148. THE DETERMINATION OF MINUTE AMOUNTS OF LEAD IN URINE

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FOR a comparative study of lead and porphyrin metabolism in lead workers a rapid and accurate method for the determination of urinary lead was necessary. Ross & Lucas [1935] separated lead by entrainment with calcium oxalate and subsequent colorimetric estimation with dithizone, as described by Fischer [1930]. Tompsett & Anderson [1935; 1939] have extracted lead from biological materials as a complex either with sodium diethyldithiocarbamate or with dithizone, both substances being stable in the presence of ammoniacal cyanide. Hubbard [1937] prefers to use dithizone throughout for extraction and analysis; modifications have been introduced [cf. Bambach, 1939], such as the use of hydroxylamine hydrochloride to prevent the oxidation of dithizone in the initial extraction.

Employing Tompsett's method, the writer experienced losses during the recovery of lead from urine owing to its entrainment with phosphate and finely divided silica. Unavoidable excess of perchloric acid often led to the formation of a yellow oxidation product from dithizone. Digestion with delead H_2SO_4 to destroy organic material and the use of perhydrol overcame these difficulties. Lead was quantitatively removed from the H_2SO_4 digest as the complex with sodium diethyldithiocarbamate in ether. 100 ml. urine required only 5 ml. pure H_2SO_4 and approximately the same volume of ammonia (sp. gr. 0.880), both rendered lead-free. The blank was $3.4 \mu\text{g}$. The extracted lead was digested with 1 ml. H_2SO_4 with a final addition of 4 or 5 drops of perhydrol. Excess of perhydrol was easily removed and no trouble was experienced from the oxidation of dithizone. For the final comparison as dithizonate the technique of King *et al.* [1937] was used with spectrum green filter 604 and neutral grey screen density 0.74 as standard.

PROCEDURE

Pyrex glassware and glass-distilled water were used throughout. Reagents were purified as follows.

2*N* H_2SO_4 was twice shaken with CCl_4 -ether mixture (1:20) and 20 mg. Na diethyldithiocarbamate and run off. The acid was then shaken with ether alone and the latter removed by careful warming on the water bath.

20% Na citrate and ammonia were delead in a similar manner.

Merck's perhydrol and 10% KCN were obtained lead-free.

5 mg. dithizone dissolved in 5 ml. CCl_4 were shaken with 5% NH_4OH . The aqueous layer was acidified with *N* HCl and the dye taken up in CCl_4 , leaving traces of lead in the aqueous phase. Finally, the purified dithizone was extracted with 5% NH_3 just before use.

100 ml. urine were evaporated with 100 ml. 2N H_2SO_4 in a 350 ml. Kjeldahl flask and digested with the addition of 4 drops of perhydrol until clear. The digest was diluted with water, 5 ml. 20 % Na citrate were added and the solution was made alkaline to litmus with ammonia (sp. gr. 0.880). After the addition of 5 ml. 10 % KCN, lead was removed by shaking twice with ether and approximately 20 mg. Na diethyldithiocarbamate. The ethereal extract was washed with water, the solvent evaporated and the residue digested with 1 ml. H_2SO_4 . 3-4 drops of perhydrol were added and heating was continued for 5 min. after clearing.

The acid was diluted with 4 ml. water and 0.2 ml. CH_3COOH ; 3 ml. ammonia (sp. gr. 0.880) were added to make the reaction alkaline. This solution was washed out into a 50 ml. separating funnel (with very short stem) with 5 ml. 1 % KCN, and 5 ml. CCl_4 were added. After vigorous shaking with slight excess of dithizone solution (2-3 drops) the aqueous phase was drawn off with a Pasteur pipette and the remaining dithizone washed out with 1 % KCN. The coloured CCl_4 layer was run off and compared with a standard (lead acetate) in a Klett microcolorimeter, using photometric technique.

DISCUSSION

Known quantities of lead covering a wide range were added to normal urine to test the accuracy of the method. The data are presented in Table 1 and it is evident that the recovered amounts agree to within 3 $\mu\text{g.}$ with the amounts added.

Table 1. *Recovery of lead added to normal urine*

Vol. of urine ml.	Amount of lead added $\mu\text{g.}$	Total lead recovered $\mu\text{g.}$	Reagent blank $\mu\text{g.}$	Total lead corrected for blank $\mu\text{g.}$	Amount of added lead recovered $\mu\text{g.}$
50	0	5.6	3.4	2.2	—
	10	15.6		12.2	10.0
	20	25.7		22.3	20.1
	30	33.8		30.4	28.2
	40	46.9		43.5	41.3
100	0	11.2	3.4	7.8	—
	20	33.1		29.7	21.9
	30	43.6		40.2	32.4
	40	52.6		49.2	41.4
100	0	11.1	3.4	7.7	—
	20	33.6		27.7	20.0
	30	28.6			
	40	44.1		40.7	33.0
		53.6		50.2	42.5

The method was now applied to the determination of the lead excretion in normal adult urine and urine from lead workers. For a series of 10 normal adults with no known exposure to lead the figures varied from 0.038 to 0.080 mg./l. with an average of 0.057 mg./l. These values are in close agreement with those reported by Kehoe *et al.* [1935] and by Ross & Lucas [1935].

Samples of urine from lead workers exposed to materials containing negligible amounts of tin, antimony and bismuth were collected throughout working hours. No bismuth could be detected in the samples by extracting with dithizone at pH 2 after treatment with 1 % HNO_3 . 120 determinations were made over a period of several months and representative results are shown in Table 2. The urinary excretion of this group of lead workers varied from 0.1 to 0.35 mg./l., and 100 ml. duplicates agreed within 0.004 mg.

Table 2. *Urinary lead excretion in lead workers*

Urine no.	Lead recovered from 100 ml. urine	Corrected reading for 100 ml. urine
	$\mu\text{g.}$	$\mu\text{g.}$
1	28.0	24.6
3	36.8	33.4
	37.9	34.5
5	25.0	21.6
	29.1	25.7
7	17.6	14.2
11	16.2	12.8
	15.5	12.1
13	18.8	15.4
	20.0	16.6
	19.4	16.0
17	17.8	14.4
	18.4	15.0
22	16.3	12.9
	17.0	13.6
23	25.4	22.1
27	17.7	14.3
41	22.2	18.8
51	13.7	10.3
86	25.4	22.0
112	24.3	10.9
114	19.0	15.6

SUMMARY

A method is described for the determination of lead in urine. 50–100 ml. samples are adequate and the method is economical in both time and reagents.

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