[CONTRIBUTION FROM THE DIVISION OF INDUSTRIAL HYGIENE, NATIONAL INSTITUTE OF HEALTH, U. S. PUBLIC HEALTH SERVICE]

A Rapid Method for the Microanalysis of Lead*

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The lead content of urine as a diagnostic sign in lead poisoning has become so important that scores of methods have been developed mostly aimed at simplifying or speeding up the procedure. Unfortunately, the amount of lead of interest in urine is so small and the volume of fluid, as well as the bulk of interfering organic and inorganic constituents, so large that the problem is one of peculiar difficulty. Practically every means of attack afforded by analytical chemistry has been successively applied or advocated in order to establish a procedure of sufficient merit to be at once rapid and accurate. The most promising from the former point of view is the diphenylthiocarbazone (dithizone) method originally advanced by Fischer,[1] which has since undergone a number of different modifications. While the coloration produced by lead with this reagent is not specific, it has been found possible in testing this procedure to so de-limit other interfering metals, chiefly by the use of ammoniacal cyanide, that a reasonable approximation of the amount of lead added to urine could be made by ashing, extraction and reading in a photometer provided with an appropriate filter.[2] In using this method, it is necessary to destroy the organic material in urine by ashing, as otherwise undesirable emulsions are formed in extracting the lead. Unfortunately, in attempting to increase the speed of the method, the quantities of urine used for analysis have been so reduced in volume that variations in color corresponding to the order of 1×10^{-3} mg. become of significance and very little attempt has been made to make a study of the natural lead content of urine parallel with the colorimetric evaluation. The great merit of diphenylthiocarbazone as a reagent for lead lies in the fact that a chloroform solution of this substance removes the lead quantitatively—along with a number of other metals, such as copper, zinc, and tin—from an aqueous solution when made faintly ammoniacal. It thus provides a means of separation and elimination of great convenience.

In the following method the addition of ammonia until the urine is sufficiently alkaline precipitates the lead as phosphate along with the alkaline earth phosphates. This precipitate is subsequently dissolved in nitric acid, and the lead collected from the neutralized solution by the use of dithizone, prior to its determination as lead chromate.

Drinking water presents less difficulty than urine with respect to the determination of its lead content and the method developed for urine can be conveniently adapted to this end.

Experimental

A study of the natural lead content of urine sufficiently large in amount so that it could be determined by standard chemical procedure, as compared with dithizone readings with small individual samples, indicates a notable disparity between the apparent quantity as measured colorimetrically and the actual lead content (Table I).

TABLE I

COMPARISON OF THE ACTUAL LEAD CONTENT OF URINE WITH THE APPARENT (COLORIMETRIC) CONTENT

Total vol. of urine analyzed, liters	Lead content per liter based upon analysis of the total volume, mg.	Lead content per liter based upon dithizone values per 100 cc. portions of original urine, mg.	Ratio of colorimetric to chemical values
40	0.025	0.092	3.6
10	.009	.047	5.2
105	.002	.027	13.5
100	.010	.043	4.3
14	.030	.090	4.5
18.2	.020	...	..
100.7	.023	.084	3.6
5	.013	.050	3.8

Each of these large amounts of urine, after being sampled for the determination of lead by the usual diphenylthiocarbazone colorimetric procedure, was completely ashed either by dry ashing or by means of nitric acid, the ash dissolved in water acidified with nitric acid, citric acid added to hold back the calcium and magnesium salts and finally all extractable material removed from the faintly ammoniacal solution by means of a chloroform solution of diphenylthiocarbazone as in the usual

(1) Fischer, *Wiss. Veröffent. Siemens-Konzern*, **4**, 11, 158 (1926); Fischer and Leopoldi, *Z. anorg. Chem.*, **47**, 90 (1934).

(2) Clifford and Wichmann, *J. Assoc. Official Agr. Chem.*, **19**, 130 (1936).

colorimetric procedure. The solution from which the lead had thus been removed was additionally carried through a chemical procedure to verify the complete removal of lead and finally all portions of the segregated metals were combined and the lead separated and determined as chromate.[3] While the investigation of such large quantities of urine was very time consuming, care was used throughout to guard against the possibility of loss due to incomplete precipitation or possible volatilization in ashing and to ensure that the amount of lead determined represented incontestably the total lead content of the original lot of urine.

It is apparent from Table I that the usual diphenylthiocarbazone procedure as carried out with small samples of urine gives high results. Liebhafsky and Winslow[4] have also noted high values with the diphenylthiocarbazone method for minute amounts of lead, particularly in the presence of copper and suggest that this "lead" may be due to diphenylthiocarbazone oxidation products.

The disparity noted between the apparent and true values for the lead content of normal urine as shown above was incidental to the development of a method of analysis of urine for lead that would be at once concise and accurate. To this end, advantage was taken of the co-precipitation of lead when the phosphates are precipitated from ammoniacal urine[5] since this avoids the time-consuming ashing of urine. It was found that by dissolving the phosphate precipitate in nitric acid and extracting with dithizone, the lead—together with certain other accompanying metals—could be rapidly and completely separated from the great bulk of urine without the necessity of ashing. The lead may be readily removed from the diphenylthiocarbazone extract by shaking with 2% nitric acid, and may then be completely separated from contaminating metals by precipitation as chromate following neutralization and acidification with acetic acid. The lead is then determined directly by filtration and titration of the insoluble chromate. As a further check on this procedure, urine filtrates from the phosphate precipitation stage were ashed and analyzed for lead and indicated that only an insignificant trace had escaped precipitation.

Co-precipitation of lead from urine by the addition of ammonia is particularly effective since adsorption increases with decreasing solubility and because of the bulk and type of the coagel of earthy phosphates. Lead phosphate is quite insoluble and the surface effect of the amorphous precipitate of earthy phosphates is pronounced. Co-precipitation as the oxalate gave an average recovery of only 58% of the lead. The phosphate and alkaline earth ions are normally present in sufficient amount in urine to ensure complete precipitation of the lead upon addition of the proper amount of ammonia.

Evaluation of the precipitated lead chromate may be conveniently done by filtering and washing on a microfunnel, dissolving the minute amount of lead chromate in a few drops of dilute hydrochloric acid and, following the addition of potassium iodide, titrating with 0.002 *N* sodium thiosulfate solution in a 5-cc. glass-stoppered flask. The end-point was found to be more conveniently and accurately determined by the use of carbon disulfide than with the usual starch suspension. If preferred, the washed lead chromate can be read colorimetrically following the usual dithizone procedure. While the colorimetric values obtained in the latter case are still slightly high, the error is much less than that arising from the usual direct colorimetric procedure with small samples of urine because interfering metals have been removed.

It should be emphasized that the volume of urine used for analysis should be one liter, or preferably the entire 24-hour output, in order to secure a sufficient amount of lead for objective identification and evaluation. When this is done, the values obtained by the above method compare closely with those values obtained by analysis of very large volumes. A further check on the initial lead content of a number of urines was established in the application of this amount to the correction of analyses of "unknowns" where the amounts of added lead were of the order of a few hundredths of a milligram of lead per liter of urine.

The two stages of (a) adsorption of the lead by precipitation of the earthy phosphates, and (b) extraction of the lead from the latter precipitate by means of dithizone, so concentrate the lead and separate it from interfering substances that an accurate analysis of urine for lead can be completed in a short period of time.

The accuracy of this method was shown in the analysis of one liter lots of urine containing

(3) Fairhall, *J. Ind. Hyg.*, **4**, 9 (1922).
(4) Liebhafsky and Winslow, THIS JOURNAL, **59**, 1966 (1937).
(5) Fairhall, *J. Biol. Chem.*, **60**, 485 (1924).

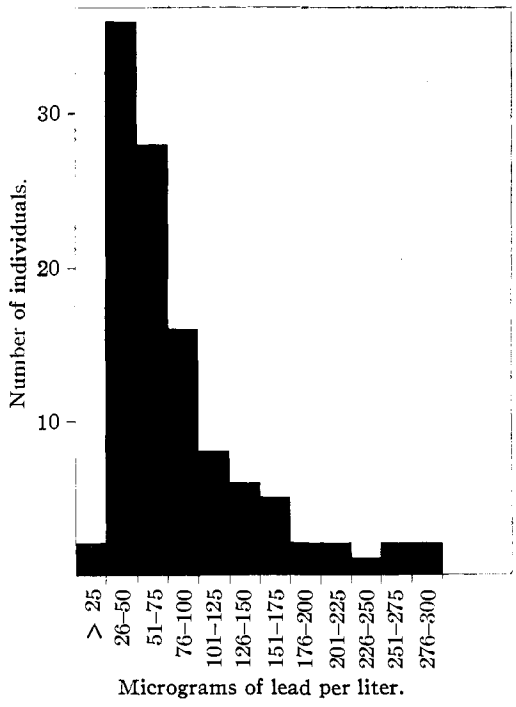

Fig. 1.

added—but unknown to the analyst—amounts of lead of a low order of magnitude. Two such sets of recovery experiments are shown in Table II.

The range 0 to 0.05 mg. of lead per liter is not of any particular significance in relation to lead absorption, but quantities above this amount constitute a level worthy of attention with reference to the symptomatology of lead poisoning.

Comparison of different groups of individuals show differences which can be related to lead absorption. Thus, while examination of 73 individuals in this Laboratory showed an average excretion of only 15 micrograms of lead per liter, a recent practical field test of this method of the daily output of lead of a second group of 110 individuals subjected to a possible lead hazard showed a distribution of lead values (Fig. 1).

TABLE II

ANALYSIS OF ONE LITER VOLUMES OF MIXED URINE

Lead added, mg.	Sodium thiosulfate solution ($N = 4.96 \times 10^{-3}$ corrected for e. p.), cc.	Total lead recovered, mg.	Evaluation of the amount of lead added, mg.
...	0.04	0.014	...
0.010	.07	.024	0.010
.020	.105	.036	.022
.030	.13	.044	.030
.040	.16	.055	.041
.060	.22	.075	.061
.080	.28	.096	.081
.100	.34	.116	.102
.200	.60	.205	.191
.500	1.50	.513	.499
	($N = 2.11 \times 10^{-3}$)		
...	0.10	0.015	...
0.010	.175	.026	0.011
.020	.24	.035	.020
.030	.29	.042	.027
.040	.38	.056	.041
.050	.47	.069	.054

Method of Analysis

The detailed procedure is as follows. To the one-liter sample of urine, or preferably the 24-hour output, in a glass-stoppered mixing cylinder add ammonium hydroxide in small amounts until, on shaking, the froth colors blue with thymolphthalein indicator (*p*H 10). Allow the precipitate to settle. This occurs so rapidly that the clear liquid may be decanted after fifteen minutes, but it is usually preferable to allow it to stand somewhat longer. Filter with suction in a Büchner funnel, wash with 25 to 50 cc. of alcohol—which permits the precipitate to be detached easily from the filter paper—transfer to a beaker with about 50 cc. of water and dissolve the precipitate in a small amount of nitric acid. Boil gently for fifteen minutes. Add 30 cc. of 50% citric acid, neutralize with ammonium hydroxide and extract with a 100 mg. per liter chloroform solution of dithizone. Wash the extract at least three times with distilled water to remove all trace of citric acid, which otherwise interferes with later precipitation, remove the lead from the chloroform layer by means of 10 cc. of 2% nitric acid, transfer to a 25-cc. Erlenmeyer flask, neutralize, re-acidify with acetic acid, precipitate as chromate, boil, filter through a small filter,[6] wash, dissolve in a few drops of dilute hydrochloric acid and wash into a 5-cc. glass-stoppered flask, add potassium iodide, and titrate with 0.002 *N* sodium thiosulfate solution using a micro-buret following the addition of one or two drops of carbon disulfide to indicate the end-point.

For the determination of lead in drinking water, five or six liters of the water may be extracted with

(6) The microfunnels used were made by sealing a length of 2 mm. capillary tubing to a 6 or 7 cm. length of 1 cm. tubing and internally grinding flat at the junction. One centimeter discs of filter paper resting on the flat surface filtered these minute amounts of lead efficiently.

dithizone in one-liter portions and the procedure followed as indicated above from this point.

Summary

The apparent lead content of normal urine as indicated by dithizone measurements is greater than the true lead content as measured by chemical separation of the total lead from large volumes of such urine. A rapid micromethod for the determination of lead is described which permits the objective separation and precise evaluation of minute amounts in drinking water and in urine without the necessity of ashing.

WASHINGTON, D. C. RECEIVED JUNE 16, 1941

[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, DUKE UNIVERSITY]

Electric Polarization and Association in Solution. III. The Dipole Moments of Some Alcohols in Very Dilute Benzene Solutions

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Numerous determinations of the electric polarization of alcohols in non-polar solvents have been made previously. Hennings,[1a] and more recently Hückel,[2] have made rather complete studies of the polarization of various alcohols over a wide concentration range. They used benzene, hexane, and several other non-polar solvents. The polarization as a function of concentration exhibited quite complex behavior, showing maxima and minima which were interpreted by assuming the existence of a number of associated alcohol complexes. Goss[3] has made use of the partial molar polarizations to interpret the behavior of alcohols in solution and Rodebush[4] has examined several alcohols in carbon tetrachloride and interpreted the data on the basis of the Onsager theory.[5]

In none of the above-mentioned work, however, did the concentration range studied extend sufficiently low to eliminate entirely any association effects; consequently, any calculation of the dipole moment of the unassociated alcohol would necessarily involve some uncertainty. In cases where dilution sufficient to eliminate association was obtained,[6] the polarization values appeared to be quite anomalous. Apparently, there exist no data in the literature at either low enough concentration or of sufficient reliability to furnish very dependable dipole moments for the alcohols from solution measurements.

In the present work the polarizations and dipole moments of methyl, ethyl, isopropyl, and *t*-butyl alcohols have been determined in benzene at concentrations ranging from approximately 10^{-4} to 10^{-2} mole fraction with the object of obtaining accurate solution values for the moments of these alcohols and to study association effects if they existed at these low concentrations.

Materials

Methyl Alcohol.—Merck absolute methanol was dried over magnesium ribbon and distilled in a 180-cm. Dufton column. The boiling point of the middle fraction was 64.51° (cor.). The critical solution temperature in carbon disulfide was found to be 35.2°. This value is slightly lower than that found for anhydrous methyl alcohol by McKelvy and Simpson.[7] Since drying with magnesium is superior to the method used by the latter workers, it is quite probable that our alcohol is more nearly anhydrous than that used by these authors.

Ethyl Alcohol.—A best grade absolute ethanol was purified in the same manner as methanol, b. p. 78.34° (cor.). The critical solution temperature in carbon disulfide was −23.5°, indicating less than 0.1% moisture.[8]

Isopropyl Alcohol.—Eastman Kodak Company best grade alcohol was purified in the same manner as methyl and ethyl alcohol. As a check on the purity, a part of the best fraction was again refluxed over magnesium ribbon and distilled. Polarization measurements on this repurified sample agreed with those of the other fraction. The b. p. was 82.33–82.39° (cor.).

***t*-Butyl Alcohol.**—Eastman best grade *t*-butanol was first distilled over sodium. The best fraction was then fractionally recrystallized from its own melt until no change in melting point occurred upon further recrystallization; m. p. 24.85–25.0°.

Experimental

The experimental procedure and most of the apparatus have been described previously.[9] An entirely new hetero-

(1) The author is indebted to the Ethyl-Dow Chemical Company for the grant of a Post-Doctorate fellowship which enabled this problem to be undertaken.

(1a) Hennings, *Z. physik. Chem.*, **B28**, 267 (1935).

(2) Hückel and Schneider, *ibid.*, **B47**, 227 (1940).

(3) Goss, *J. Chem. Soc.*, 888 (1940).

(4) Rodebush, Eddy and Eubank, *J. Chem. Phys.*, **8**, 889 (1940).

(5) Onsager, THIS JOURNAL, **58**, 1486 (1936).

(6) Hoecker, *J. Chem. Phys.*, **4**, 431 (1936).

(7) McKelvy and Simpson, THIS JOURNAL, **44**, 105 (1922).

(8) Schoorl and Regenbogen, *Rec. trav. chim.*, **41**, 125 (1922).

(9) Pohl, Hobbs and Gross, *J. Chem. Phys.*, **9**, 408 (1941).