 59] could indicate a metabolic disorder or dysfunction of the distal tubule. It is noteworthy that kallikrein excretion is decreased in most types of hypertension and the kallikrein kinin system is considered as a hypotensive function of the kidney.[61] In the present study a decreased kallikrein activity in urine was more prevalent in workers with Hg-U above 50 μg/g creatinine (figure) but whether this change reflects a toxic injury to the distal tubules or is an indication of a disturbance in renal haemodynamics remains to be determined.

Dose-effect and dose-response relations (table 5 and figure) suggest that with the exception of reduced prostaglandin excretion, the renal changes found in this study mainly occur when the Hg-U exceeds 50 μg/g creatinine. This confirms our previous estimate[12 17] of the threshold limit value for the risk of nephrotoxicity due to Hg vapour. None of the renal changes found in workers exposed to Hg was related to the duration of exposure. This is of special interest because it supports the view that the renal changes induced by moderate exposure to Hg[15 17] are reversible and mainly the consequence of recently absorbed Hg.

Apart from a slight increase in the serum anti-DNA concentration and a positive association between total IgE concentration in serum and Hg-B, no clear evidence of polyclonal activation was found in workers exposed to Hg.[62] It should be noted, however, that the workers examined in this study had a comparatively low exposure to Hg and none showed signs of glomerular dysfunction. Furthermore, as autoimmune reactions are linked to factors of individual susceptibility,[63] it is also possible that none of the workers examined in the present study had the required genetic predisposition.

In conclusion, although exposure to Hg vapour was low, workers showed significant changes in urinary excretion of prostaglandins and tubular antigens. These changes were related to Hg-B and Hg-U but not to duration of exposure. This supports the view that the changes may be reversible and caused only by recent exposure. Furthermore, as

Table 7 Prevalences of abnormal values of immunological markers in controls and workers exposed to Hg

Marker (serum)	*Cut off value†*	*Control workers (n = 49) No %*	*Exposed workers (n = 44) No %*	*p Value**
Anti-GBM	> 36·7	2 (4·1)	5 (11·4)	NS
Anti-DNA	> 4·0	2 (4·1)	8 (18·2)	<0·05
IgE complexes	> 316	2 (4·1)	3 (6·8)	NS
Total IgE	>1259	2 (4·1)	3 (6·8)	NS
Rheumatoid factor	>2755	2 (4·1)	3 (6·8)	NS

*Fisher's exact test.
†For units see table 6.

they were not associated with changes in the renal handling of proteins, they may not necessarily lead to significant alterations in renal function, although the risk of idiosyncratic reactions or of interaction—for example, with other diseases—cannot be completely ruled out.

Appendix

CONVERSION OF UNITS

Lead 1 μg = 4·83 nmol
Cadmium 1 μg = 8·90 nmol
Mercury 1 μg = 4·99 nmol
Creatinine 1 g = 8·84 mmol

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