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THE DETERMINATION OF SMALL QUANTITIES OF BISMUTH IN TISSUE, EXCRETA, BLOOD AND BONE.

BY JAMES A. SULTZABERGER.

There are a number of methods for the determination of bismuth in small quantities. These methods are mostly based on the determinations in colloidal solutions by using an alkaloid with the sulphide, the oxide or some other form of bismuth. As the accuracy of methods based upon colloidal suspensions is questionable, they are not to be recommended.

In 1887, Stone¹ found that when small quantities of bismuth in solution were treated with an excess of potassium iodide in the presence of sulphuric acid and sulphurous acid, a characteristic greenish yellow color was obtained. This was due to the formation of a complex bismuth potassium iodide having the formula $\text{BiI}_3 \cdot 2\text{KI} \cdot 4\text{H}_2\text{O}$. Rowell² made use of this compound in analyzing ores and alloys containing copper, lead, etc., for small quantities of bismuth. He found that alkalis in excess, ammonium acetate, hydrochloric acid and chlorides bleached the color. The heavy metals such as arsenic, lead and copper form color and must be removed. Iron in the absence of sulfurous acid liberates free iodine.

C. A. Hill³ recently in analyzing urine digested the organic matter by a method of wet ignition using nitric acid (Sp. gr. 1.42). He developed the color for comparison, however, by the aid of an alkaloid. Caille and Viel⁴ in determining small quantities of antimony and bismuth ignited their samples in a muffle furnace.

Soon after the method to be described in this paper had been perfected, C. S. Leonard⁵ published a method in which samples were digested by heating in a

¹ F. B. Stone, *J. Soc. Chem. Ind.*, 6, 416 (1887).

² H. W. Rowell, *Ibid.*, 27, 102 (1908).

³ C. A. Hill, *Lancet*, 209, Part 2, 1281 (1925).

⁴ Caille and Viel, *Compt. rend.*, 176, 1759-1761 (1925).

⁵ C. S. Leonard, *J. Pharmacol.*, 28, Part 2, 81 (1926).

flask with concentrated nitric and sulphuric acids (20:1). The readings were made from bismuth potassium iodide solutions acidified with sulphuric acid instead of hydrochloric acid. He also reiterated Rowell's statement that chlorides and hydrochloric acid affect the color.

Autenreith and Meyer¹ digested organic matter by the use of potassium chlorate and hydrochloric acid. Bismuth was precipitated in the usual way and compared in bismuth potassium iodide solution acidified with hydrochloric acid.

Studies in this laboratory² on the Absorption, Distribution and Elimination of Bismuth yielded samples for the determination of bismuth in amounts from 50 mg. to nil. The method first adopted in the analysis of these samples was that of Autenreith and Meyer.¹ This method proved unsatisfactory because too much time was required for digestion and precipitation. Small amounts of iron in the reagents also caused trouble after the precipitation of bismuth, by constantly liberating free iodine which could not be all removed with chloroform. As no other satisfactory method was found in the literature, it was deemed advisable to work out the details of a satisfactory method, particularly in regard to a simplified procedure of digestion.

PROCEDURE.

Ten to 50 Gm. of tissue or feces were placed in a small porcelain dish, moistened with concentrated nitric acid and warmed over night on a steam-heated sand bath. The sample was then ignited over a Bunsen burner with a low flame which was gradually increased to its full intensity for three or four hours. If the residue still contained carbon particles, nitric acid was added to the cooled dish and heated until the brown fumes were expelled. This operation was repeated until all the carbon was digested.

Urine was digested in samples of not less than 25 cc., in 50-cc. Pyrex beakers in the same manner. Larger samples were evaporated to dryness, treated with nitric acid and transferred to 50-cc. beakers. Most urine residues turned white after igniting only a short time.

The ignited residues were boiled with concentrated hydrochloric acid, evaporated to dryness, treated with 0.5 cc. of concentrated hydrochloric acid and 5 cc. of water, filtered and diluted to about 15 cc. in a graduated cylinder. To each cylinder 0.75 cc. of 25 per cent potassium iodide and 0.5 cc. of 1 per cent sodium bisulphite were added. The solution was diluted to exactly 20 cc. and compared with a standard in the Duboscq colorimeter.

The standard was made from a bismuth nitrate solution containing 0.05 mg. of bismuth per cc. In the test 2 cc. or 0.1 mg. was usually taken, acidified with 0.5 cc. of concentrated hydrochloric acid and diluted to 15 cc. Potassium iodide and sodium bisulphite were added and the solution was diluted to 20 cc. as in the unknown. If the color in the unknown indicated more than 0.1 mg., an aliquot of the unknown was diluted and compared using the same concentrations of reagents as in the standard. If the color in the unknown indicated less than 0.1 mg., a standard was made to match the unknown as nearly as possible.

In the digestion of bone, the precipitation of the metal with hydrogen sulphide

¹ Autenreith and Meyer, *Münch. med. Wochschr.*, 71, 601 (1924).

² O. M. Gruhitz, F. H. Tendick and J. A. Sultzaberger, Parts I and II, *Am. J. Syphilis*, Vol. 11 (January 1927).

was necessary because of the excess amount of salts present. Nitric acid was added after ignition to dissolve most of the salts. The undigested carbon particles were filtered off, digested separately and added to the nitric acid solution which was evaporated to dryness. The bismuth was precipitated from hydrochloric acid solution in the ordinary way with hydrogen sulphide. The precipitate was filtered on a Gooch, dissolved in concentrated hydrochloric acid, filtered to remove the asbestos and the bismuth was determined as stated above.

In the present work, it was found that sodium chloride by itself does bleach the bismuth potassium iodide color to a marked degree, but with the addition of hydrochloric acid the color returns and no difference could be detected between solutions of bismuth potassium iodide containing sodium chloride and hydrochloric acid, and hydrochloric acid alone. In sulphuric acid solution the color was very slightly more intense but as hydrochloric acid was added to all the standards, the same conditions were maintained in the standard as in the unknown.

Blanks run by the above method were water white when all the reagents were added.

The use of the muffle furnace was eliminated by the following tests. Known quantities of bismuth were added to six samples of meat weighing 10 Gm. each. Three samples were ignited over Bunsen burners and three in the muffle furnace.

TABLE I.
IGNITED OVER BUNSEN BURNERS.

Bi added in mg.	Bi found in mg.	Per cent recovered.
0.2	0.196	98
0.4	0.365	91
0.8	0.770	96

IGNITED IN MUFFLE FURNACE.

Bi added in mg.	Bi found in mg.	Per cent recovered.
0.2	0.960	48
0.4	0.235	59
0.8	0.455	57

No tests were made to determine whether the metal was fused into the porcelain dishes or was volatilized.

VERIFICATION OF THE METHOD.

Various quantities of bismuth, which were unknown to the author, were added to four samples of urine containing 25 cc. and to four samples of meat weighing 10 Gm. each.

TABLE II.

URINE.

Bi added in mg.	Bi found in mg.	Per cent recovered.
2.0	2.0	100
0.5	0.44	88
0.75	0.73	97
0.15	0.12	80

MEAT.

Bi added in mg.	Bi found in mg.	Per cent recovered.
0.50	0.48	92
0.30	0.26	87
0.75	0.67	89
0.15	0.15	100

Known amounts of bismuth were added to 25 Gm. samples of fresh ground bone. As certain animal studies gave evidence of bismuth in the bones, it was deemed advisable to make these tests.

TABLE III.

Bi added in mg.	Bi found in mg.	Per cent recovered.
0.2	0.200	100
0.2	0.188	94

Two small young rats were injected with amounts of bismuth unknown to the analyst, and killed immediately. Two more rats were killed and known amounts of bismuth solution were added to the dishes.

TABLE IV.

Bi injected in mg.	Bi found in mg.	Per cent recovered.
1.0	1.000	100
1.5	1.492	99

Bi added in mg.	Bi found in mg.	Per cent recovered.
0.5	0.482	96
1.0	1.000	100

SUMMARY.

A method is described for the rapid determination of small quantities of bismuth in body tissues, excreta, blood and bone. The advantages of this method are as follows:

1. Digestion is accomplished by dry ashing over the Bunsen burner in Pyrex beakers and porcelain dishes. It has been shown that none of the bismuth is lost at the temperature of the Bunsen burner while the carbon is, of course, oxidized rapidly.

2. No precipitation is necessary, except in analyzing bone where the amount of salts is too great to be dissolved in a small quantity of solution.

3. Chlorides do not interfere with the accuracy of the method.

4. The bismuth is determined colorimetrically in hydrochloric acid solution with an excess of potassium iodide. The liberation of free iodine by oxidation in the presence of iron is prevented by the addition of sodium bisulphite to the acid solution.

ARTICLE No. 38,
CHEMICAL RESEARCH DEPT.,
PARKE, DAVIS AND CO.

WASICKY'S REAGENT FOR THE IDENTIFICATION OF ALKALOIDS.

Wasicky's reagent is prepared by dissolving para-aminodimethylbenzaldehyde, 6 Gm. in strong H_2SO_4 , 2 Gm., and adding 0.04 Gm. of water. The author enumerates the color reactions obtained with a number of alkaloids, in the cold, on warming and on diluting the reaction mixture with water. Several of these are characteristic, such as those of the ergot alkaloids. The reagent also serves to

distinguish sabadine from veratrine and morphine or codeine from the other opium alkaloids. It also serves to distinguish the three chief quebracho alkaloids, to identify ibogaine and yohimbine, and esters of tropic or taropic acids. He does not agree with Field Stedman that the reaction obtained with it with quebrachamine is due to an indole group in that base, since he finds that pure indole does not give the same reaction.—Raymond Hamet (*Bull. sci. pharmacol.*, 447 (1926); through *Pharm. J.* (Feb. 12, 1927)).