Determination of Cadmium in Urine and Observations on Urinary Cadmium and Protein Excretion in Men Exposed to Cadmium Oxide Dust

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Prolonged exposure of workmen in the alkaline-accumulator industry to cadmium oxide dust led in many cases to the appearance of protein in the urine (Friberg, 1950). As part of a study of the proteinuria of cadmium poisoning an accurate method for the measurement of cadmium in urine was obligatory. This paper describes a sensitive and specific dithizone procedure and presents data on the incidence and degree of proteinuria in relation to cadmium exposure and excretion.

MATERIALS AND METHODS

Fresh urinary specimens of 120 employees in an alkaline-accumulator factory were examined for the presence of protein as follows: 2 ml. 25% (w/v) trichloroacetic acid were added to 2 ml. urine that had first been filtered until clear. A just perceptible opalescence was recorded as a trace ($\pm$), definite opacity as positive (+) and a precipitate as strongly positive (++). Twenty-five urinary specimens gave evidence of the presence of protein by this sensitive test, and the positive specimens also showed heat coagulation, precipitation by 25% (w/v) salicylosulphonic acid and Esbach's and Heller's Tests.

Urinary specimens from twelve workmen offered the possibility of more detailed study. Mid-morning specimens from these men were transported weekly to the laboratory with minimal delay for quantitative evaluation of cadmium and protein. A group of 100 workmen of similar age range without known exposure to cadmium were used as controls.

Total urinary protein. Total urinary protein was determined by the biuret method of Hiller, McIntosh & Van Slyke (1927), using 40 ml. urine instead of 2 ml. as in the original procedure.

Concentration of the urine. Creatinine concentration was determined by the alkaline picrate method of Bonsnes & Taussky (1945). Specific gravity was measured by weighing the urine in a calibrated 50 ml. volumetric flask corrected for temperature.

Cadmium in urine

Earlier reports of cadmium excretion (Princi, 1947; Cotter & Cotter, 1951) indicated that a suitable method should measure 0–20 μg . Cd in 50 ml. urine and be sensitive to 0.1 μg . Cd. A colorimetric procedure using diphenylthiocarbazone appeared most likely to fulfil these conditions.

Numerous dithizone methods for Cd have been described, based on the original observation of Fischer & Leopoldi

(1937) that the cadmium–dithizone complex in CHCl_3 solution is stable on treatment with 6% NaOH. The procedure of Setterlind & Krause (1943) for determination of Cd in air appeared capable of application to biological material without major modification.

In early trials with this method it was found that 0–40 μg . Cd in aqueous solution could be recovered quantitatively in the presence of 1000 μg . of each of the following metal ions: Ag^+ , Al^{3+} , Ba^{2+} , Bi^{3+} , Ca^{2+} , Co^{2+} , Cr^{3+} , Fe^{3+} , Mn^{2+} , Pb^{2+} , Sn^{2+} , and Sn^{4+} , but that quantities of Cu^{2+} and Hg^{2+} greater than 200 and 30 μg . respectively prevented recovery of Cd. Interference from Cu and Hg could be avoided by a preliminary extraction of these metals from approximately 1% HNO_3 with CHCl_3 and dithizone.

Further experience in the application of the method to measurement of Cd in urine showed that a number of changes were necessary. The following points are worthy of special mention: (1) For final measurement of Cd the pink dithizone complex is to be preferred to the green dithizone solution in CHCl_3 regenerated from the complex, since the dithizone solution is photolabile. (2) Considerable losses of Cd occurred during the two washings with 6% NaOH. Such losses could be prevented by dropwise addition of a 1.5% (w/v) NH_3 solution of dithizone to maintain an excess of dithizone in the aqueous layer. This effect could be ascribed to an equilibrium between dithizone in the aqueous phase and the complex in CHCl_3 . Great excess of dithizone resulted in the passage of Pb along with Cd as dithizonates into the CHCl_3 layer. Eventually the dithizone was employed entirely in NH_3 solution and the CHCl_3 added without dithizone in solution. In addition, the blank colour was thereby reduced considerably having been formerly somewhat yellow due to the presence of a decomposition product of dithizone. (3) The amount of KCN (0.25%) used by Setterlind & Krause in the second alkaline washing was insufficient to prevent interference by a large excess of Pb. The observation of Saltzman (1953) that the presence of cyanide in the first alkaline washing generally reduced interference from other metals, mainly Pb, was confirmed. The concentrations of cyanide employed by Saltzman (0.05 and 1.0% for the first and second washing respectively) were adopted and were found to be satisfactory. (4) The acid digests of urine contained much $(\text{NH}_4)_2\text{SO}_4$ formed from urea and other urinary nitrogen compounds. When NaOH was added to the neutralized digest in the first alkaline washing, NH_3 was evolved with equivalent loss of alkali and no control over the pH of the aqueous phase was possible. This difficulty was avoided by neutralizing the digest with conc. NH_3 (sp.gr. 0.880) and making a preliminary extraction of trace metals from weakly ammoniacal solution with dithizone and CHCl_3 .

The reagents and method finally employed are as follows.

Reagents. Water distilled and stored in Pyrex glassware was used in the preparation of all aqueous solutions. All the reagents used were of Analar quality.

Sodium hydroxide 40%-potassium cyanide 0.05%: 80 g. NaOH pellets and 0.1 g. A.R. KCN were dissolved in 200 ml. distilled water.

Sodium hydroxide 40%-potassium cyanide 1.0%: 80 g. NaOH pellets and 2.0 g. KCN were dissolved in 200 ml. distilled water; both these solutions are renewed after 7 days.

Sodium potassium tartrate solution 25% (w/v); this is also renewed after 7 days.

Ammonia solution 1.5% (w/v): 25 ml. conc. NH_3 (sp.gr. 0.880) were diluted to 500 ml. with distilled water.

Nitric acid solution 1% (w/v): 20 ml. HNO_3 (sp.gr. 1.42) were diluted to 2000 ml. with distilled water.

Ammoniacal dithizone was prepared by dissolving approximately 0.1 g. diphenylthiocarbazon

in 10 ml. CHCl_3 and extracting the solution with 20 ml. 1.5% NH_3 solution. The CHCl_3 layer was run off completely from the orange-red ammoniacal layer.

A stock standard Cd solution was prepared by dissolving 0.2032 g. $\text{CdCl}_2 \cdot 2.5 \text{H}_2\text{O}$, previously dried in a desiccator, in 1000 ml. 1% nitric acid. This solution contained 100 μg . Cd/ml. A working standard solution containing 1 μg . Cd/ml. was prepared as required by diluting 10 ml. of the stock solution to 1000 ml. with 1% nitric acid.

The reagent blank for the complete procedure was found to be so low (0.3 μg . Cd) that removal of Cd from the reagents was unnecessary.

Three 250 ml. Pyrex pear-shaped separatory funnels were employed. These were cleaned twice with hot tap water and rinsed twice with distilled water after each analysis.

Method. 50 ml. urine were measured in a graduated flask and transferred to a 100 ml. Pyrex Erlenmeyer flask. Any deposit was stirred thoroughly into the urine before measuring. Transfer was completed by two washings with distilled water. The urine was evaporated gently to dryness on a sand bath. The flask was then allowed to cool and 5 ml. conc. HNO_3 were added, the flask being further warmed on the sand bath until the evolution of brown fumes had ceased. After cooling, 4 ml. conc. H_2SO_4 were added and the flask was heated more strongly to evaporate the HNO_3 . At this stage the blackened digest was allowed to cool, treated with 0.5 ml. 100-vol. H_2O_2 and then warmed gently. The addition of H_2O_2 was repeated two or three times until the digest remained clear after strong heating.

The digest was allowed to cool and diluted with 10 ml. distilled water and approx. 1 g. sodium citrate added. The sodium citrate prevented the formation of a precipitate (presumed to be calcium phosphate) which otherwise formed on neutralization. The contents of the flask were rendered just alkaline to litmus by addition of conc. NH_3 (sp.gr. 0.880). The neutralized digest was cooled and transferred to a separatory funnel. The flask was rinsed out with two 5 ml. portions of 1% HNO_3 and the washings were added to the funnel. A further 5 ml. 1.5% NH_3 solution were added, together with 10 ml. CHCl_3 and ten drops of

ammoniacal dithizone. The funnel was shaken and more dithizone was added until, after shaking, the CHCl_3 layer became dark blue in colour. The coloured extract, containing the dithizonates of most of the trace metals including Cd together with excess of dithizone, was run off into a second funnel containing 25 ml. 1% HNO_3 . Further,

Fig. 1. Relation between the amount of cadmium and the optical density of its derived dithizonate, measured in chloroform (10 ml.) at 506 $\mu\mu$.

Table 1. Recovery of cadmium added to normal urine

The cadmium was added to 50 ml. samples of urine. Columns I and II show duplicate values.

Added	Amount of cadmium (μg .)			
	Found		Recovered	
	I	II	I	II
0.0	0.5	0.5	0.0	0.0
1.0	1.6	1.4	1.1	0.9
2.0	2.6	2.5	2.1	2.0
3.0	3.7	3.5	3.2	3.0
4.0	4.5	4.3	4.0	3.8
5.0	5.3	5.4	4.8	4.9
10.0	10.8	10.7	10.3	10.2

similar extractions were made until the CHCl_3 became green. After shaking the pooled CHCl_3 extracts with 1% HNO_3 , the CHCl_3 layer, containing Cu and Hg, was discarded. The last traces of green dithizone colour were removed with 5 ml. CHCl_3 , added gently and run off without shaking.

The aqueous layer, which contained Cd and the other trace metals whose dithizonates were unstable in the presence of dilute acid, was rendered strongly alkaline

with 5 ml. 40% NaOH-0.05% KCN, and 2 ml. 25% sodium potassium tartrate, 10 ml. CHCl_3 and five drops of ammoniacal dithizone were added. The mixture was shaken for 1 min. and then allowed to stand. If the aqueous layer was colourless or only pale yellow on settling further dithizone was added and the funnel was shaken again. The pink CHCl_3 layer containing only Cd and traces of Pb as their dithizonates was run into a third separatory funnel containing 25 ml. 1% HNO_3 . The contents of the second funnel were further extracted with 10 ml. portions of CHCl_3 until the extracts were colourless, three such extractions being normally sufficient to remove the largest quantity of Cd encountered.

The third funnel was then shaken for 1 min. to decompose the complexes, the Cd and Pb entering the aqueous phase. The pale green CHCl_3 layer was discarded and the last traces of dithizone removed by adding 5 ml. CHCl_3 , which were run off without shaking. To the aqueous layer were added 5 ml. 40% NaOH-1% KCN and 5 ml. CHCl_3 . Three drops of ammoniacal dithizone were added and the mixture was shaken for 1 min. With large quantities of Cd further addition of dithizone was necessary to ensure that the aqueous layer remained yellow in colour. The pink CHCl_3 layer, containing only cadmium dithizonate, was withdrawn into a graduated tube and the extraction was repeated with a further 5 ml. CHCl_3 . A few drops of CHCl_3 were used to remove the last traces of complex. The volume in the tube was brought to 10 ml. with CHCl_3 and the tube was stoppered and allowed to stand, when most of the water droplets entrained in the CHCl_3 settled out on the walls of the tube. The optical density of the cadmium dithizonate was measured at its maximum, 506 $\text{m}\mu$., in a Unicam spectrophotometer SP. 500. The time of standing was not critical as the cadmium complex was photostable.

Normally twelve urines with two blanks were digested and analysed together and the final CHCl_3 solutions stood for about an hour before the measurement of the absorption. Cadmium contents of the various specimens were read from a standard curve prepared by passing accurately measured volumes of the working standard Cd solution through the analytical procedure. The standard curve is shown in Fig. 1. The reagent blank for the complete determination was consistently 0.3 μg . Recoveries of Cd added to normal urine are presented in Table 1.

RESULTS

The data on the incidence of proteinuria in this study are presented in Table 2. Proteinuria was observed in 5% of the control group of workers, whereas 20% of the workers exposed to cadmium showed urinary protein. The normal range of urinary cadmium was found to be 0-20 $\mu\text{g}/\text{l}$., and the range in the workmen exposed to cadmium was 10-580 $\mu\text{g}/\text{l}$.

The results of cadmium and protein determinations over a period of 6 weeks on weekly spot samples from twelve workers are shown in Table 3. Specific gravity and creatinine values are included as a guide to the concentration of the specimens. No attempt has been made to employ these values

to correct the cadmium and protein excretions for variations from normal urinary concentration.

Cadmium in air determinations in the plant were made during the period February-July 1952. The mean values for the two main processes carried out in the plant were 0.33 and 0.55 mg. Cd/ m^3 with the highest figure 1.9 mg./ m^3 . Many of the workmen studied had been subjected for many years to

Table 2. *Incidence of proteinuria in workers exposed to cadmium and in workers without known exposure*

The total number of exposed workers tested was 120 and the total number tested, but not exposed, was 100. The amount of protein excreted is designated as follows: + + +, approx. 5 g./l.; + +, approx. 1 g./l.; +, 0.1-1 g./l.; $\pm$, 25-100 mg./l.; negative, <25 mg./l.

Degree of proteinuria	Number of workers	
	Exposed	Not exposed
+ + +	0	1
+ +	1	0
+	17	1
$\pm$	7	3
Negative	95	95

conditions worse than this. The various processes were graded 0-4, and an exposure index, a measure of the atmospheric cadmium in the individuals working life, was computed by summation of the products of process grading and time of exposure (in years) for all the work done.

DISCUSSION

The subjects of this study have been exposed to cadmium oxide dust for periods varying from 0 to 30 years, the severity of exposure likewise differing widely throughout the group. There was no clear relationship between grading of exposure and urinary cadmium, especially on the least hazardous operation (Table 3).

Proteinuria occurred more frequently in cadmium workers than in the control group (Table 2), although in all exposed cases the quantity of protein was small. Urinary cadmium and excreted protein appeared to vary independently, but workman A, who worked in a severely contaminated atmosphere, consistently excreted most cadmium and protein in his urine. Currently exposed workmen (D, H, J, K, L, P and R) generally exhibited higher rates of urinary cadmium excretion than those not currently exposed (M, O and Q), whilst the values for N, who had not been in contact with cadmium oxide dust, were within the normal range. The nature of the proteinuria in this intoxication is the subject of continued study.

Table 3. *Urinary excretion of cadmium and protein in workmen exposed to cadmium oxide dust*

The urine of each workman was tested weekly for six successive weeks (urines 1-6). For definition of exposure rating and exposure index see text.

Workman	A	D	H	J	K	L	M	N	O	P	Q	R
Exposure												
Current rating	4	2	2	1	1	1	0	0	0	1	0	2
Time (years)	15	30	15	26	30	25	22	0	14	30	25	10
Index	53	83	28	44	53	43	66	0	22	30	16	16
Urine 1												
Cd ($\mu\text{g./l.}$)	578	98	122	52	90	114	36	22	40	76	10	42
Protein (mg./l.)	1138	170	—	383	738	575	400	0	10	15	5	25
Sp.gr.	1.020	1.021	1.023	1.028	1.020	1.022	1.017	1.027	1.024	1.023	1.015	1.023
Urine 2												
Cd ($\mu\text{g./l.}$)	500	56	80	41	54	20	32	10	44	52	—	28
Protein (mg./l.)	925	320	13	40	195	103	125	10	15	8	—	183
Sp.gr.	1.021	1.019	1.020	1.027	1.012	1.009	1.028	1.019	1.024	1.019	—	1.023
Creatinine (mg./100 ml.)	—	98	82	44	62	32	160	99	—	153	—	125
Urine 3												
Cd ($\mu\text{g./l.}$)	534	50	40	42	66	76	32	12	30	84	15	37
Protein (mg./l.)	575	5	25	55	83	75	393	0	0	45	15	10
Sp.gr.	1.019	1.014	1.013	1.030	1.018	1.019	1.019	1.024	1.021	1.023	1.019	1.025
Creatinine (mg./100 ml.)	97	61	20	58	122	109	116	154	111	174	92	129
Urine 4												
Cd ($\mu\text{g./l.}$)	335	20	139	36	62	38	—	10	—	26	12	18
Protein (mg./l.)	1283	48	43	500	1300	508	563	0	—	25	10	0
Sp.gr.	1.019	1.017	1.024	1.024	1.019	1.010	—	1.022	—	1.021	1.017	1.023
Creatinine (mg./100 ml.)	76	80	134	34	128	30	112	122	—	144	91	105
Urine 5												
Cd ($\mu\text{g./l.}$)	—	56	38	36	78	104	24	9	37	27	13	16
Protein (mg./l.)	—	100	28	638	1005	678	1128	0	10	10	10	0
Sp.gr.	—	1.017	1.011	1.026	1.015	1.017	1.020	1.011	—	1.017	1.021	1.017
Creatinine (mg./100 ml.)	—	56	19	20	72	86	50	26	168	135	82	43
Urine 6												
Cd ($\mu\text{g./l.}$)	—	76	132	34	76	44	26	12	20	48	10	28
Protein (mg./l.)	—	80	0	600	1270	520	780	0	15	10	0	0
Sp.gr.	—	1.017	1.025	1.024	1.019	1.010	—	1.010	1.020	1.020	1.012	1.019
Creatinine (mg./100 ml.)	—	148	164	86	159	84	132	174	120	160	79	180

SUMMARY

1. An accurate, sensitive and reliable method for the determination of cadmium in urine has been evolved.

2. The incidence of proteinuria in a group of workers exposed to cadmium oxide dust was higher than in controls.

3. Urinary cadmium and protein appeared to vary independently.

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