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Urinary Protein Excretion in Workers Exposed to Low Doses of Cadmium

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In 1950, Friberg reported on a comprehensive study into the effects of long-term cadmium exposure via air. It was discovered that cadmium was associated with proteinuria, a finding which has been corroborated in a number of studies since then (FRIBERG et al. 1974). Olhagen (1950) showed that the chief component of the urinary proteins excreted had a molecular weight in the range of 20,000-30,000. Studies performed by Piscator (1962b and 1966b) further demonstrated that the proteinuria seen in cadmium exposure was characterized by a predominance of low molecular weight proteins in the beta globulin class.

Renal tubular dysfunction is a predictable result of long-term exposure to cadmium oxide dust or cadmium oxide fumes. This is considered to be due to decreased tubular reabsorption of filtered proteins (FRIBERG et al. 1974).

An increase in low molecular weight proteins in the urine is regarded as constituting the first sign of cadmium-induced renal damage and has increased the diagnostic sensitivity (BROWN 1977). Quantitative determinations of urinary β_2-microglobulin have revealed highly increased amounts of this protein in persons with tubular damage (BERGGARD and BEARN 1968) while total protein was not increased to the same extent in a group of patients with renal disease linked to cadmium toxicity (KANAI et al. 1971).

The possibility that cadmium produces cardiovascular disease, especially hypertension, has attracted much attention. The fact that hypertension has been associated with high kidney levels of cadmium in certain studies cannot be used for firm conclusions concerning casuality (FRIBERG et al. 1974).

Cadmium plays an important role in turf management when integrated with other fungicides according to the U. S. Golf Association. The greens keepers represent a population of workers who have low-dose exposure to this chemical over an extended period of time.

The basic objective of the study was to measure the excretion of specific proteins in the urine of these workers as a measure of cadmium toxicity at this level of exposure. The question of whether cadmium may have an effect on blood pressure

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was also addressed.

METHODS

There were 49 male subjects tested in the study, 18 of which were black and 31 white. They were divided into 4 groups - 14 with an exposure of less than 5 years, 22 with an exposure of 5 years or more, 3 formulators who worked with the chemical the same day the urine collection was begun, and 10 controls. The cadmium-exposed participants were greens keepers who regularly sprayed the greens with cadmium to control the growth of fungi. Their ages ranged between 21 and 62 years. The 10 control subjects ranged between 22 and 62 years of age and were selected from faculty members and other employees of the Medical University of South Carolina. Emphasis was placed on selecting healthy males who had with certainty not been exposed to any significant levels of cadmium. The exposure history was elicited by interview and a general medical history was obtained. It was assumed that routine procedures in maintaining greens would insure a good degree of consistency among the workers regarding levels of exposure.

A 12-hour overnight urine was collected and analyzed for specific gravity, PH, creatinine concentration, cadmium, total protein, and B_2-microglobulin as an index of tubular damage. Other determinations included urinary lysozyme as a second index of tubular damage and sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), a technique that allows the screening of tubular proteinuria through separation according to molecular size (PIRES et al. 1975). Other parameters which were measured in the specified groups were weight and blood pressure. With the exception of blood pressure readings, only those measurements which were germane to answering the hypothesis that kidney damage results from long-term exposure to low doses of cadmium were included in the tables.

Standard laboratory techniques were employed for these determinations. The supplied urines were filtered and divided into two aliquots: a small aliquot of 10 ml was kept unconcentrated, to be used in the determination of urinary total proteins, beta-2 microglobulin, and lysozyme; a second aliquot of 100 ml was concentrated by negative pressure ultrafiltration, to be used in the separation of urinary proteins by SDS-PAGE. Total urinary proteins were directly assayed on filtered, unconcentrated urines by using the Bio-Rad protein assay method.[1] Beta-2 microglobulin was assayed by radioimmunoassay[2] and the lysozyme assay was done using the lysoplate method (OSSERMAN and LAWLOR 1966). Cadmium levels in the urine were measured by atomic absorption.

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[1]Bio-Rad, Richmond, CA. 94804. This assay can detect protein concentration of 0.1 mg/ml.

[2]Pharmacia Fine Chemicals, Piscaraway, New Jersey 08854.

RESULTS AND DISCUSSION

Cadmium levels are found in Table 1. Normal values are provided at the top of the table where appropriate. The results of our determinations are detailed in Table 2. They include all the essential indices of possible kidney damage from cadmium and also systolic and diastolic BP. Although cadmium is not a normal constituent of the urine, the levels which were detected were far below any level which would be considered hazardous.

TABLE 1 - CADMIUM LEVELS

All in exposure group 1 (controls)	- negative
All in exposure group 2 (<5years)	- negative
All in exposure group 3 (5 years or more)	- negative
Group 4 (formulators)	CM - 9.5 ppb
	WM - 3.0 ppb
	WK - 10.7 ppb

Among the exposed individuals, two had increased proteinuria (total protein). One (T.G.), age 50, had increased proteinuria, classified as glomerular by SDS-PAGE, but he also eliminated detectable amounts of lysozyme, suggesting a mild compromise of both his glomerular and tubular functions. The second patient with increased proteinuria (W.M.) had predominant elimination of low molecular weight proteins as seen by SDS-PAGE, and as such the proteinuria would be classified as tubular. However, the urine did not contain detectable lysozyme and the concentration of beta-2 microglobulin was normal. A third patient (A.G.) had an upper-limit of normal value for total protein but had normal values for beta-2 microglobulin and lysozyme, and by SDS-PAGE the only abnormality detected was increased amounts of albumin. In this group of cadmium-exposed individuals, we found one with elevated concentration of beta-microglobulin in the urine. Although the assayed concentration would be considered within normal limits by some authors, it was well above the levels assayed in any other individual in this series or in any of the controls. SDS-PAGE of the urine of this subject (O.L.) also showed tubular proteinuria. SDS-PAGE showed abnormalities in one additional cadmium exposed subject who had no other abnormal parameter. The electrophoretic separation showed mixed proteinuria with a predominantly tubular pattern (A.McW.), meaning that his urine, which was the most abnormal of all those included in the present study as seen by SDS-PAGE, contained predominantly low molecular weight fractions in addition to increased amounts of high molecular weight proteins. It must be pointed out that, at the present state of knowledge, the finding of abnormality on SDS-PAGE can only be interpreted as indicating abnormal proteinuria but is not indicative of a compromise of kidney function unless there are other abnormal indices present. Furthermore, this sensitive technique may detect a transient proteinuria in healthy individuals or be reflective of other disease states in the body which affect the kidney.

TABLE II

CADMIUM EXPOSURE 1977

Exposure Code:
1=control
2=<5 yrs
3=5 yrs or more
4=formulator

	NAME	AGE	EXPO-SURE	nl=150-200mg/l TOTAL PROTEIN (mg/l)	nl=4-370ug/l Mean=82.5ug/l B2 MICRO-GLOBULIN (Hg/l)	nl=0 LZM (Hg/ml)	SDS-PAGE	BP SYS	BP DIAS
CONTROLS	EB	25	1	110	52	0	Normal	150	90
	STC	27	1	100	64	0	Normal	140	75
	CMC	61	1	120	28.5	0	Normal	124	76
	CDG	60	1	100	39	0	Tubular	130	80
	JWK	54	1	80	32	0	Normal	130	90
	RR	22	1	250	60	0	Tubular	130	80
	SHS	62	1	110	36	0	Normal	125	86
	APS	23	1	100	54	0	Normal	110	70
	NHW	59	1	90	Tr.	0	Normal	140	80
	RTW	27	1	90	<20	0	Mixed	120	80
PATIENTS	AA	62	3	70	93	0	Normal	110	70
	HB	40	3	100	Tr.	0	Normal	115	80
	RB	36	3	60	Tr.	0	Normal	125	80
	LB	41	2	50	<20	0	Normal	145	85
	SB	38	3	90	43	0	Normal	160	110
	JMC	27	3	40	Tr.	0	Normal	140	80
	HC	36	3	80	Tr.	0	Normal	120	85
	TD	48	3	50	37.5	0	Normal	160	100
	JD	56	2	130	Tr.	0	Normal	170	110
	JE	48	3	50	<20	0	Normal	130	85
	JF	35	2	40	Tr.	0	Normal	135	70

HF	45	3	70	Tr.	0	Normal	190	110
AG	31	2	190	Tr.	0	Normal(Alb)	150	110
TG	46	3	80	39	0	Mixed	170	95
HG	40	2	50	Tr.	0	Normal	140	80
SG	41	3	40	43	0	Normal	125	90
TG	58	3	240	Tr.	1.9ug/ml	Glomerular	180	115
HH	48	3	50	Tr.	0	Normal	140	80
JGi	35	3	45	Tr.	0	Normal	130	85
JHu	46	3	43	26	0	Normal	150	90
JaJ	27	3	30	Tr.	0	Normal	150	60
JoJ	29	2	40	Tr.	0	Normal	150	70
EdK	53	2	30	Tr.	0	Normal	160	80
EuK	31	2	20	25	0	Normal	125	80
OL	52	2	130	165	0	Tubular	120	75
AMcW	31	3	61	Tr.	0	Mixed(Tubular)	150	90
CM	--	4	90	Tr.	0	Normal	130	80
KM	26	2	80	31.5	0	Normal	160	80
LM	50	2	65	Tr.	0	Normal	125	80
WM	--	4	230	51	0	Tubular	150	90
CJP	53	3	90	<20	0	Normal	150	85

B2-Microglobulin:

<20 indicates values inferior to those of the calibration point for 20 ug/l, but that would correspond to levels between 10 - 20 ug/l if calibration curve was extrapolated.

Trace = values lower than 10 ug/l.

All quantifiable parameters measured were compared statistically by the specified cohorts or exposure level using one-way analysis of variance. A few cases are worth mentioning in which one group had significantly higher values than the other group. The most obvious one was in the Cd level of the formulators who averaged 7.7 ppb as compared to an 0 level for all other groups. For total protein, the control group had a significantly higher level primarily due to (R.R.) whose value was extremely high. Considering the measurement of B_2-microglobulin among the various groups, the mean value for the control group was statistically higher than the other groups. However, it is not meaningful in that all the values are well within normal limits. When the mean systolic and diastolic blood pressures were compared among the various cohorts with separation by race, there was no difference statistically. However, the combining of the races raised the mean values of systolic BP for groups 2 and 3 to a significantly higher level than the all-white control and was attributed to a tendency among blacks generally to have a higher BP than whites. The readings seen were not inconsistent with this hypothesis and were not thought to be due to an effect of cadmium.

Otherwise, no specific exposure group had values for the variables measured which were significantly higher than any other group according to the statistical method employed. Those variables that could legitimately qualify as covariates were considered as such. No significant ($P<.05$) relationship resulted and therefore an analysis of covariance was not done. Multivariate analysis techniques were considered both for the index as well as the response variables. However, the individual response variables were of specific interest and the univariate analysis of these was emphasized.

CONCLUSIONS

There was evidence of mild renal dysfunction in three of the cadmium-exposed individuals out of the total of 39. However, in none of them did we find a fully developed tubular proteinuria in which moderate increases of total proteinuria, increased lysozymuria and beta-2 microglobulinuria, and excretion of low molecular weight proteins by SDS-PAGE would also be found.

Out of the four parameters determined, only one patient had three of them in the abnormal range, but this patient showed predominant elimination of high molecular weight proteins, compatible with glomerular damage, an unexpected finding in cadmium poisoning. Renal dysfunction was detected in one control subject who had increased total protein classified as tubular. There were two abnormal SDS-PAGE patterns found in the control group in which the other parameters measured were normal as was the case in the cadmium exposed patients.

Therefore, in reviewing the determinations of the indices of renal function which were tested for, it was concluded that there was essentially no difference statistically between the frequency of abnormalities of the control group and the group of cadmium-exposed subjects. While there were demonstrated some statistically significant higher average values in the controls for some of the indices, namely B_2-microglobulin and total protein, this was due to an unexplained trend of higher measurements which were still in the normal range. Blood pressure readings were likewise considered to show no significant statistical variation among the various cohorts since the slightly higher mean values in two of the exposed groups could easily be accounted for by the epidemiological documented tendency among the black population to have higher blood pressure.

In conclusion, there was one control subject who had some degree of evidence of a compromise of renal function as compared with three in the study group. As such it is not legitimate to attribute the exposed group to cadmium toxicity. It appears that the long-term use of this pesticide by golf course workers is not associated with a demonstrable health hazard in either the application or formulation process as judged by renal function assayed by specific methods for proteinuria.

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