

Assessment of renal function in workers previously exposed to cadmium

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ABSTRACT Cadmium induced renal effects were examined in 60 workers (58 men, 2 women) previously exposed to cadmium. Tubular damage in the form of β_2 -microglobulinuria was found in 40%, and urinary albumin and orosomuroid increased significantly with increasing urinary cadmium and increasing relative clearance of β_2 -microglobulin. It is suggested that increased albumin excretion is secondary to the tubular damage. In no case was typical glomerular proteinuria found that could be related to cadmium. Histories of renal stones were more common among the workers with high urinary cadmium concentrations. The glomerular filtration rate was measured in 17 of the workers who had pronounced tubular dysfunction. The average glomerular filtration rate for these men was less than the age adjusted predicted value ($\bar{x} = 84\%$). Furthermore, there was a significant ($p < 0.05$) correlation ($r = -0.47$) between tubular reabsorption loss and a decreased glomerular filtration rate.

Cadmium is well known as a nephrotoxic agent^{1,2} and several groups of investigators have described the urinary findings in cadmium workers.^{1,3-11} The most typical finding is an increased urinary excretion of low molecular weight serum proteins such as β_2 -microglobulin, whereas the excretion of larger proteins, such as albumin, increases much less.^{1,2,13} This is evidence of tubular damage or dysfunction.^{1,2,14-16} The cadmium induced tubular dysfunction may also lead to an increased urinary excretion of other substances that are filtered through the glomerulus and normally reabsorbed in the tubular cells—for example, glucose, aminoacids, and calcium.^{2,17} An increased prevalence of renal stones among cadmium workers has been reported from Sweden¹⁸ and from the United Kingdom,^{19,21} but not from other countries.²

Bernard and his co-workers found that some cadmium workers also have an increased urinary excretion of larger proteins; albumin, transferrin, and IgG, and based on this observation suggested that cadmium may also induce a glomerular type of proteinuria.^{10,11}

In severe cases of renal cadmium poisoning glomerular filtration may also be impaired. Friberg found that the average inulin clearance was lowered among workers who had cadmium induced proteinuria¹ and Adams *et al* reported that most of the men with cadmium induced proteinuria also had decreased creatinine clearance rates.¹⁹ In a few cases the cadmium induced renal damage eventually led to uraemia and death.^{2,22,23}

The objective of the present study was to provide additional information on the nature of the cadmium induced renal effects. Is there a higher prevalence of renal stone disease? Is there evidence for the glomerular and the tubular type of proteinuria? What is the significance of a raised urinary β_2 -microglobulin concentration? Is there any relation between tubular damage and a decrease in the glomerular filtration rate (GFR)?

Subjects

Sixty eight workers who had been exposed for at least five years before 1978 in a factory using cadmium containing solders were invited to take part in a health examination. Six refused and two had died. The remaining 60 (58 men aged 27-78 and 2 women

aged 29–30) took part. The mean age at the time of the health examination was 48.

Exposure

The factory which produces coolers, radiators, and heat exchangers used cadmium containing solders between 1955 and 1978. The whole production hall was heavily contaminated with cadmium, and measurements carried out in 1976 showed atmospheric cadmium concentrations ranging from 0.01 to 2 mg/m³.²⁴ During 1977 and 1978 the environmental conditions were improved, but it was not until the middle of 1978, when cadmium solders were abandoned, that exposure to cadmium ceased completely. Further data about the exposure as well as dose response analyses will be published elsewhere.

Health examination

Each participant was asked to answer a self administered questionnaire that included questions about present and previous diseases, history of renal stones, use of drugs, and smoking habits. The answers given in the questionnaire were compared with those of a reference group of 127 industrially employed men working in the same district but not occupationally exposed to cadmium. The mean age of the reference group was 38.

In acidic urine (pH < 5.6) β_2 -microglobulin may be degraded.²⁵ To prevent this, each person ingested about 4 g of sodium bicarbonate (Samarin) the night before morning urine was collected for protein analyses.

Urine was sampled in carefully washed polyethylene bottles with a small addition (about 10 mg) of sodium azide to prevent bacterial growth. Venous blood samples were taken in 10 ml evacuated test tubes without additives. Serum was obtained after coagulation and centrifugation. Serum and urine samples were kept at a temperature close to 0°C until frozen. The physical examination included measurement of systolic and diastolic blood pressure in the left arm with the patient in the horizontal position. The lung function, as measured by spirometry and single breath nitrogen washout, will be presented elsewhere.

Methods

All chemical analyses were carried out at the Chemical Division, National Board of Occupational Safety and Health. Albumin, β_2 -microglobulin, orosomucoid, and creatinine were determined in serum and urine. Cadmium, specific gravity, and pH were also determined in urine. In addition, all urine sam-

ples were screened for the occurrence of glucose, red blood cells, and bacteria, using indicator paper. Albumin and orosomucoid were measured by zone immunoelectrophoresis²⁶ and β_2 -microglobulin by a radioimmunoassay (Phadebas, Pharmacia, Sweden). Creatinine was determined using a colorimetric method and a reaction rate analyser, LKB 8600.²⁷ Cadmium in urine was measured by atomic absorption spectrophotometry using electrothermal atomisation.²⁸ The urinary excretion of cadmium and proteins was related to the urinary excretion of creatinine, and reported as mg or nmol/mmol of creatinine.

The relative clearance of β_2 -microglobulin was estimated according to the formula²⁹

$$\frac{Cl_{\beta_2}}{Cl_{\text{creatinine}}} (\%) = 100 \times \frac{U-\beta_2 \times S-\text{creatinine}}{S-\beta_2 \times U-\text{creatinine}}$$

The GFR was measured in 17 men who had raised urinary β_2 -microglobulin excretion exceeding 0.06 mg β_2 /mmol creatinine at the Department of Clinical Chemistry in Linköping, using a CrEDTA plasma clearance technique as described by Bröchner-Mortensen.³⁰

Data on urinary albumin excretion from one person (35.8 mg/mmol creatinine) were excluded. This 63 year old man had been diagnosed in 1982 as having an interstitial nephritis or nephrosclerosis of unknown aetiology. His glomerular filtration rate was found to be reduced, 53 ml/min, and he had obtained a sickness pension. In view of his comparably low urinary excretion of cadmium (5 nmol/mmol creatinine) and normal urinary excretion of β_2 -microglobulin he probably suffers from a renal disease not primarily related to, or caused by, occupational exposure to cadmium.

Normal values

The normal urinary excretion of cadmium, albumin, and β_2 -microglobulin has been well documented. The upper 95 or 97.5 percentile for normal urinary cadmium, albumin, and β_2 -microglobulin excretion is about 2 nmol Cd/mmol creatinine,³¹ 1.5 mg albumin/mmol creatinine^{26,32,33} and 0.034 mg β_2 -microglobulin/mmol creatinine.^{32,34} The normal urinary excretion of orosomucoid in one report ranged up to about 0.5 mg/mmol creatinine.³²

Another group of industrial workers (n = 32) not exposed to cadmium was examined shortly after the exposed group in this study. Using the same methods and techniques as for the cadmium exposed group, the urinary cadmium excretion was found to be less than 1.0 nmol/mmol creatinine. The median protein concentrations were: 0.4 mg albumin/mmol creatinine (range 0.1–8.4), 0.02 mg orosomucoid/mmol

creatinine (range <0.003–0.28), and 0.004 mg β_2 -microglobulin/mmol creatinine (range 0.002–0.020).

Normally, about 99.96% of the filtered amount of β_2 -microglobulin is absorbed in the tubules and thus the urinary excretion is about 0.04%.^{15,29} If, for example, the β_2 -microglobulin clearance is 10% of the creatinine clearance this may be regarded as a 10% decrease in the normal tubular reabsorption of β_2 -microglobulin.

CrEDTA clearance was corrected to a body surface area of 1.73 m² based on the height and body weight of the person examined. As GFR decreases with age a ratio was calculated between the observed and predicted GFR according to the age of the subject. Age related changes in GFR when determined by the CrEDTA technique used in the present study have been reported by Granerus and Aurell.³⁵

Results

The completed questionnaires showed no significant differences between the exposed and control groups with regard to subjective complaints of ill health, kidney stones, or other diseases of the kidney. Eight (14%) of the exposed men had a history of kidney stone (stones) compared with 12 (9%) of the controls ($p > 0.10$). Within the exposed group a history of renal stones was, however, significantly ($p = 0.025$ using one tailed exact probability test) more common among those who had had urinary cadmium excretion exceeding the median value for the whole group (6.3 nmol/mmol creatinine) compared with those who excreted less cadmium (table 1). Furthermore, four of the eight men with renal stones had urinary β_2 -microglobulin concentrations exceeding 0.1 mg/mmol creatinine. Only 10 of the remaining 50 men without renal stones had urinary β_2 -microglobulin excretion exceeding the concentration ($\chi^2 = 3.39$ without Yates's correction, $p = 0.07$ two tailed).

Twenty four (40%) of the exposed workers had urinary β_2 -microglobulin concentrations exceeding 0.034 mg/mmol creatinine and 14 (23%) had urinary β_2 -microglobulin concentrations exceeding 0.1 mg/mmol creatinine. The prevalence of proteinuria increased with increasing urinary cadmium (table 2).

The increase was more pronounced for β_2 -microglobulin and orosomuroid than for albumin. At urinary cadmium concentrations exceeding 10 nmol/mmol creatinine the prevalence of raised β_2 -microglobulin, orosomuroid, and albumin was 88%, 88%, and 31% respectively.

The urinary excretion of β_2 -microglobulin, orosomuroid, and albumin correlated significantly ($p < 0.05$) with cadmium (figs 1, 2, and 3). It may be seen in figs 1 and 2 that there is no real linear relation between urine cadmium and β_2 -microglobulin or orosomuroid. In these figures we have therefore also included lines that describe the "floating average" of urine proteins (β_2 -microglobulin and orosomuroid) for the nearest five urine cadmium data, that is

$$y_{x_{i-2}} - x_{i+2} = (y_{x_{i-2}} + y_{x_{i-1}} + \dots + y_{x_{i+2}}) : 5.$$

For urinary β_2 -microglobulin there appears to be a threshold around 9 nmol Cd/mmol creatinine. Above this concentration the average β_2 -microglobulin increases appreciably.

Urinary albumin (fig 3) increases only slightly, but still correlates significantly, with increasing urinary cadmium. The slope constant is, however, considerably lower than those for β_2 -microglobulin and orosomuroid.

Urinary orosomuroid and albumin excretion have been depicted in figs 4 and 5 as a function of increasing relative β_2 -microglobulin clearance

$$\left(\frac{\text{Cl } \beta_2}{\text{Cl creatinine}} \% \right)$$

The urinary excretion of both proteins increases, but not to the same extent. The relative increase in orosomuroid (fig 4) is more pronounced than that for albumin (fig 5).

The GFR was measured in 17 of the workers and the values ranged from 41 to 127 ml/min/m² body surface. For each individual the ratio between observed GFR and the age and sex predicted GFR was estimated. The ratio varied between 57% and 127% with an overall average of 84% (SD = 18.4). This is significantly less ($p < 0.05$) than 100%. In fig 6 the ratio between observed and predicted GFR has been related to the relative clearance of β_2 -microglobulin. A weak but significant ($p < 0.05$) negative linear or rank correlation was found; obs

Table 1 Number of exposed men with a history of renal stones, in relation to urinary cadmium

	U-Cd \leq 6.3 (nmol/mmol creatinine)	U-Cd $>$ 6.3 (nmol/mmol creatinine)	All
History of renal stone(s)	1	7	8
No history of renal stone(s)	28	22	50
All	29	29	58

$\chi^2 = 5.19$ without Yates's correction.
 $p = 0.025$ using one sided exact probability test.

Table 2 Prevalence of β_2 -microglobulinuria, orosomuroiduria, and albuminuria in relation to urinary cadmium

U-Cd (nmol/mmol creatinine*)	U- $\beta_2 > 0.034$ (mg/mmol creatinine†)	U-orosomuroid > 0.5 (mg/mmol creatinine‡)	U-albumin > 1.5 (mg/mmol creatinine§)
≤ 2	1/14 (7%)	1/14 (7%)	2/14 (14%)
2- ≤ 5	3/12 (25%)	0/12 (0%)	1/12 (8%)
5- ≤ 10	6/18 (33%)	4/18 (22%)	1/17 (6%)
10- ≤ 15	4/5 (80%)	4/5 (80%)	1/5 (20%)
> 15	10/11 (91%)	10/11 (91%)	4/11 (36%)
All	24/60 (40%)	19/60 (32%)	9/59 (15%)

*1 nmol Cd/mmol creatinine = 1 μ g Cd/g creatinine.

†0.034 mg β_2 /mmol creatinine = 0.3 mg/g creatinine.

‡0.5 mg orosomuroid/mmol creatinine = 4.4 mg/g creatinine.

§1.5 mg albumin/mmol creatinine = 13.2 mg/g creatinine.

$$\text{GFR/pred GFR (\%)} = 90\% - 1.1 \times \frac{\text{Cl } \beta_2}{\text{Cl creat}} (\%).$$

The slope remained essentially unchanged even when the subjects with the most severe tubular impairment were excluded from the calculation ($a = 90$, $b = 1.2$) but the correlation is no longer significant.

Discussion

This study confirms that occupational exposure to cadmium may cause renal impairment, and that the first sign is an increased urinary excretion of low molecular weight proteins such as β_2 -microglobulin and orosomuroid (figs 1 and 2). A relation was found between urinary cadmium and albumin (fig 3), similar to that previously reported for cadmium exposed workers in Belgium.^{11,32}

A history of renal stones was more common in the exposed group than in the reference group, although this did not attain statistical significance. The refer-

ence group was, however, younger than the exposed group, and as the prevalence of renal stones increases with age³⁶ this difference may be artificial. On the other hand, several workers in the exposed group had relatively low urinary cadmium concentrations and were thus not at the same risk of developing cadmium induced renal effects. Within the exposed group ($n = 58$) men with a history of renal stones had a significantly higher urinary cadmium concentration (table 1) and tended to have a higher urinary β_2 -microglobulin excretion compared with those who had no history of stones. The mean age of the men with stones ($\bar{x} = 49$) was similar to that of the whole group ($\bar{x} = 48$). Thus the present results give some support to the earlier findings of an increased frequency of renal stones in cadmium workers.¹⁸⁻²¹

Table 2 presents the prevalence rates for raised urinary β_2 -microglobulin, orosomuroid, and albumin in relation to urinary cadmium. As no large control group was examined in parallel with the exposed

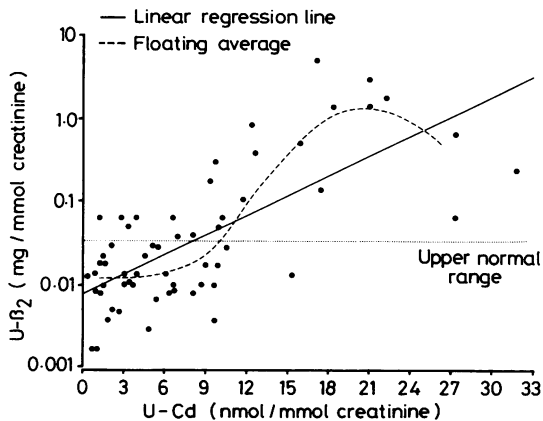


Fig 1 Urinary excretion of β_2 -microglobulin in relation to urinary excretion of cadmium. Linear regression line; $^{10}\log U-\beta_2$ (mg/mmol creatinine) = $0.008 + 0.080 \times U-Cd$ (nmol/mmol creatinine).

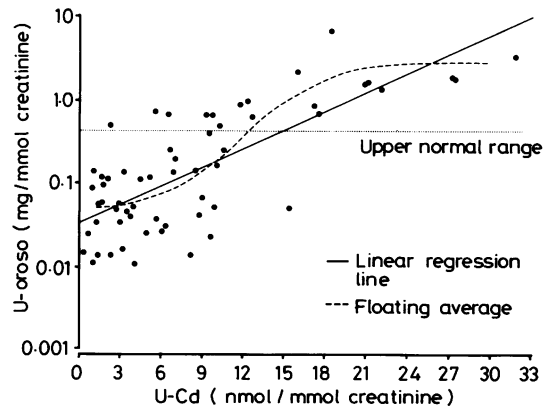


Fig 2 Urinary excretion of orosomuroid in relation to urinary excretion of cadmium. Linear regression line; $^{10}\log U$ -orosomuroid (mg/mmol creatinine) = $0.033 + 0.076 \times U-Cd$ (nmol/mmol creatinine).

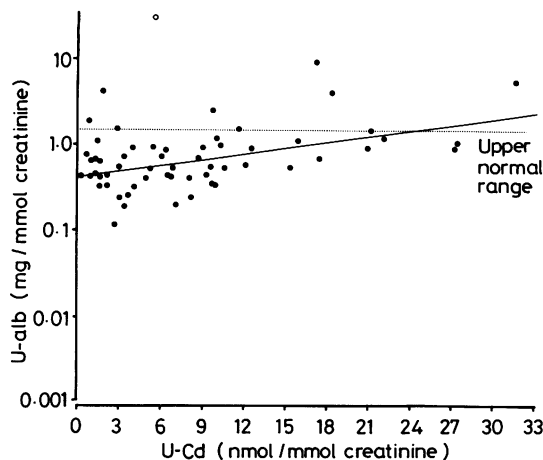


Fig 3 Urinary excretion of albumin in relation to urinary excretion of cadmium. Linear regression line; $^{10}\log$ U-albumin (mg/mmol creatinine) = $0.43 + 0.023 \times$ U-Cd (nmol/mmol creatinine). Unfilled dot depicts data from one excluded person (see methods).

group the upper normal ranges for the proteins in urine were derived from published data.²⁶³²⁻³⁴ If other cut off levels are chosen the prevalence data will obviously be somewhat different, but as may be deduced from figs 1, 2, and 3, the general appearance of the data will be similar to those in table 2. If, for example, the cut off points for raised urinary

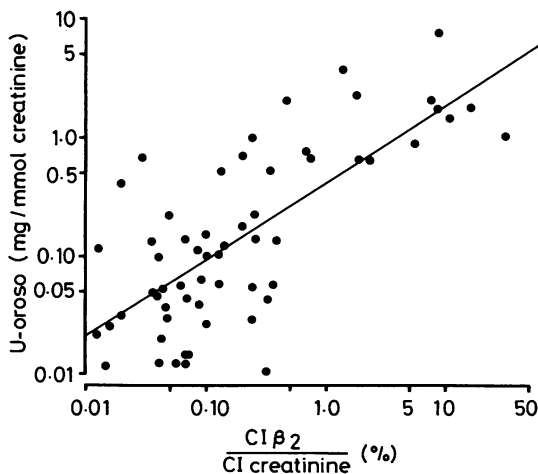


Fig 4 Urinary excretion of orosomucoid in relation to relative clearance of β_2 -microglobulin. Linear regression line; $^{10}\log$ orosomucoid (mg/mmol creatinine)

$$= 0.41 + 0.65 \times ^{10}\log \left(\frac{CI \beta_2}{CI creat} \% \right).$$

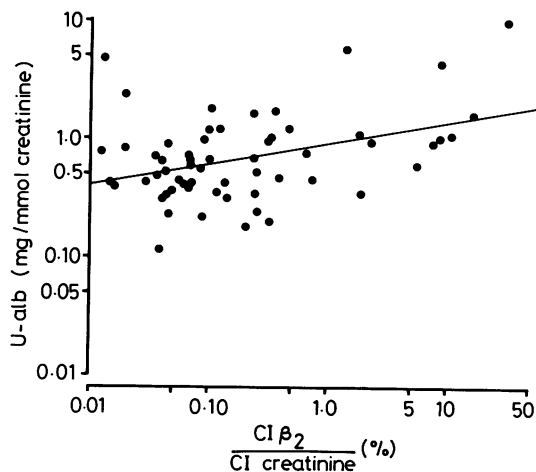


Fig 5 Urinary excretion of albumin in relation to relative clearance of β_2 -microglobulin. Linear regression line; $^{10}\log$ albumin (mg/mmol creatinine) = $0.91 + 0.17 \times ^{10}\log$

$$\left(\frac{CI \beta_2}{CI creat} \% \right).$$

β_2 -microglobulin, orosomucoid, and albumin were set at 0.1, 1, and 2 mg/mmol creatinine, respectively, the prevalence figures would become; 0/14, 0/12, 2/18, 3/5, and 9/11 for β_2 -microglobulinuria, 0/14, 0/12, 0/18, 1/5, and 9/11 for orosomucoiduria, and 1/14, 0/12, 1/17, 0/5, and 3/11 for albuminuria.

All the urinary proteins studied correlated

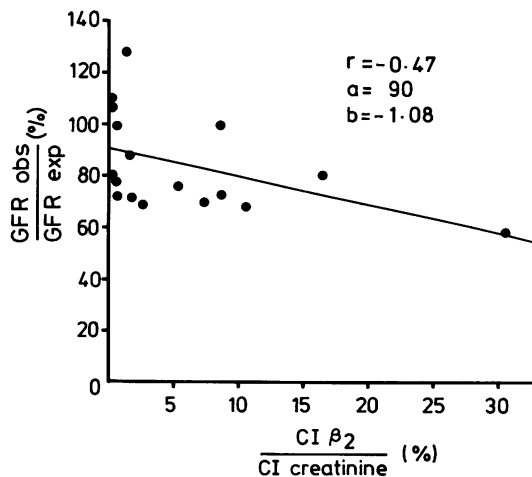


Fig 6 Ratio (%) between observed and predicted glomerular filtration rate (GFR) by 17 men in relation to relative clearance of β_2 -microglobulin. Linear regression line;

$$\frac{Obs GFR}{Pred GFR} (\%) = 90 - 1.08 \times \left(\frac{CI \beta_2}{CI creat} \% \right), r = 0.47.$$

significantly with urinary cadmium. The increase was much clearer for β_2 -microglobulin (fig 1) and orosomuroid (fig 2) than for albumin (fig 3), however. In figs 4 and 5 the urinary excretion of orosomuroid and albumin have been related to the relative clearance of β_2 -microglobulin. Both urine orosomuroid and albumin increase significantly with decreasing tubular reabsorption capacity. For orosomuroid this is easy to understand as it is another low molecular weight protein filtered through the glomeruli and reabsorbed in the tubules. Of greater interest is the tendency towards higher urinary albumin excretion among people with tubular dysfunction. None of those examined, however, with the exception of the 63 year old man discussed above, had urinary albumin concentrations exceeding the concentration of about 10 mg/mmol creatinine, which has been used as a limit for the diagnosis of glomerular proteinuria.¹²³⁷

Our interpretation is that the tendency towards higher urinary concentrations of albumin among workers with tubular impairment is related to a decreased tubular reabsorption capacity rather than to an increased glomerular permeability for larger proteins as was suggested by Bernard and co-workers.¹⁰¹¹ We believe that the increased urinary albumin excretion seen among those with tubular impairment simply constitutes further evidence of tubular damage as larger proteins, such as albumin, are also filtered through the glomerulus, although to a much lesser extent than are low molecular proteins.¹²¹⁵ Thus tubular impairment will also result in an increased urinary excretion of larger proteins.

On the other hand, measurements of the GFR using CrEDTA clearance showed that, on average, people with tubular damage also had a reduction in GFR (84% of age predicted value) and, furthermore, there was a significant correlation between tubular reabsorption loss and GFR (fig 6). In support of these findings a negative relation between tubular damage and decrease in GFR, as measured by endogenous creatinine clearance, was recently reported from environmentally exposed Japanese farmers.³⁸

Taken together, these findings strengthen the value of measuring low molecular weight proteins, such as β_2 -microglobulin, in urine in order to identify the early adverse effects on health caused by exposure to cadmium. If the tubular proteinuria is prevented it is also likely that glomerular effects will be avoided.

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