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Chronic Renal Effects in Three Studies of Men and Women Occupationally Exposed to Cadmium

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Abstract. We measured sensitive indicators of renal damage in three different populations occupationally exposed to cadmium, and examined the degree of variation in damage and the relative sensitivity of different types of indicators. The three studies included (1) men exposed in a cadmium recovery plant, (2) men exposed in a nickel/cadmium battery plant, and (3) women exposed in the latter plant. The indicators of renal damage were urinary proteins in three categories: (1) the high molecular weight enzymes alanine aminopeptidase (AAP) and N-acetyl-β-D-glucosaminidase (NAG), (2) the intermediate molecular weight protein albumin (ALB), and (3) the low molecular weight proteins retinol-binding protein (RBP) and β_2-microglobulin (B2M). These tests indicate that exposed groups with higher urine cadmium levels had varying degrees of renal damage. All exposed groups showed evidence of renal damage when compared with their respective control groups. A higher percentage of elevated protein levels was noted in the exposed group of Study 1 than in the exposed groups of Studies 2 and 3. In Study 1, the means of all five protein levels and ALB, RBP, and B2M fractional clearances were significantly elevated in the group with higher urine cadmium concentrations when compared with the groups with lower urine cadmium concentrations. Highly significant dose-response relationships for all of the urinary protein tests, including fractional clearances, were found. All of the tests were more sensitive in detecting evidence of subclinical renal damage than serum creatinine, a commonly used indicator of renal function. The order of test sensitivity in men was determined by considering three factors: (1) the magnitude of the correlation coefficient between the test and the urine cadmium concentration in the study with the most advanced damage, (2) the relative cadmium level predicted by the dose-response model at which there is a 10% chance of observing an elevated test value, and (3) the ability of the tests to detect renal effects in the population with less advanced damage. The tests in order of decreasing sensitivity in men are ALB, AAP, NAG, RBP ≈ B2M. The women with higher urine cadmium levels in Study 3 had a higher percentage of elevated AAP and NAG values when compared with the control group.

In cases of long-term chronic occupational exposure to cadmium, the kidneys are usually the most critically affected organs (World Health Organization Study Group 1980). Various factors can affect the extent of uptake, severity of toxicity, and initial site of damage. Length and type of exposure, route of exposure, gender, and nutritional status (calcium, iron, zinc, vitamins, protein, and other trace metals) can all play a role in determining the nature of the renal toxicity of cadmium (Friberg *et al.* 1986; Bernard *et al.* 1981; Nath *et al.* 1984). In some studies relating elevated renal parameters to urine cadmium levels, investigators report that renal dysfunction begins when urine cadmium levels reach 10 μg Cd/g creatinine (Bernard *et al.* 1979; Roels *et al.* 1983; Jakubowski *et al.* 1987; Shaikh *et al.* 1987). Other investigators, however, report evidence of adverse renal effects below this urine cadmium level (Elinder *et al.* 1985; Verschoor *et al.* 1987; Chia *et al.* 1989). Although early renal damage caused by cadmium is assumed to be primarily tubular (Piscator 1984; Elinder *et al.* 1985; Friberg *et al.* 1986), results of other studies suggest that glomerular damage can accompany (Bernard *et al.* 1979; Bernard 1980; Elinder *et al.* 1985; Lauwerys and Bernard 1986), and under certain circumstances occur independently of, tubular damage (Bernard *et al.* 1979; Roels *et al.* 1981; Bernard *et al.* 1981; Lauwerys and Bernard 1986).

The relative sensitivity of the tests used to detect renal damage is important to studies of toxicity and to the monitoring of potentially exposed people. The number of people determined to be affected by a given level of toxic exposure is directly related to the sensitivity of the test used to detect the damage.

We used sensitive renal tests to examine both the variations in renal damage among different occupationally exposed populations and the tests' relative sensitivity. The three studies in-

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Table 1. Sensitive Renal Tests[a]

Category	Example	Primarily Indicates	Properties
High molecular weight protein (enzymes)	AAP NAG	Direct release of tubular tissue into urine	Not normally filtered through the glomerulus
Intermediate molecular weight protein	ALB	Glomerular damage[b]	A small amount normally filtered through the glomerulus and reabsorbed by the tubules
Low molecular weight protein	RBP B2M	Damaged tubular reabsorption	Freely filtered by the glomerulus and reabsorbed by the tubules

[a] Urine tests for AAP: alanine aminopeptidase, NAG: N-acetyl-β-D-glucosaminidase, ALB: albumin, RBP: retinol-binding protein, and B2M: β_2-microglobulin. In addition to the primary indications of elevations of these proteins in urine, elevations can occur in cases of bacterial infection and damage to other parts of the urinary tract

[b] Can also be elevated when tubular reabsorption is damaged

cluded (1) men with long-term occupational cadmium fume exposure in a cadmium recovery plant, some of whom had not been exposed recently, (2) men employed at a nickel/cadmium battery plant and exposed primarily to cadmium and nickel dust, and (3) women with high cadmium exposure and less nickel exposure at the same nickel/cadmium battery plant. The tests used to evaluate these different populations included the measurement of three classes of urinary proteins (Table 1). Each class of tests provided different information about the specific location of cadmium nephrotoxicity. These three classes included (1) alanine aminopeptidase (AAP) and N-acetyl-β-D-glucosaminidase (NAG), high molecular weight enzymes that are not normally filtered through the glomerulus and are elevated in urine when there is direct release of tubular tissue into the urine (Mattenheimer 1977; Vanderlinde 1981); (2) albumin (ALB), an intermediate molecular weight protein that is normally filtered in small amounts, is reabsorbed by the tubules, is elevated in cases of glomerular damage, and can also be elevated when tubular reabsorption is compromised; and (3) retinol-binding protein (RBP) and β_2-microglobulin (B2M), low molecular weight proteins that are freely filtered by the glomerulus, are normally reabsorbed by the tubules, and are elevated when tubular reabsorption is compromised. In addition to these primary interpretations, some of these proteins can be elevated in bacterial infections and when other parts of the urinary tract are damaged. Urine cadmium levels were used as a surrogate measure of exposure because of the relation of the urine levels to cumulative cadmium body burden (World Health Organization Study Group 1980; Roels *et al.* 1981; Nordberg (1982).

Methods

Study Stubjects

The groups of study subjects included those persons for whom there was sufficient data to analyze the relationship between the excretion of proteins used as indicators of renal damage and urine cadmium levels.

Study 1: The first study consisted of 76 men separated into two groups, each with different urine cadmium levels. Details of the patient cohort have been previously described (Smith *et al.* 1980a, 1980b; Ellis *et al.* 1985; Thun *et al.* 1989). For the purpose of examining protein excretion at different cadmium levels the cutoff between the groups was set at 2.0 μg Cd/L. All nonoccupationally exposed subjects except one had urine cadmium concentrations below this level. These were hospital workers with no known cadmium exposure. The one exception (urine cadmium = 3.4 μg/L) was not included in the data set because samples were not available for urine protein analysis. The group with higher cadmium levels (≥ 2.0 μg/L) had been occupationally exposed to cadmium dust and fume by recovering cadmium from waste material that had been captured by emission control devices in primary smelters. Some of the workers were still exposed at the time of the study, and some were no longer working. The length of exposure ranged to 38 years. Some of those removed from exposure had had higher cadmium levels previously. The higher cadmium group (urine Cd ≥ 2.0 μg/L) consisted of 40 men (Group 1MH: mean age = 56.7 years, SD = 13.9), and the low cadmium group (urine Cd < 2.0 μg/L) consisted of 36 men (Group 1ML: mean age = 48.0 years, SD = 14.4).

Study 2: The second study consisted of 75 men. The process and exposures have been described in detail by the National Institute for Occupational Safety and Health (NIOSH, 1990). The 39 occupationally exposed men were exposed to cadmium and nickel dusts in the platemaking and pressed plate areas of a cadmium/nickel battery manufacturing plant (Group 2MH: mean age = 42.0 years, SD = 7.4). The nonoccupationally exposed comparison group contained 36 adult male employees of the same plant (Group 2ML: mean age = 49.9 years, SD = 7.9). The exposed group had at least 7.9 years of experience with the plant.

Study 3: The third study consisted of 109 women working in the same battery plant as the men in Study 2. They had been occupationally exposed to various levels of cadmium and minimum levels of nickel. Exposures of these participants have also been described in detail by NIOSH (1990). In this study, the numbers of participants with a large range of urine cadmium levels were sufficient to separate the women into three groups based on urine cadmium level. The first group had cadmium concentrations > 10.0 μg/g creatinine (Group 3FH: mean age = 42.9 years, SD = 6.5), the second group had cadmium concentrations > 2.0 μg/g creatinine and ≤ 10.0 μg/g creatinine (Group 3FM: mean age = 45.8 years, SD = 7.6), and the third group had cadmium concentrations ≤ 2.0 μg/g creatinine (Group 3FL: mean age = 46.0 years, SD = 8.9). In the groups with medium and high urine cadmium concentrations the minimum estimated exposure was 9.6 years.

Samples

First morning urine samples were mixed, aliquoted and stored at −20°C. Sample collection and treatment was the same for all studies except the urine sample for B2M in Study 1 was preserved with 0.1% sodium azide, 0.1% sodium carbonate, and 58.5 KIU aprotinin (Sigma Chemical Co.). AAP samples were preserved with 10% glycerol

Table 2. Cadmium, lead, and nickel levels

	Study 1		Study 2		Study 3		
Metal	1MH	1ML	2MH	2ML	3FH	3FM	3FL
Cadmium (urine)							
Mean (μg/g creat)	10.16[a]	1.00	5.88[a]	0.84	16.28[a]	4.98[a]	1.15
SD	6.94	1.09	4.81	0.53	5.43	2.15	0.58
Number of subjects	40	34	39	36	32	51	26
5th percentile	2.22	0.00	0.98	0.31	10.74	2.12	0.16
95th percentile	22.68	3.83	14.70	2.18	29.42	9.05	2.00
Lead (blood)							
Mean (μg/L)	134.8[b]	97.3	—	—	—	—	—
SD	74.4	43.6	—	—	—	—	—
Number of subjects	40	35	—	—	—	—	—
5th percentile	43.3	40.2	—	—	—	—	—
95th percentile	311.2	202.0	—	—	—	—	—
Nickel (urine)							
Mean (μg/g creat)	—	—	7.19[a]	1.64	4.17[c]	3.67	2.46
SD	—	—	5.90	0.80	2.52	3.81	1.44
Number of subjects	—	—	39	36	32	51	26
5th percentile	—	—	1.28	0.60	1.46	0.98	0.54
95th percentile	—	—	20.90	3.72	11.04	10.51	5.87

[a] Significantly different from low cadmium group, $p = 0.0001$, Kruskal-Wallis test
[b] Significantly different from low cadmium group, $p = 0.0425$, Kruskal-Wallis test
[c] Significantly different from low cadmium group, $p = 0.0022$, Kruskal-Wallis test

(Mueller *et al.* 1986). Samples for NAG and AAP analysis were centrifuged at 3,000 rpm for 10 min at 5°C after reaching room temperature. Urine cadmium samples were preserved with 120 μL of concentrated nitric acid/12 mL of urine. Blood for cadmium and lead determinations was anticoagulated with Na_2EDTA and stored at 4°C. Serum samples were prepared by allowing whole blood to clot at room temperature 20–30 min. and centrifuging for 10 min. at 1000 × g.

Assays

ALB was assayed in urine by a modification (Whitfield and Spierto 1986) of the enzyme-linked immunosorbent assay procedure of Fielding *et al.* (1983). This method has a routine lower detection limit of 0.75 mg/L at dilutions of 1:250 which allows quantification of normal levels. ALB was assayed in serum by the standard DuPont ACA procedure (DuPont Co., Wilmington, DE). RBP was assayed in urine (after concentration) and in serum using Radial Immunodiffusion Kits (Calbiochem-Behring, LaJolla, CA) (Studies 1 and 2), and in urine and serum by an enzyme immunoassay modification of Topping *et al.* (1986) (Study 3). B2M was assayed in urine and serum using Pharmacia Diagnostics Phadebas β_2-microglobulin Test Kits (Uppsala, Sweden). AAP was assayed by adaptation of the method of Jung and Scholz (1980), and modified for analysis on the Cobas Bio centrifugal analyzer (Roche Diagnostic Systems) (Mueller *et al.* 1986). NAG activity was measured by a modification of the fluorimetric assay of Leaback and Walker (1961). Creatinine was assayed in urine and serum by using standard DuPont ACA procedures (DuPont Co., Wilmington, DE).

Blood cadmium levels were determined by a graphite furnace atomic absorption method based on the method of Stoeppler and Brandt (1980), and urine cadmium levels were determined by a modification of the method of Pruszkowska *et al.* (1983). Blood lead levels were determined by the atomic absorption method of Paschal and Bell (1981). Urine nickel concentrations were measured by the method of Paschal and Bailey (1989).

Statistical Methods

All statistical analyses, including comparisons of means and correlations, were performed by using the Statistical Analysis System (SAS Institute, Cary, NC). Means were compared by using the nonparametric Kruskal-Wallis test. Corrections for urine flow were made by calculating the ratio of a protein concentration, activity, or metal concentration to the urine creatinine concentration (UCREAT > 50 mg creatinine/dL for studies 2 and 3). All concentrations of urine parameters were based on these ratios to creatinine or estimates of fractional clearances calculated according to the following equations:

1. CALB = (UALB/SALB)/(UCREAT/SCREAT)
2. CRBP = (URBP/SRBP)/(UCREAT/SCREAT)
3. CB2M = (UB2M/SB2M)/(UCREAT/SCREAT)

where C refers to fractional clearance, U to urine test value, and S to serum test value, and CREAT to creatinine. In these calculations the values were adjusted so that the units of both urine and serum results for a given test were the same. The number and percent elevated (or low) in Tables 3–6 are based on the number of test values above the 95th percentile of the low cadmium group for that study (or below the 5th percentile of the low cadmium group).

Probit analyses were performed on the urine protein and cadmium data of Study 1. The data were grouped on the basis of cadmium range and were represented by rthe mean cadmium level. The response was defined as the number of samples within a specified cadmium range with protein: creatinine ratios (or fractional clearances) greater than the 95th percentile of the low cadmium group. Parameters were estimated by iteration based on the probit equation:

$$\Phi^{-1}[(y-C)/(1-C)] + 5 = A + Bx,$$

where Φ is the cumulative distribution function of the standard normal distribution, x is the level of the dose, y is the probability of a response (effect), A is the intercept, B is the slope, and C is the natural (thresh-

Table 3. Albumin levels

	Study 1		Study 2		Study 3		
Test[a]	1MH	1ML	2MH	2ML	3FH	3FM	3FL
Albumin (urine)							
Mean (mg/g creat)	30.8[b]	10.7	4.0	2.7	2.3	2.0	2.4
SD	41.7	8.0	5.7	1.5	2.5	2.2	2.6
Number of subjects	40	34	39	36	31	49	19
5th percentile	3.2	5.0	0.9	1.2	0.0	0.0	0.0
95th percentile	114.2	31.7	21.2	6.1	9.5	7.1	9.5
No. elevated	11/40	1/34	5/39	1/36	1/31	0/49	1/19
% elevated	27.5	2.9	12.8	2.8	3.2	0.0	5.3
Albumin fractional clearance							
Mean ($\times 10^5$)	0.99[c]	0.27	0.12	0.06	0.04	0.04	0.05
SD ($\times 10^5$)	1.34	0.21	0.17	0.04	0.05	0.04	0.06
Number of subjects	40	33	38	36	31	49	19
5th percentile ($\times 10^5$)	0.07	0.11	0.03	0.03	0.00	0.00	0.00
95th percentile ($\times 10^5$	4.76	0.87	0.75	0.16	0.17	0.13	0.21
No. elevated	12/40	1/33	6/38	1/36	0/31	0/49	1/19
% elevated	30.0	3.0	15.8	2.8	0.0	0.0	5.3

[a] No. and % elevated are defined as the fraction and percent of values ≥ the 95th percentile of the low cadmium group
[b] Significantly different from low cadmium group, p = 0.0003 Kruskal-Wallis test
[c] Significantly different from low cadmium group, p = 0.0006, Kruskal-Wallis test

old) response rate. Estimated doses were calculated with 95% fiducial limits for various probabilities of observing a response. The model is considered to fit the data if χ^2 is low as defined by $p > 0.1$. The probit analysis yields an estimated cadmium level at which 5% or 10% of the exposed group have elevated test values relative to the 95th percentile of the control group. The upper fiducial limit corresponds to an estimated 95% upper probability limit to the estimated cadmium level associated with the elevated test values in 5% or 10% of the exposed group.

Results

In Vivo Metal Levels

Study 1: The mean urine cadmium levels (expressed as μg Cd/g creatinine) for the men in the high and low cadmium groups in Study 1 differed by more than a factor of 10 (Table 2). Study participants in the high cadmium group also had a statistically

Table 4. Urinary enzyme levels

	Study 1		Study 2		Study 3		
Test[a]	1MH	1ML	2MH	2ML	3FH	3FM	3FL
Alanine aminopeptidase							
Mean (U/g creat)	13.83[b]	6.58	4.68[c]	5.51	5.30	4.89	4.18
SD	12.34	3.47	3.13	2.10	2.46	2.38	1.56
Number of subjects	38	31	38	34	32	49	19
5th percentile	2.54	1.20	1.91	1.93	2.22	1.98	2.06
95th percentile	36.43	13.42	12.43	10.53	11.49	9.82	7.68
No. elevated	13/38	1/31	3/38	1/34	4/32	4/49	1/19
% elevated	34.2	3.2	7.9	2.9	12.5	8.2	5.3
N-acetyl-β-D-glucosamididase							
Mean (U/g creat)	2.95[d]	0.92	0.73	0.96	0.86	0.71	0.58
SD	4.37	1.03	0.46	0.86	0.64	0.44	0.40
Number of subjects	40	34	39	36	31	49	19
5th percentile	0.11	0.00	0.22	0.28	0.26	0.10	0.16
95th percentile	13.50	3.11	2.07	3.46	2.48	1.55	1.73
No. elevated	11/40	1/34	0/39	1/36	2/31	0/49	1/19
% elevated	27.5	2.9	0.0	2.8	6.4	0.0	5.3

[a] No. and % elevated are defined as the fraction and percent of values ≥ the 95th percentile of the low cadmium group
[b] Significantly different from low cadmium group, p = 0.0018, Kruskal-Wallis test
[c] Significantly different from low cadmium group, p = 0.0105, Kruskal-Wallis test
[d] Significantly different from low cadmium group, p = 0.0083, Kruskal-Wallis test

Table 5. Low molecular weight protein levels

	Study 1		Study 2		Study 3		
Test[a]	1MH	1ML	2MH	2ML	3FH	3FM	3FL
Retinol-binding protein (urine)							
Mean (mg/g creat)	7.16[b]	0.10	0.08[c]	0.11	0.09	0.07	0.09
SD	24.58	0.07	0.06	0.07	0.08	0.05	0.09
Number of subjects	40	34	39	35	30	46	23
5th percentile	0.02	0.00	0.00	0.02	0.02	0.01	0.01
95th percentile	96.11	0.26	0.25	0.27	0.28	0.17	0.35
No. elevated	13/40	1/34	1/39	1/35	0/30	0/46	1/23
% elevated	32.5	2.9	0.0	2.8	0.0	0.0	4.3
Retinol-binding protein fractional clearance							
Mean ($\times 10^5$)	243.3[d]	2.2	2.0	2.4	3.2	2.3	2.9
SD ($\times 10^5$)	844.9	1.8	1.6	1.9	2.7	1.7	2.8
Number of subjects	40	33	38	35	30	45	23
5th percentile ($\times 10^5$)	0.4	0.0	0.0	0.4	0.5	0.6	0.3
95th percentile ($\times 10^5$)	3062.9	7.2	6.0	7.4	9.9	6.1	10.0
No. elevated	11/40	1/33	0.38	1/35	1/30	0/45	1/23
% elevated	27.5	3.0	0.0	2.8	3.3	0.0	4.3
β_2-microglobulin (urine)							
Mean (mg/g creat)	6.98[e]	0.25	0.09	0.11	—	—	—
SD	20.45	0.17	0.12	0.18	—	—	—
Number of subjects	40	34	39	36	—	—	—
5th percentile	0.11	0.08	0.00	0.01	—	—	—
95th percentile	65.93	0.73	0.26	0.35	—	—	—
No. elevated	12/40	1/34	1/39	1/36	—	—	—
% elevated	30.0	2.9	2.6	2.8	—	—	—
β_2-microglobulin fractional clearance							
Mean ($\times 10^3$)	16.2[f]	1.9	—	—	—	—	—
SD ($\times 10^3$)	38.5	1.5	—	—	—	—	—
Number of subjects	40	33	—	—	—	—	—
5th percentile ($\times 10^3$)	0.6	0.6	—	—	—	—	—
95th percentile ($\times 10^3$)	156.9	6.5	—	—	—	—	—
No. elevated	10/40	1/33	—	—	—	—	—
% elevated	25.0	3.0	—	—	—	—	—

[a] No. and % elevated are defined as the fraction and percent of values $\geq$ the 95th percentile of the low cadmium group
[b] Significantly different from low cadmium group, p = 0.0092, Kruskal-Wallis test
[c] Significantly different from low cadmium group, p = 0.0062, Kurskal-Wallis test
[d] Significantly different from low cadmium group, p = 0.0058, Kruskal-Wallis test
[e] Significantly different from low cadmium group, p = 0.0087, Kruskal-Wallis test
[f] Significantly different from low cadmium group, p = 0.0194, Kruskal-Wallis test

significant higher mean blood lead concentration than those in the low cadmium group.

Study 2: The predominant exposures for the men in Study 2 were to cadmium and nickel. The mean urine cadmium level of the high cadmium group was 7 times that of the low cadmium group and 58% that of the high group in Study 1. There were also significantly higher nickel levels in the high cadmium group of Study 2 compared with the low cadmium group. The mean nickel level in the high cadmium group was over 4 times that of the low group.

Study 3: The predominant exposures for the women in Study 3 were also to cadmium and nickel. In the high cadmium group the mean urine cadmium level was 14 times that of the low cadmium group, and in the medium cadmium group the mean urine cadmium level was 4 times that of the low group. The mean urine cadmium level of the high group was above that of the high group in Study 1 and considerably above that of the high group in Study 2. There was also a slight increase in mean nickel levels with increasing urine cadmium levels in the three groups of Study 3.

Protein Levels

Study 1: The urine ALB, RBP, and B2M values and fractional clearances for the men in Study 1 are shown in Figure 1. For each of these tests of renal damage, there were elevated values in the high cadmium group (elevated above the 95th percentile of the test values for the control group). This is the case when the test values were calculated as a ratio to creatinine and when the fractional clearances of these proteins were estimated relative to creatinine clearance. The ALB, RBP, and B2M values were elevated in 28% to 32% of the workers in the high cadmium group (Tables 3 and 5), and ALB, RBP, and B2M fractional clearances were elevated in 25% to 30% of the workers in this group. The means of ALB, RBP, B2M, and their

Table 6. Serum parameters

Test[a]	Study 1		Study 2		Study 3		
	1MH	1ML	2MH	2ML	3FH	3FM	3FL
Creatinine							
Mean (mg/dL)	1.21	1.05	1.18[b]	0.99	0.81	0.80[c]	0.86
SD	0.39	0.17	0.25	0.13	0.14	0.13	0.11
No. of subjects	40	35	38	36	32	50	26
5th percentile	0.80	0.78	1.00	0.68	0.60	0.60	0.64
95th percentile	2.46	1.42	1.46	1.2	1.14	1.04	1.06
No. elevated	4/40	1/35	8/38	0/36	2/32	2/50	1/26
% elevated	10.0	2.8	21.0	0.0	6.2	4.0	3.8
Albumin							
Mean (g/dL)	4.23	4.31	4.16[d]	4.32	4.19	4.19	4.27
SD	0.32	0.30	0.22	0.19	.28	0.27	0.24
No. of subjects	40	35	38	36	32	50	26
5th percentile	3.59	3.84	3.74	3.97	3.74	3.80	3.62
95th percentile	4.70	4.84	4.55	4.67	4.63	4.64	4.58
No. low	6/40	1/35	8/38	1/36	0/32	0/50	1/26
% low	15.0	2.8	21.0	2.8	0.0	0.0	3.8
Retinol-binding protein							
Mean (mg/dL)	5.58	5.24	4.70	5.18	2.47	2.77	2.67
SD	1.21	1.09	0.82	1.52	0.72	0.86	0.69
No. of subjects	40	35	38	36	32	51	26
5th percentile	3.90	3.68	3.18	3.31	1.31	1.29	1.56
95th percentile	8.00	7.26	6.22	8.69	3.89	4.37	3.71
No. elevated	5/40	2/35	0/38	1/36	2/32	9/51	1/26
% elevated	12.5	5.7	0.0	2.8	6.2	17.6	3.8
β-microglobulin							
Mean (mg/L)	2.90[e]	1.50	—	—	—	—	—
SD	2.98	0.38	—	—	—	—	—
No. of subjects	40	35	—	—	—	—	—
5th percentile	1.01	0.98	—	—	—	—	—
95th percentile	10.34	2.31	—	—	—	—	—
No. elevated	10/40	1/35	—	—	—	—	—
% elevated	25.0	2.9	—	—	—	—	—

[a] No. and % elevated are defined as the fraction and percent of values > the 95th percentile of the low cadmium group and No. and % low are defined as the fraction and percent of values < the 5th percentile of the low cadmium group
[b] Significantly different from low cadmium group, $p = 0.0001$, Kruskal-Wallis test
[c] Significantly different from low cadmium group, $p = 0.0266$, Kruskal-Wallis test
[d] Significantly different from low cadmium group, $p = 0.0013$, Kurskal-Wallis test
[e] Significantly different from low cadmium group, $p = 0.0072$, Kruskal-Wallis test

respective fractional clearances in the high cadmium group were significantly higher than those of the low group.

The urinary enzyme levels of Study 1 have been previously described (Mueller *et al.* 1989). In the high cadmium group results of the AAP and NAG tests were elevated for 28% to 34% of the workers (Table 4). The mean values of these tests in the high cadmium group were significantly higher than the mean values of the low group.

Serum creatinine concentrations were elevated in 10% of the high cadmium group in Study 1, and serum ALB concentrations were lower in 15% of this group when compared with the low cadmium group (Table 6). The serum RBP and B2M concentrations were elevated in 12% and 25%, respectively, of the high cadmium group. The means of the first 3 serum parameters were not statistically different in the 2 cadmium groups, but the mean serum B2M of the high cadmium group was significantly higher than that of the low cadmium group ($p = 0.0072$).

Study 2: The ALB values and ALB fractional clearances were elevated in 13% and 16%, respectively, of the high cadmium group of Study 2 (Table 3). No elevations in RBP values, RBP fractional clearances, or B2M values were seen in this group (Table 5). The mean protein and fractional clearance levels in the high cadmium group were not significantly higher than the means of the low cadmium group.

The AAP values were elevated in 8% of the high cadmium group, but the mean AAP was not significantly higher than that of the low cadmium group (Table 4). The NAG values were not elevated in the high group when compared with the low group, and the means were not significantly different.

Serum creatinine concentrations were elevated in 21% of the high cadmium group and serum ALB concentrations were lower in 21% of this group when compared with the low cadmium group (Table 6). The means of both parameters in the high cadmium group were statistically different from the means of the low cadmium group (serum creatinine, $p = 0.0001$; serum albumin, $p = 0.0013$). Although the serum creatinine values were elevated above the 95th percentile of the control group

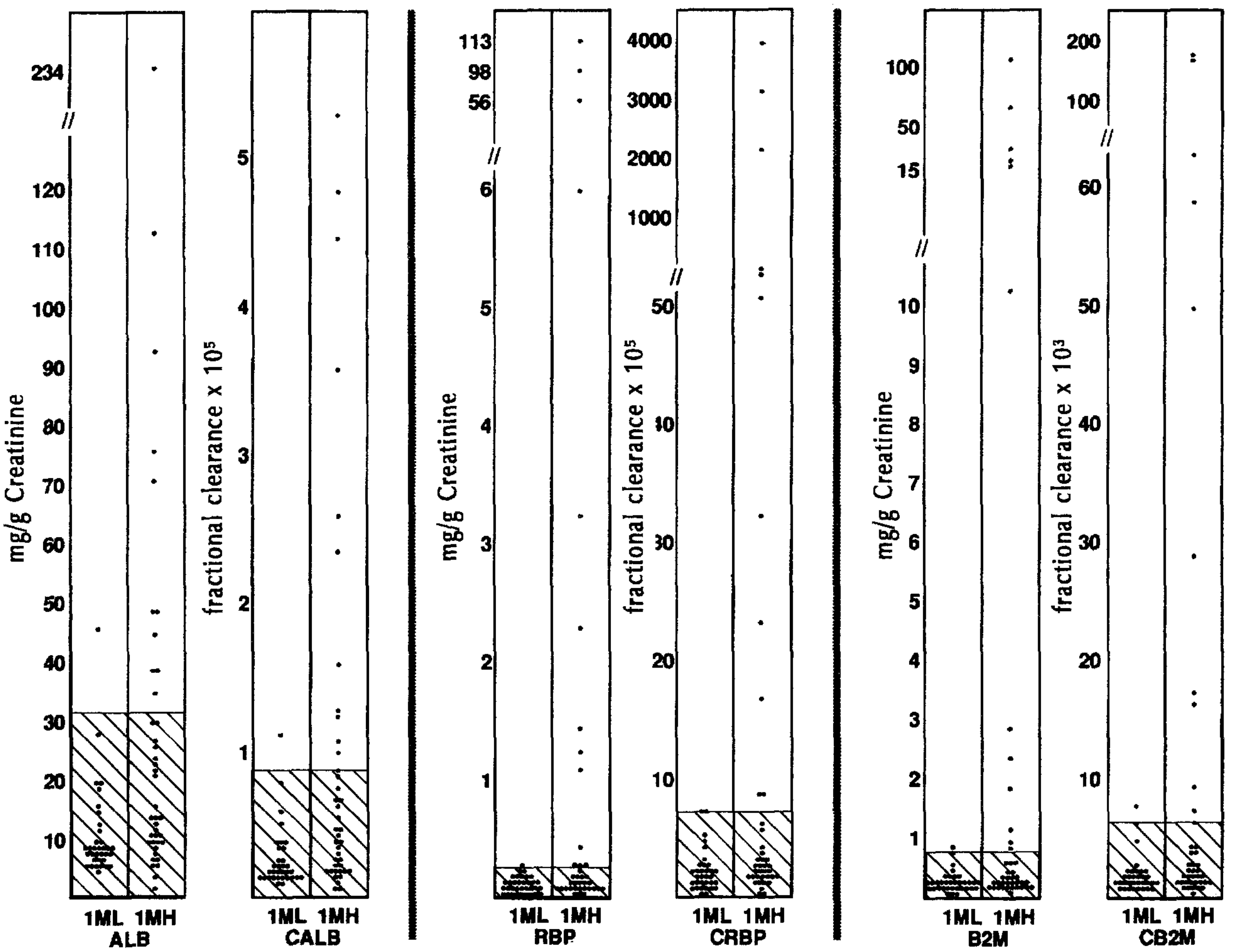

Fig. 1. Urine protein levels and fractional clearances in Study 1 for men with low urine cadmium concentrations (urine cadmium < 2.0 μg/L, 1ML) and higher urine cadmium concentrations (urine cadmium ≥ 2.0 μg/L, 1MH). The proteins measured included albumin (ALB), albumin fractional clearance (CALB), retinol-binding protein (RBP), retinol-binding protein fractional clearance (CRBP), β_2-microglobulin (B2M), and β_2-microglobulin fractional clearance (CB2M). The shaded areas represent the 95th percentile of the low cadmium group (1ML)

in 21% of the high cadmium group, as discussed below, serum creatinine does not correlate with urine cadmium in this study. The serum RBP concentrations do not vary significantly between the 2 cadmium groups.

Study 3: In Study 3 none of the medium or low molecular weight proteins or their fractional clearances were elevated in the high or medium cadmium groups when compared with the low cadmium group (Tables 3 and 5). There were also no statistically significant differences in the means of the protein values or their fractional clearances among the three groups.

The AAP values were elevated in 12% of the high cadmium group and 8% of the medium cadmium group. The means were higher than those for the low cadmium group, but these differences in means were not statistically significant (Table 4). The NAG values were elevated in a slightly larger percentage of the high cadmium group (6%) but not in the medium cadmium group. The mean NAG values in the high and medium cadmium groups were also higher than the mean of the low cadmium group, and, as with AAP, the differences were not statistically significant.

Serum creatinine concentrations were elevated in 6% of the high cadmium group and 4% of the medium cadmium group, but the means were not higher than the mean for the low cadmium group (Table 6). There were no significant differences in serum ALB concentrations among the three groups. The mean serum RBP concentrations did not vary significantly in the 3 cadmium groups, but 6% were elevated in the high cadmium group and 18% were elevated in the medium cadmium group.

Correlations

Study 1: Of the five urine protein tests in Study 1 using the total data set, ALB gives the best correlation with urine cadmium ($r = 0.70$, $p = 0.0001$, $n = 74$). ALB fractional clearance was also highly correlated with urine cadmium ($r = 0.69$, $p = 0.0001$, $n = 73$), and serum albumin was negatively correlated with urine cadmium ($r = -0.50$, $p = 0.0001$, $n = 73$). AAP and NAG gave the next best correlations with urine cadmium (AAP: $r = 0.56$, $p = 0.0001$, $n = 69$; NAG: $r = 0.51$, $p = 0.0001$, $n = 74$) (Mueller *et al.* 1989). RBP and B2M also gave statistically significant correlations with urine cadmium (RBP: $r = 0.31$, $p = 0.0071$, $n = 74$; B2M: $r = 0.33$, p = 0.0038, n = 74), as did RBP and B2M fractional clearances (RBP fractional clearance: r = 0.30, p = 0.0100, n = 73; B2M fractional clearance: r = 0.38, p = 0.0009, n = 73). Serum RBP concentrations were not correlated with urine cadmium, but serum B2M concentrations were (r = 0.36, p = 0.0015, n = 73). Serum creatinine gave a low statistically significant correlation with urine cadmium (r = 0.29, p = 0.0132, n = 73). None of these correlations were significantly improved in the high cadmium group except for the negative correlation between serum albumin and urine cadmium (r = −0.75, p = 0.0001, n = 40). None of the urine or serum protein tests correlated significantly with blood lead except for a seemingly paradoxical negative correlation of AAP in the high cadmium group (r = −0.37, p = 0.0213, n = 38).

Study 2: Of the five urine protein tests in Study 2 using the total data set, ALB and ALB fractional clearance gave the best correlations with urine cadmium (ALB: r = 0.36, p = 0.0013, n = 75; ALB fractional clearance: r = 0.40, p = 0.0004, n = 74). Serum albumin was negatively correlated with urine cadmium (r = −0.47, p = 0.0001, n = 74). AAP gave a low correlation of borderline statistical significance with urine cadmium (r = 0.22, p = 0.06, n = 72) and none of the other tests, including serum creatinine, gave statistically significant correlations with urine cadmium. When the data in the high cadmium group were analyzed separately the correlations of AAP and NAG with urine cadmium became statistically significant (AAP: r = 0.44, p = 0.0058, n = 38; NAG: r = 0.41, p = 0.0087, n = 39), and the correlation of RBP and urine cadmium improved (r = 0.30, p = 0.0666, n = 39).

AAP and B2M also gave low statistically significant correlations with urine nickel when the total data set was used (AAP: r = 0.29, p = 0.0142, n = 72; B2M: r = 0.28, p = 0.0144, n = 75). None of the other tests gave significant correlations with urine nickel. AAP and B2M gave better correlations with the product of cadmium and nickel values than with either cadmium or nickel alone (AAP: r = 0.46, p = 0.0001, n = 72; B2M: r = 0.37, p = 0.0010, n = 75). In the high cadmium group AAP, NAG, and B2M gave significant correlations with urine nickel (AAP: r = 0.53, p = 0.0007, n = 38; NAG: r = 0.52, p = 0.0006, n = 39; B2M: r = 0.65, p = 0.0001, n = 39) and even better correlations with the product of urine cadmium and nickel (AAP: r = 0.68, p = 0.0001, n = 38; NAG: r = 0.56, p = 0.0002, n = 39; B2M: r = 0.73, p = 0.0001, n = 39).

Study 3: The only urine protein or enzyme test in Study 3 to give a statistically significant correlation with urine cadmium when the total data set was used was NAG (r = 0.22, p = 0.026, n = 99). None of the serum parameters, including creatinine, gave significant correlations with urine cadmium, and none of these serum or urine tests gave a statistically significant correlation with urine nickel except serum RBP, which gave a low negative corrlelation (r = −0.20, p = 0.0342, n = 109). Again in this study, the correlation of AAP was better with the product of cadmium and nickel values than with either cadmium or nickel alone (r = 0.23, p = 0.0217, n = 100).

The correlations of urine ALB, ALB fractional clearance, urine RBP, and RBP fractional clearance with urine cadmium improved slightly in the medium cadmium group, but did not reach statistical significance. In this group RBP fractional clearance is significantly correlated with urine nickel (r = 0.36, p = 0.0150, n = 45) and better correlated with the product of urine cadmium and nickel (r = 0.45, p = 0.0020, n = 45).

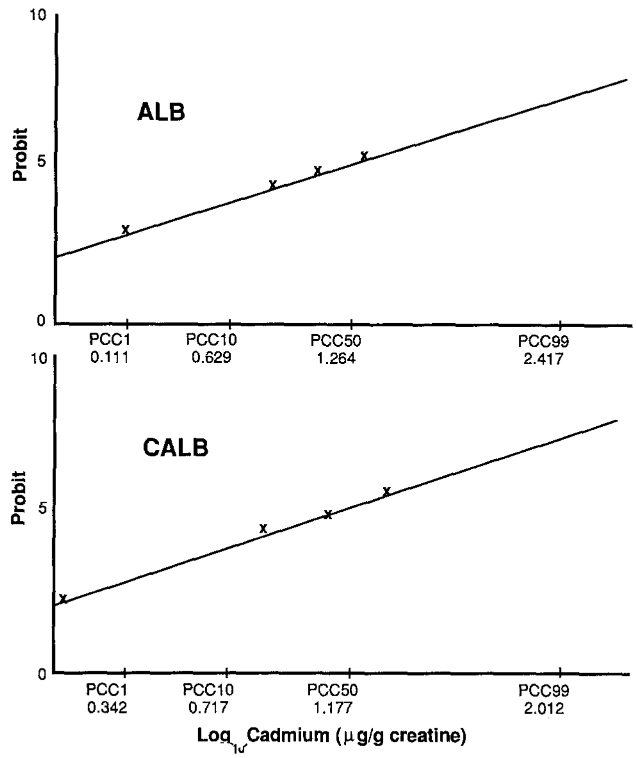

Fig. 2. Probit analysis of albumin (ALB) data corrected for creatinine and albumin fractional clearances (CALB). Probit values on the vertical axis reflect transformed percentages of response. The values on the horizontal axis are the logs of cadmium concentrations corrected for creatinine. PCC = Population critical concentration, the terminology of Friberg (1984). PCC1, PCC10, PCC50, and PCC99 indicate the log of the cadmium level at which there is a 1, 10, 50, and 99% chance of observing an elevated ALB or CALB value. The cadmium levels corresponding to PCC5 and PCC10 are listed in Table 7

Dose-Response Relationships

The first of these three studies had sufficient numbers of elevated renal test values in the high cadmium group to allow us to analyze for a dose-response relationship between urinary cadmium levels in men and the prevalence of elevated urinary protein test values. The probit analyses of the AAP and NAG values in Study 1 are described in Mueller *et al.* (1989), and the same type of analysis of ALB, ALB fractional clearance, RBP, RBP fractional clearance, B2M, and B2M fractional clearance yielded similar results (Figures 2–4). Each of these parameters yielded highly significant fits to the model (Table 7). The probit analysis model of these parameters for men predicted a 5% chance of observing an elevated test value at urine cadmium levels from 3 to 7 μg cadmium/g creatinine (upper 95th fiducial limits from 7 to 10 μg cadmium/g creatinine) and a 10% chance of observing an elevated test value at urine cadmium levels from 4 to 8 μg cadmium/g creatinine (upper 95th fiducial limits from 8 to 12 μg cadmium/g creatinine). The men in the high cadmium group of Study 2 had a lower mean cadmium level (5.88 μg/g creatinine) than the high cadmium group of Study 1 (10.16 μg/g creatinine), and the results of Study 2 generally agreed with the predictions of the model based on Study 1 (Tables 3, 4, 5, and 7).

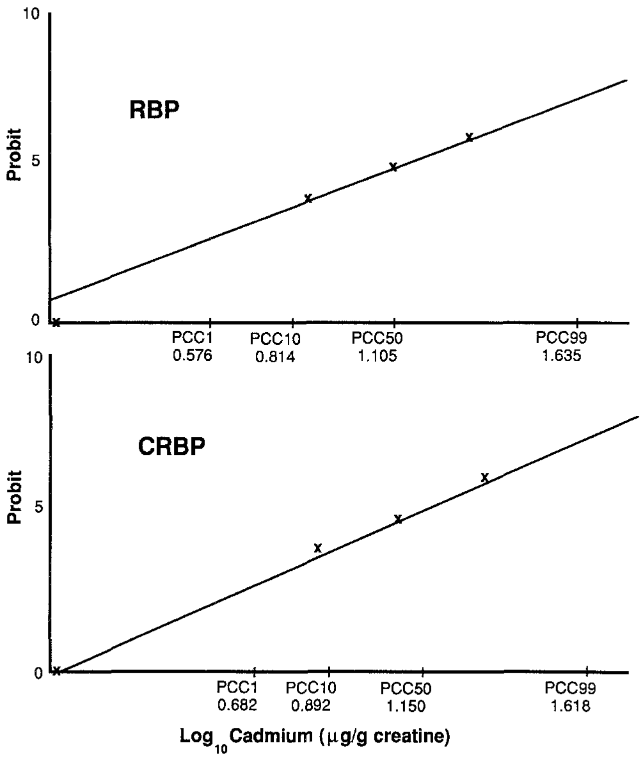

Fig. 3. Probit analysis of retinol-binding protein (RBP) data corrected for creatinine and retinol-binding protein fractional clearances (CRBP). Probit values on the vertical axis reflect transformed percentages of response. The values on the horizontal axis are the logs of cadmium concentrations corrected for creatinine. PCC = Population critical concentration, the terminology of Friberg (1984). PCC1, PCC10, PCC50, and PCC99 indicate the log of the cadmium level at which there is a 1, 10, 50, and 99% chance of observing an elevated RBP or CRBP value. The cadmium levels corresponding to PCC5 and PCC10 are listed in Table 7

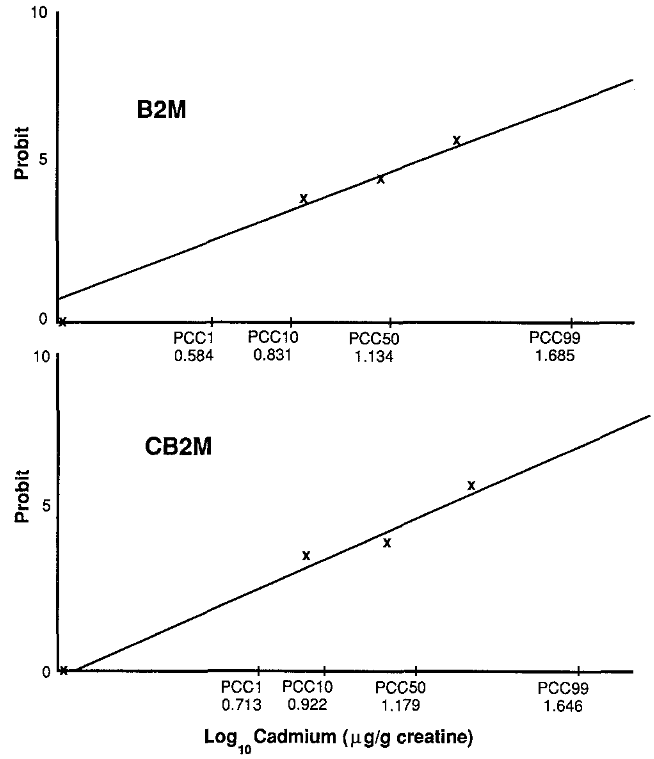

Fig. 4. Probit analysis of β_2-microglobulin (B2M) data corrected for creatinine and β_2-microglobulin fractional clearances (CB2M). Probit values on the vertical axis reflect transformed percentages of response. The values on the horizontal axis are the logs of cadmium concentrations corrected for creatinine. PCC = Population critical concentration, the terminology of Friberg (1984). PCC1, PCC10, PCC50, and PCC99 indicate the log of the cadmium level at which there is a 1, 10, 50, and 99% chance of observing an elevated B2M or CB2M value. The cadmium levels corresponding to PCC5 and PCC10 are listed in Table 7

Discussion

In Study 1, the study with the most advanced renal damage, the means of all five urine protein tests were significantly elevated (ALB, AAP, NAG, RBP, and B2M) in the group with high urine cadmium concentrations when compared with the group with low urine cadmium concentrations. All five urine protein tests were related to the urine cadmium concentrations in a significant dose-response manner, and all of the urine protein levels were elevated in approximately the same percentage of exposed participants. In addition, all the urine protein tests were more sensitive than the serum creatinine test that is commonly used to measure renal function. In Studies 2 and 3, where the renal damage was less advanced, particular urinary protein tests were elevated in some individuals in the groups with elevated cadmium concentrations and other tests were not.

The results of Study 2 generally agree with the predictions based on the men in Study 1. The mean cadmium level in the high cadmium group of Study 2 was 5.88 μg/g creatinine (Table 2). At this cadmium level, the model based on Study 1 data (Table 7) predicts that approximately a 10% prevalence of elevated results would be seen in the ALB, ALB fractional clearance, and AAP tests. The model predicts that the other urinary protein tests would require a higher cadmium level for a 10% prevalence of elevated values. In the high cadmium group of Study 2, 12.8% of ALBs, 15.8% of ALB fractional clearances (Table 3), and 7.9% of AAPs (Table 4) were elevated above the 95th percentile of the low cadmium group. None of the other urinary proteins showed elevations.

The relative sensitivity of urine protein tests used to detect renal damage in cases of cadmium exposure is important both for toxicity studies and for monitoring those persons potentially exposed. To judge the relative sensitivity of the urinary protein tests in men, we considered three factors: 1) the magnitude of the correlation coefficient between the urinary protein level and the urine cadmium concentration in Study 1, $r_{ALB} > r_{AAP} \approx r_{NAG} > r_{RBP} \approx r_{B2M}$; 2) the relative cadmium level predicted by the dose-response model where there is a 10% chance of observing an elevated urinary protein level (PCC10), $PCC10_{ALB} < PCC10_{AAP} < PCC10_{NAG} < PCC10_{RBP} < PCC10_{B2M}$; and 3) the ability of the urinary protein tests to detect renal effects in the study with less advanced damage, ALB > AAP > NAG ≈ RBP ≈ B2M. These criteria taken together showed that ALB was found to be the most sensitive urine protein test in men, followed by AAP, NAG, RBP, and B2M. AAP and NAG were the only urine protein tests with a higher prevalence of elevated values in Study 3 women who had elevated urine

Table 7. Dose-response predictions from Study 1[a]

	Cadmium level (μg/g creatine)[b]					
Test	5%	Upper 95th fiducial limit	10%	Upper 95th fiducial limit	χ^2	p
Albumin	2.8	7.3	4.2	9.0	0.0025	0.96
Albumin fractional clearance	3.8	7.1	5.2	8.6	0.1571	0.69
Alanine aminopeptidase[c]	3.8	6.8	5.0	8.1	2.6345	0.45
N-acetyl-β-D-glucosaminidase[c]	5.0	7.9	6.3	9.2	0.7395	0.86
Retinol-binding protein	5.4	7.8	6.5	8.9	0.0045	0.95
Retinol-binding protein fractional clearance	6.6	9.2	7.8	10.5	0.1344	0.71
β_2-microglobulin	5.6	8.1	6.8	9.4	0.3878	0.53
β_2-microglobulin fractional clearance	7.1	10.2	8.4	11.5	0.8650	0.35

[a]Based on probit analyses of data from Study 1. The model is considered to fit the data if χ^2 is low as defined by $p > 0.1$
[b]Cadmium levels at which there is a 5% or 10% chance of observing an elevated protein test value
[c]Mueller et al. 1989

cadmium levels. Low molecular weight protein tests (RBP and B2M) have been traditionally used in the assessment of renal damage due to cadmium exposure, and they provide unique information about tubular reabsorption capability. However, they are less sensitive to the renal effects of cadmium than the other three tests.

It is important to note that the elevated urinary protein levels that were seen in the cadmium exposed groups were mostly in the upper part of, or just above, the normal range. Therefore, when using these tests in epidemiologic studies, a control population is essential.

It is also possible that these tests will be used for monitoring individuals exposed to cadmium. When so used, baseline values to establish an individual's range of biological variation and long-term standardization of assays to validate any changes seen (Mueller *et al.* 1991) are essential.

The findings from the first two studies of men were consistent with reports that glomerular damage can occur in the presence of minimal tubular damage (Lauwerys *et al.* 1974; Roels *et al.* 1975; Bernard *et al.* 1979; Roels *et al.* 1981; Bernard *et al.* 1981; Lauwerys and Bernard 1986). In Study 1, all parameters in the three classifications of tests indicate renal damage. In Study 2 only urinary ALB, ALB fractional clearance and, to a lesser extent, AAP (not urinary RBP or B2M, markers of loss of tubular reabsorption capability) had a higher prevalence of values elevated above the 95th percentile of the low cadmium group. Explanations for a mechanism for glomerular damage include the finding that exposure to cadmium in men is linked to a loss of membrane negative charge as measured by cationic dye binding to the surface of red blood cells (Bernard *et al.* 1988). Loss of negative charge on the glomerular capillary wall is widely proposed as a mechanism for subclinical increases in urinary albumin.

Another interesting finding was the decrease in serum albumin that was related to urine cadmium levels in Studies 1 and 2. In both studies there were highly significant negative correlations between serum albumin and urine cadmium. Of the 11 men in Study 1 with urine albumin levels above the 95th percentile of the low cadmium group, 4 had low serum albumin levels (below the 5th percentile of the low cadmium group). All the men with low serum albumin values had urine albumin value above 20 mg/g creatinine. In Study 2 the low serum albumins were not as low as those in Study 1, and 6 of the 8 low serum albumins were not associated with high urinary albumins. This may reflect less advanced damage in this group of men, but other causes cannot be ruled out.

In environmental studies, renal damage in women exposed to cadmium is mainly seen after menopause (Lauwerys *et al.* 1980). Women (age > 50 years) living in cadmium-polluted areas of Japan, who have established renal damage consistent with high levels of such indicators as serum creatinine and blood urea nitrogen, are reported to excrete elevated levels of markers of compromised tubular reabsorption (Tohyama *et al.* 1986). Women (age > 60 years) living in cadmium polluted areas of Belgium are reported to excrete significantly higher levels of ALB than a control population. The B2M excretion rate of this group is greater, but not statistically significantly greater, than that of the control group. The women in Study 3 were younger than the women in these studies; thus fewer would be postmenopausal. In an occupational study of younger Malay, Chinese, and Indian women (ages 30 to 39 years) exposed to cadmium in a nickel/cadmium battery factory, the authors conclude that NAG detects the largest proportion of abnormalities in the exposed group when compared with B2M (Chia *et al.* 1989). In another study of younger women (mean age = 30.5 years) occupationally exposed to cadmium dust, the authors find no evidence of abnormal urinary protein by electrophoresis in the exposed group (Lauwerys *et al.* 1974). In our Study 3, only the indicators of direct release of tubular tissue (AAP and NAG) had a higher prevalence of elevated test values in the higher urine cadmium groups, not the indicator of glomerular damage (ALB) nor the indicator of tubular reabsorption capability (RBP). The mean age of menopause is 51 years (Rebar 1988). Of the 9 women in Study 3's medium and high cadmium groups who had elevated AAP or NAG values, 5 were older than 50 years and 2 were ages 47 and 49 years (21.9% of the total number of women in these groups ages ⩾ 47 years). Of the 13 women ages 47 and older in the group with low urine cadmium levels, none had an elevated AAP and 1 had an elevated NAG (7.7%). These results are consistent with the expectation of higher rates of elevated renal test parameters in women with higher cadmium levels after age 47.

In addition to variations in cadmium level and gender among the studies, three additional differences in the study populations are important to note. First, the individuals with high urine cadmium levels in Study 1 were exposed to cadmium fume and those with elevated cadmium levels in Studies 2 and 3 were exposed only to cadmium dust. Second, some of the individuals in Study 1 had carried their cadmium body burden for a long time, with more opportunity for a retained cadmium body burden to cycle through the kidney. The high cadmium group of Study 1 was also older than the other groups. Third, there was an accompanying exposure to nickel in Studies 2 and 3. Animal experiments show that pretreatment with nickel protects against cadmium-induced nephrotoxicity in rats (Tandon *et al.* 1984), but administration of cadmium to rats followed by nickel enhances nephrotoxicity (Khandelwal and Tandon 1984). It is not known if these effects occur in humans. In this regard, better correlations were seen between several renal tests and the product of urine cadmium and nickel levels in Studies 2 and 3 than between the renal tests and either metal alone, indicating the possibility of an interaction between these two metals as they affect the kidney.

Although NIOSH recommends that cadmium exposure be reduced to the fullest extent feasible, cadmium body burdens are retained for years because of the 10- to 30-year half-life of cadmium (Friberg *et al.* 1986). It would be useful to follow the individuals in Studies 2 and 3 who were exposed in the nickel/cadmium battery plant to determine if their renal damage progresses. This is particularly important for the women in Study 3 as their high cadmium body burdens continue past menopause.

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