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Renal Function in Relation to Low Levels of Cadmium Exposure in a Group of Smelter Workers

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Blood and urine samples were obtained from 274 smelter workers and urine samples from 48 controls. Cadmium, β_2-microglobulin, and creatinine were estimated in blood and urine, and total protein in urine. Concentrations of cadmium in urine (mean 2.0 nmole/mmole creatinine) and blood (mean 21.8 nmole/L) observed in the smelter workers confirmed that this group had absorbed more cadmium than the general population, but less than most other occupationally exposed groups studied. Mean β_2-microglobulin in urine was not significantly different in the smelter workers and the controls. The mean total protein in urine was 20% higher in the smelter workers, a difference which was significant (p=0.01). There was no consistent picture within the smelter workers of a relationship between history of cadmium exposure and the effect measures of β_2-microglobulin in urine and blood, total protein in urine, creatinine clearance and relative β_2-microglobulin clearance. Small but significant positive correlation coefficients were observed between cadmium in urine and β_2-microglobulin in urine (r = 0.13), total protein in urine (r = 0.23) and β_2-microglobulin clearance (r = 0.15), although these may be artifactual.

Introduction

While adverse health effects resulting from heavy past occupational and environmental exposure to cadmium have been amply documented, the relationship between long-term, low-level exposure to cadmium and early effect is less clear. With regard to occupational exposure, both tubular and glomerular dysfunction have been observed, mainly after more than 25 years exposure in workers whose cadmium concentration in blood exceeded 10 μg (90 nmole)/L and whose cadmium concentration in urine exceeded 10 μg/g (or nmole/mmole) creatinine (*1*). A potential health risk for the general population has been suggested by a higher mortality from nephritis and nephrosis in long-term residents of a cadmium-polluted urban area with evidence of an increased body burden of cadmium (*2*). However, in England, no significant health effects were found in long-term residents in an area with heavy cadmium pollution of soil and evidence of increased cadmium absorption (*3*).

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Cadmium concentrations in blood and urine are the only readily available indicators of absorption and accumulation, for in an equilibrium situation, blood cadmium is considered to be an indicator of average intake over recent months, while urinary cadmium with low or moderate exposure relates more closely to cadmium body burden. Tubular dysfunction, evidenced by low molecular weight (LMW) proteinuria is considered to be an early indicator of effect, and the radioimmunoassay method developed for the estimation of β_2-microglobulin (*4*) has made this the most commonly estimated of the LMW proteins. However, β_2-microglobulin is a fragile protein which degrades rapidly in acid urine (pH below 5.6) both when stored in the bladder and following collection. Different procedures for collection and handling urine samples prior to analysis have been responsible, at least in part, for variation in results between laboratories. In a study based on almost 1000 urine samples, probably the most comprehensive to be performed in the general population, urinary cadmium increased significantly with age, while the relationship between β_2-microglobulin and age was of borderline significance, with no increase in urinary β_2-microglobu-

lin with increasing urinary cadmium concentration (*5*).

In the present study, evidence of an early renal effect in relation to occupational cadmium exposure was sought by estimation of the concentration of β_2-microglobulin and of creatinine in blood and urine and of total protein concentration in the urine. Exposure was assessed by study of the work environment, and indicators of recent absorption and of body burden were obtained by estimation of blood and urinary concentrations of cadmium, respectively.

Methods

The sample was selected from the current work force of a large primary smelter for the extraction and refining of lead, zinc and cadmium. This smelter is included in the national mortality study of cadmium workers in England now in progress (*6*). All hourly paid male workers who were first employed before 1972, together with those currently employed on a cadmium process since that date, were requested to participate. A control group of workers without previous occupational exposure to cadmium but comparable in terms of age and length of employment was drawn from post office workers residing in the same region. All participants completed a questionnaire giving a full occupational and smoking history.

Timed urine samples were collected on a single occasion from all workers and on a second occasion from the controls after an interval of 3 days. A venous blood sample was taken from the exposed workers only. All samples were collected between 9 a.m. and midday. The pH of the urine samples was recorded and the urine immediately diluted to 1 in 10 with Barbitone buffer containing sodium azide as a preservative. An aliquot of each blood sample was centrifuged and the plasma diluted 1 in 200 with Barbitone buffer. All samples were refrigerated before transport to the laboratory.

The β_2-microglobulin concentration in plasma and in urine was measured by radioimmunoassay using the Phadebas β_2-micro test (*4*). All determinations were performed in duplicate. Accuracy and precision were within the limits given by Schaller (*7*). Creatinine concentrations in plasma and urine were estimated on the Autoanalyser by using the standard Jaffe's method. Total urine protein concentration was estimated by a modified Bio Rad dye-binding method (*8*). Cadmium concentrations in blood and urine were determined by electrothermal atomization and atomic absorption spectrophotometry. The procedure described by Stoeppler and Brandt (*9*) was followed. The laboratory participated in a national quality control scheme and in the IUPAC interlaboratory comparison exercise (*10*). Blood lead estimations were performed by atomic absorption spectrophotometry by using the punched disc technique of Cernik and Sayers (*11*). Three samples were split at time of collection and each half submitted (blind) for analysis. The mean difference in estimates was 3% and 11% for β_2-microglobulin in urine and in blood, respectively, and 13% and 9% for cadmium in urine and in blood, respectively. Clearances were calculated, and the clearance of β_2-microglobulin was expressed as a ratio of the clearance of endogenous creatinine.

The jobs done by the men had been previously recorded and rated for the purpose of the national mortality study on a three-point scale into "high," "medium" and "low" exposure groups, on taking into account available environmental and biological monitoring data. Exposure assessments in the various job categories were made following interviews with the company's occupational hygienist and selected long-term employees. Environmental monitoring data were available from the 1950s and urinary cadmium levels in selected workers from 1972. Account was taken of technological changes over the years and increasing hygiene control. A more detailed twelve-point scale was also used for the present study. For each point of this scale an estimate was made of environmental exposure (in $\mu g/m^3$ of cadmium oxide). From these estimates, a current exposure and cumulative exposure (in $\mu g/m^3$-years) rating was computed for each man.

The Statistical Package for the Social Sciences (*12*) was used for analysis of the data. Where variables were more nearly lognormally than normally distributed, geometric means and standard deviations are given, and further analyses performed on logarithms of the variables.

Results

Samples were obtained from 48 controls and 274 smelter workers. These two groups are seen in Table 1 to be reasonably similar with respect to age, years in current employment and smoking habit.

Estimations from the two separate samples of urine obtained from the controls were used to determine the variation of urinary concentrations within an individual in a 4-day period. Estimations of cadmium, β_2-microglobulin and total protein in urine from the two samples were ob-

Table 1. Characteristics of smelter workers and controls.

	Controls	Smelter workers
Number in study	48	274
Age, yr	48.4	47.6
Duration of employment, yr	20.1	20.4
Ever smoked, %	77	84
Current smokers, %	35	50

Table 2. Basic urinary estimations.

	Mean ± SE	
	Controls	Smelter
pH	5.55 ± 0.07	5.61 ± 0.04
Volume, mL	121 ± 11	140 ± 5.4
Time, hrs	2.6 ± 0.2	2.6 ± 0.1
Creatinine, mmole/L	11.8 ± 0.6	11.2 ± 0.4

Table 3. Indicators of absorption.

	Mean (SE)[a]	
	Controls	Smelter
Cadmium in urine, nmole/mmole creatinine	0.69 (8%)	2.0 (5%)
Cadmium in blood, nmole/L	—	21.8 (5%)

[a]Geometric means and standard errors.

Table 4. Cadmium in urine and smoking in controls.

	Cadmium, nmole/mmole	
	Mean (SE)[a]	*n*
Current smokers	0.91 (11%)	17
Ex-smokers	0.59 (10%)	20
Nonsmokers	0.63 (14%)	11

[a]Geometric means and standard errors, nmole/mmole creatinine. Age accounted for by analysis of covariance.

served to differ by an average of 73, 139 and 44%, respectively, after correcting for creatinine concentration. Comparing these substantial differences with those observed between duplicate estimations made of split samples allows us to conclude that much the greater part of the differences are due to real within person variation. The existence of variation of this magnitude has relevance to the design and effectiveness of biological monitoring programs.

Mean urinary pH is seen in Table 2 to be low for the smelter workers and controls. Volume of sample, time over which sample was collected, and creatinine concentration are similar in the two groups. Geometric means of cadmium concentration in urine are shown in Table 3 for the controls (both samples combined) and smelter workers and of cadmium in blood for the smelter workers. The mean cadmium in urine concentration is significantly higher in the smelter workers ($p < 0.001$).

Since age, smoking and exposure are all possibly related to cadmium concentration in urine and blood, the effect of one can only be considered after accounting for the others. This was done using analysis of covariance, with exposure accounted for by indices of both current and cumulative exposure. Age is significantly positively correlated to cadmium concentration in urine in the controls ($p \simeq 0.01$), and cadmium concentration in urine ($p < 0.001$) and blood ($p < 0.001$) in the smelter workers, after accounting for all other factors.

The effect of smoking on the mean concentration of cadmium in urine in the controls is shown in Table 4. The current smokers have a signifi-

Table 5. Cadmium in urine and in blood and smoking in smelter workers.

	Cd in urine, nmole/mmole creatinine		Cd in blood, nmole/L	
	Mean (SE)[a]	*n*	Mean (SE)[a]	*n*
Current smokers	2.46 (6%)	128	33.1 (5%)	136
Ex-smokers	1.72 (8%)	89	15.3 (7%)	87
Nonsmokers	1.46 (11%)	44	11.8 (10%)	43

[a]Geometric means and standard errors. Age, cumulative exposure, and current exposure accounted for by analysis of covariance.

Table 6. Cadmium in urine and blood by last job.[a]

	Cadmium in urine, nmole/mmole creatinine		Cadmium in blood, nmole/L	
Department	Mean (SE)	*n*	Mean (SE)	*n*
Cadmium plant	6.30 (25%)	10	63.43 (20%)	11
Sinter	2.48 (13%)	36	27.66 (11%)	35
Furnace	1.65 (12%)	39	17.29 (10%)	41
Other	1.81 (5%)	175	20.45 (5%)	178

[a]Age and smoking accounted for by analysis of covariance.

Table 7. Cadmium in urine and blood by exposure group.

	Cadmium in urine, nmole/mmole creatinine		Cadmium in blood, nmol/L	
	Mean (SE)[a]	*n*	Mean (SE)[a]	*n*
Always low	1.60 (6%)	147	17.99 (5%)	149
Medium, < 10 yr	2.20 (9%)	66	25.53 (8%)	68
Medium, > 10 yr	3.42 (11%)	48	30.88 (10%)	49

[a]Geometric means.

cantly ($p \approx 0.02$) higher mean than the current nonsmokers, but the difference between the ex-smokers and lifetime nonsmokers is not significant.

For cadmium concentration in urine in the smelter workers, the pattern is similar, but the difference between cadmium concentrations in smokers and nonsmokers is much greater in the smelter workers (1.0 nmole/mmole creatinine) than in the controls (0.3 nmole/mmole creatinine), suggesting an interaction between industrial exposure and smoking. The effect of smoking on the concentration of cadmium in blood in the smelter workers presents a similar pattern (Table 5).

Mean cadmium in urine concentrations in relation to the department in which the subject worked at the time of sampling is shown in Table 6. Age and smoking have been accounted for. The cadmium plant mean was significantly higher than others ($p < 0.001$) and the sinter mean significantly higher than the expected low exposure departments such as the furnace. All groups show higher levels than the controls. The pattern is essentially similar for blood.

Examination of environmental monitoring results indicate that levels of airborne cadmium have been generally well within 50 μg/m^3 since 1970, with highest levels recorded in the cadmium plant. Measurements before 1970, when new plant was installed, suggested that concentrations considerably above this, although well below the milligram per cubic meter range, were not uncommon in the sinter and cadmium plant.

Table 8. Regression coefficients for current and cumulative exposure.[a]

	Regression coefficient (SE)	
	Log Cd (urine)	Log Cd (blood)
Current exposure, mg/m^3	43 (14)*	57 (13)†
Cumulative exposure mg/m^3-yr	0.62 (0.24)	0.38 (0.22)

[a]Each coefficient computed with all other variables in regression. Age, smoking already in regression.
†$p < 0.001$.
*$p < 0.01$.

Table 7 shows means for groups crudely classified by duration of exposure, and by intensity as defined for the mortality study. The medium group means are significantly higher than those of the "always low" group. For cadmium in urine, the medium group with more than 10 years exposure shows a significantly higher mean than does the group with less than 10 years exposure, but this difference is much smaller and not significant for cadmium in blood, to be expected if cadmium in blood reflects only recent exposure. Again the medium group means are significantly higher than those of the "always low" group.

The relationship between cadmium in urine and blood and the continuous variables of current and cumulative exposure to cadmium, computed from work histories, are best considered by means of regression analyses. Regression coefficients, their standard errors and associated p values are given in Table 8.

Current exposure is the only significant exposure measure for cadmium in blood, as we expect, but also, less expected, current exposure appears to be the stronger predictor for cadmium in urine, although here cumulative exposure is also significant.

Lead in blood in the smelter workers had an overall mean of 48 μg/100 mL, with a standard deviation of 15 μg/100 mL. Lead exposure is largely separate from cadmium exposure. Blood lead and blood cadmium levels in the entire plant were not correlated, so that cadmium and lead effects may reasonably be investigated separately.

Means of blood and urinary estimations of effect measures are shown in Table 9. The difference between the mean concentration of β_2-microglobulin in urine in the smelter workers and the controls is not significant. The small difference in the concentration of total protein is, however, significant at the 1% level.

A logarithmic plot of urinary β_2-microglobulin against pH is shown in Figure 1. The familiar pattern of low β_2-microglobulin concentrations with low pH is clear. The pH at which degradation begins to occur was estimated by likelihood-based methods (*13*) as 5.76 [95% CI (5.60, 5.95)].

Table 9. Effect measures.

	Mean (SE)	
	Controls	Smelter
β_2-M, Urine, μg/mmole creatinine[a]	6.6 (12%)	7.7 (7%)
β_2-M, blood, mg/L	—	1.95 (± 0.04)
Total protein, urine, mg/mmole creatinine[a]	7.2 (6%)	9.1 (3.6%)
Creatinine, blood, μmole/L[a]	—	99.5 (1%)
Creatinine clearance, mL/min	—	88 (5.3%)
β_2-M clearance × 10^3[a]	—	38 (8.4%)
(β_2-M clearance/creatinine clearance) × 10^5[a]	—	41 (7.3%)

[a]Geometric means.

Table 10. Correcting β_2M for pH.

	Mean (SE)[a]	
	Before correction	After correction
Controls		
β_2-M, urine, μg/mmole creatinine	6.6 (1.31)	13.3 (1.45)
Smelter workers		
β_2-M, urine, μg/mmole creatinine	7.7 (1.13)	14.6 (1.18)
(β_2-M clearance/creatinine clearance) × 10^5	41 (1.26)	77 (1.31)

[a]Geometric means.

The relationship of logarithm of urinary β_2-microglobulin concentration with pH for pH below 5.76 is linear, with slope 1.88, so that a simple correction for pH is possible. Using this correction makes a substantial difference to overall means, almost doubling them (Table 10). Analyses of the relationship between indices of cadmium exposure or absorption and β_2-microglobulin are, however, affected only in sensitivity by making this correction, since pH is not related to cadmium exposure or absorption. Correcting for pH only slightly reduces the mean percentage difference in the estimates of β_2-microglobulin concentration from the two samples taken from the controls.

Multiple regression analyses were performed to test for dose-effect relationships with continuous measures of dose. All urinary concentrations measured were significantly correlated with age (Table 11), although correlations of clearance rates with age were not significant. Age was accounted for in all regressions, the results of which are summarized in Table 12 as partial correlation coefficients.

Investigation of mean levels of the effect measures in the smelter workers according to last

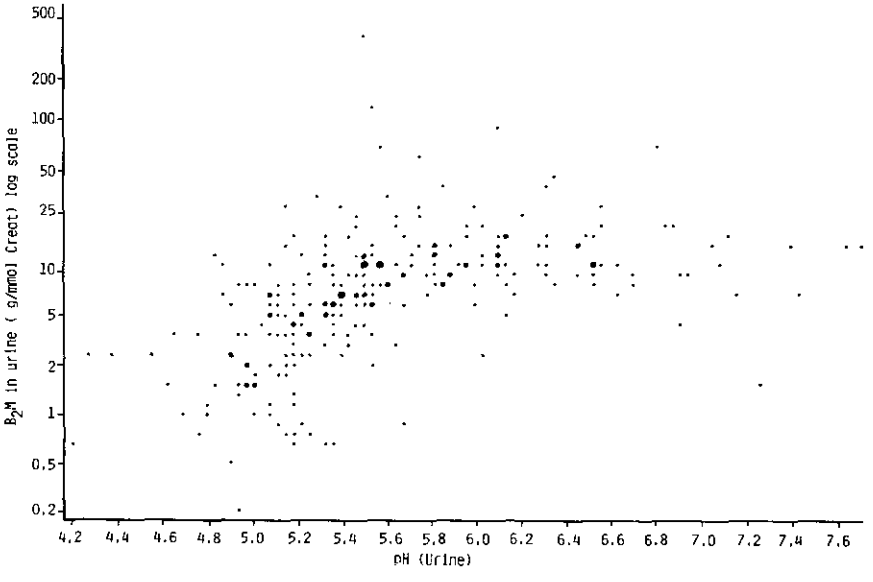

FIGURE 1. Urinary β_2-microglobulin and pH.

Table 11. Correlations with age.

Parameter	Value
Cadmium, urine nmole/mmole creatinine	0.37*
Cadmium, blood, nmole/L	0.28*
β_2-M, urine, μg/mmole creatinine	0.15†
β_2-M, blood, mg/L	0.31*
Total protein, urine, mg/mmole creatinine	0.23*
Creatinine, blood, μmole/L	0.17†
Creatinine clearance, mL/min	−0.05
β_2-M clearance, mL/min × 10^3	0.03
(β_2-M clearance/creatinine clearance) × 10^5	0.08

*$p < 0.001$.
†$p < 0.01$.

job and to the "always low" vs. medium classification suggested no pattern indicative of a cadmium effect. For example, mean β_2-microglobulin in urine, relative β_2-microglobulin clearance, and total protein concentration in urine were similar in the "always low" and "medium, > 10 yr" groups, and slightly lower in the "medium < 10 yr" group, a possible confounding effect of age. These differences are not significant.

Dose-response analyses, on taking the 97.5 percentile in the controls as cut off levels for β_2-microglobulin and for total proteinuria, were also carried out but are not reported here. Similar results to the dose-effect analyses were obtained.

Discussion

Cadmium in urine concentrations in the controls are similar to those found in unexposed populations in England (*14*), the U.S. (*5*), a group of unexposed Belgian workers (*1*), and elderly women in an unpolluted area of Belgium (*2*). The overall level for the smelter workers is low compared to most industrially exposed groups studied, but above the levels found among elderly women in Liege, a polluted area in Belgium (*2*). The mean cadmium in blood concentration in the smelter workers is higher than that found in Liege women by a factor of 1.5, but again low compared to other occupationally exposed groups studied.

From our investigation of cadmium in blood and urine we may conclude that the smelter population as a whole has evidence of cadmium absorption above that of the general population, but lower than most industrially exposed populations previously studied. Some identifiable groups, however, showed somewhat higher absorption.

The mean β_2-microglobulin concentrations uncorrected for pH found in the controls and the smelter workers in the present study are lower than those found in the U.S. population study (*5*) and the English study (*14*) quoted above. After correcting for pH our means are higher than those reported in the above studies however.

The effect of low urinary pH on β_2-microglobulin in urine will also be reflected in β_2-microglobulin clearance. Additional uncertainty in the estimation of clearances arises since the timing of urine samples could not be supervised, as a result of which the estimation of urinary flow rate in some subjects may have been inexact.

The significant positive correlations of β_2-microglobulin and total protein concentration in urine and relative β_2-microglobulin clearance with cadmium concentration in urine observed in the smelter workers (Table 12) are consistent with the presence of a cadmium effect. The significant negative correlation of creatinine clearance with urinary cadmium supports such a conclusion. However, these small correlation coefficients may be artifactual. Both effect and dose measures are corrected for concentration using creatinine excretion, which is known to be only approximately constant over time. Fluctuations in creatinine excretion would therefore cause spurious correlations between dose and effect measures. If, to avoid this problem, the cruder uncorrected cadmium in urine concentration or estimated cadmium excretion rate is taken as the dose variable,

Table 12. Partial correlations (controlling for age).

	Correlation with	
	Cadmium, urine, nmole/mmole creatinine	Cumulative exposure
β_2-M, urine, μg/mmole creatinine	0.13‡	0.10**
β_2-M, blood, mg/L	−0.13‡	−0.03
Total protein, urine, mg/mmole creatinine	0.23*	0.01
Creatinine, urine, mmole/L	0.03	−0.08
Creatinine, blood, μmole/L	−0.03	−0.01
Creatinine clearance, mL/min	−0.24*	−0.01
β_2-M clearance, mL/min × 10^3	−0.06	0.08
(β_2-M clearance/creatinine clearance) × 10^5	0.15†	0.10**

*$p < 0.001$.
†$p < 0.01$.
‡$p < 0.05$.
**$p < 0.10$.

significant correlations are no longer observed. The small negative correlation of β_2-microglobulin concentration in blood with cadmium concentration in urine may also be artifactual. The only evidence of a dose-effect relationship with cumulative exposure are weakly significant correlations with β_2-microglobulin concentration in urine and relative β_2-microglobulin clearance. Thus the search for dose-effect and some dose-response relationships within the smelter population gave suggestive but not consistent evidence for the presence of a renal effect.

We have noted that the smelter workers were also exposed to lead, but that the different distribution of lead and cadmium exposure enables the independent investigation of lead and cadmium effects. It is thus unlikely that relationships between renal function and cadmium exposure in the smelter workers would be obscured by an independent lead effect, or that spurious relationships would arise through such an effect. However, we cannot exclude the possibility that the increased mean urinary protein concentration in the smelter workers was related to lead absorption, although such an effect would not be expected at these levels of exposure. The relationship between lead exposure and renal function in the smelter population is being investigated further. We should note that the smelter workers were also exposed to zinc which has been suggested to act to protect against cadmium effects on the kidney.

Thus, the findings from this study, while suggestive, did not produce a consistent picture of an adverse renal effect as a result of low level cadmium absorption in this smelter population. If such an effect exists it is likely to be small. We plan to follow up this epidemiological study with further biochemical investigation and long-term follow-up of selected workers to delineate more precisely the presence of a renal effect at these low levels of exposure.

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