PROJECT MR-8

THE DETERMINATION OF SMALL QUANTITIES OF LEAD IN URINE

submitted by

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In order to decide upon a method for the determination of lead in urine which should be suitable for group examination, the following methods were studied, all of which are based upon the use of dithizone (diphenylthiocarbazone), which in alkaline solution forms a cherry-red colored complex with lead.

1. Behrens and Taeger.
Ztschr. ges.exptl. Med., 96, 282, 1935.

2. Ross and Lucas.
J. Biol. Chem., 111, 285, 1935.

3. Tompsett and Anderson.
Biochem. J., 29, 1851, 1935.

4. Wilkins, Willoughby, Kraemer, and Smith.
Ind. Eng. Chem. Anal. Ed., 7, 53, 1935.

5. Seelkopf and Taeger.
Ztschr. ges. exptl. Med., 91, 539, 1933.

6. Winter, Robinson, Lamb and Miller.
Ind. Eng. Chem. Anal. Ed., 7, 265, 1935

With these methods the amount of lead is either determined by comparing the intensity of the color formed by the complex compound in a colorimeter against a standard, or by determining the absorption of light in a spectrophotometer, or by determining the amount of dithizone required for the complex, the dithizone having been standardized against a known amount of lead.

The first method studied was that of Wilkins, Willoughby, Kraemer and Smith, which is based upon titrimetric extraction of lead with a standard dithizone solution. This, however, did not lend itself very favorably to our problem because its results

were not accurate enough for the quantities of lead found in urine, and because the use of a micro burette or several micro burettes would require an elaborate set up, too complicated for clinical work.

The method of Seelkopf and Taeger was not studied because it requires the use of a spectrophotometer, an instrument not available in most clinical laboratories. The methods of Behrens and Taeger, of Tompsett and Anderson, and of Winter, Robinson, Lamb and Miller, which are based on colorimetric comparison, were studied in detail and the method of Behrens and Taeger was adopted with minor modifications.

At first determinations were made to find out whether the red color of the lead complex varied directly with the concentration of lead. This was done by taking samples containing various amounts of lead as lead nitrate and comparing them against a standard containing 0.05 mg. of lead. The following results indicate that the color does vary directly with the concentration.

Standard containing 0.05 mg. of lead

Sample No.	Lead taken mg.	Lead found mg.	Error mg.
1	0.100	0.105	+ 0.005
2	0.100	0.098	- 0.002
3	0.050	0.0507	+ 0.0007
4	0.050	0.0495	- 0.0005
5	0.040	0.042	+ 0.002
6	0.030	0.031	+ 0.001
7	0.030	0.0299	- 0.001
8	0.020	0.0206	+ 0.0006

The oxidation of the urine was studied next. This was carried out by the calcination method as suggested by Behrens and Taeger. The lead is precipitated as chloride and carried down by entrainment with a precipitate of calcium oxalate. After calcination, the precipitate is dissolved in nitric acid and the lead extracted with dithizone. The following results which were obtained by adding lead as lead nitrate to the urine show that this method is quite accurate.

Standard containing 0.05 mg. of lead

Sample No.	Lead added mg.	Lead found mg.	Error mg.	Error Per cent
1	0.030	0.0242	- 0.0058	- 19.0
2	0.050	0.0497	- 0.0003	- 0.6
3	0.050	0.0485	- 0.0015	- 3.0
4	0.050	0.0492	- 0.0008	- 1.6
5	0.030	0.029	- 0.001	- 3.3
6	0.100	0.0984	- 0.0016	- 1.6
7	0.050	0.044	- 0.006	- 12.0
8	0.030	0.0315	+ 0.0015	+ 5.0
9	0.030	0.0296	- 0.0004	- 1.3
10	0.030	0.025	- 0.005	- 17.0
11	0.100	0.0995	- 0.005	- 0.5
12	0.050	0.056	+ 0.006	+ 12.0
13	0.100	0.1012	+ 0.0012	+ 1.2
14	0.030	0.0333	+ 0.0033	+ 11.0
15	0.050	0.0514	+ 0.0014	+ 2.8

It was found that the median error in these determinations was - 1.3 per cent, with maximal variations of - 19 and + 12 per cent, and an average error of about ± 6 per cent.

Another method of oxidation, as used by Kehoe and by Tompsett and Anderson, was tried. The urine is evaporated in silica dishes, calcinated, using nitric acid to remove the last traces of carbon, and then extracted by the method used above.

Two determinations gave the following results:

Sample No.	Lead taken mg.	Lead found mg.	Error mg.
1	0.050	0.034	- 0.016
2	0.050	0.0513	+ 0.0013

Since this method did not seem to offer a material advantage over the procedure used by Behrens and Taeger, its use was discontinued in this study.

The method for the determination of small amounts of lead with dithizone, according to Behrens and Taeger, with some modifications, is as follows:

Reagents used

1. Redistilled water. Prepared in a pyrex all glass still. Used for all operations except preliminary washings.
2. Nitric acid. Concentrated and 50 per cent.
3. Hydrochloric acid. 1 Normal.
4. Glacial acetic acid.
5. Ammonium hydroxide. Concentrated and 0.5 per cent.
6. Purified carbon tetrachloride.
7. Potassium cyanide. 20 per cent.
8. Sodium citrate. 10 per cent.
9. Ammonium oxalate. Saturated solution.
10. Calcium chloride. 5 per cent.
11. Brom-cresol purple or brom-thymol blue.
12. Purified dithizone solution in carbon tetrachloride; about 1 mg. per 100 cc. Dissolve about 4 mg. of

commercial dithizone (diphenylthiocarbazone) in 25 cc. of carbon tetrachloride. Extract with three 25 cc. portions of 0.5 per cent ammonium hydroxide. This dissolves the dithizone but not the impurities. Reject the carbon tetrachloride layer and neutralize the aqueous layer to litmus with 1 N hydrochloric acid. Extract the purified dithizone with several 15 cc. portions of carbon tetrachloride and dilute the total extract to 100 cc. The solution should be kept in a pyrex flask in the refrigerator and made up fresh every third day.

13. Lead standards. 0.16 gm. of recrystallized lead nitrate is made up to one liter with redistilled water, two drops of lead free nitric acid are added to a 100 cc. aliquot which is stored in a pyrex vessel.

Standard I 1 cc. contains 0.1 mg. of lead.

Standard II 1 cc. contains 0.01 mg. of lead. Made by diluting 10 cc. of Standard I to 100 cc.

Reagents containing more than 0.001 to 0.002 mg. of lead must be rejected.

Procedure

Place a suitable quantity of urine (100 cc. to 1000 cc. depending upon the concentration of lead) in a beaker and acidify with acetic acid. Then add 5 cc. of a 5 per cent calcium chloride solution and with stirring slowly add 10 cc. of a saturated solution of ammonium oxalate. In this way the lead is precipitated by entrainment with the calcium oxalate precipitate. Let it

stand over night and remove the supernatent liquid by suction the next day. The oxalate precipitate is collected by centrifuging and is washed once with redistilled water containing a few drops of saturated ammonium oxalate solution. The precipitate is transferred to a porcelain crucible, or better a silica dish, although favorable results were obtained with porcelain dishes. The precipitate is then dried on a sand bath at 120° to 140° C. and then calcined in a muffle furnace at 500° to 550°C. until the residue is entirely white (carbonate and oxide). The ashes are dissolved with the least possible amount of hot 20 per cent nitric acid and the solution mixed with 10 cc. of a 10 per cent sodium citrate solution. 1 to 2 drops of an aqueous solution of bromocresol purple or bromthymol blue are added and the solution is neutralized with ammonia. The solution is transferred to a separatory funnel and 5 drops of a 20 per cent potassium cyanide solution are added. The mixture is then shaken vigorously for 3 to 5 minutes with about 3 cc. of purified dithizone solution. The carbon tetrachloride is drawn off and the extraction repeated with diminished portions of the dithizone until no further red color appears in the carbon tetrachloride. The lead in this way is completely extracted from the aqueous solution and the yellow supernatent liquid is discarded.

The red dithizone solution, after being washed with about 10 cc. of a 0.5 per cent ammonium hydroxide solution containing 2 to 3 drops of potassium cyanide (to remove any excess of the reagent) is made up to a definite volume with carbon tetrachloride and compared in a colorimeter against a standard prepared in the

same way. The standard should be extracted at the same time as the sample, from a suitable quantity of lead standard. If the solution is not clear, the suspension of water can be removed by filtering through a dry filter paper. The samples should be compared within 1 hour after extraction because the complex decomposes upon standing.

All equipment must be first cleaned with chromic-sulfuric acid (cleaning solution) followed by tap water, dilute nitric acid, and then thoroughly rinsed with redistilled water.

The time required to run several samples simultaneously does not differ materially from that required for one sample:

Preparation of the precipitation - - 5 to 10 minutes on the evening of the first day.

Precipitation - - over night.

Centrifugation and transfer to evaporating dishes - - 15 min. on the second day.

Evaporation - - 3 to 4 hours.

Transfer to muffle furnace - - 5 minutes.

Calcination in the muffle furnace until next morning.

Dissolving residue and colorimetric determination - - 1 to 2 hours on third day.

If the reagents and containers are prepared in the proper way and are ready for use, the determination requires, therefore, from 1½ to 2½ hours of actual time expended and can be finished within 3 days, starting on the evening of the first day.

We believe that any person with training in analytical chemistry should be able to do the determination of lead as outlined above and that the method is suitable for the routine analysis of lead in urine.