[CONTRIBUTIONS FROM THE CHEMICAL LABORATORY OF THE AMERICAN MEDICAL ASSOCIATION.]

THE DETECTION AND ESTIMATION OF HEXAMETHYLENAMINE IN PHARMACEUTICAL MIXTURES.

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The increasing popularity of hexamethylenamine as a therapeutic agent and its appearance in pharmaceutical mixtures under fanciful and misleading names has made its detection and estimation an interesting and necessary subject of investigation. In the examination of a preparation suspected to contain hexamethylenamine the existing methods of estimation were found inadequate and a new method had to be developed.

Hexamethylenamine was for a long time of theoretical interest only, until introduced in 1904 by Nicolaier. Since then hexamethylenamine has appeared under a host of names, although the substance in each case is the same. As a chemical substance, synthetic hexamethylenamine has received considerable attention, especially as regards structural constitution, which, it was thought, once known would give some idea of the chemical action as a therapeutic agent. The structural formulas suggested for hexamethylenamine are quite numerous but the one that has been received with the greatest favor is that of Lösekann.[1] Lösekann's formula is, $N\equiv(CH_2{-}N:CH_2)_3$. With this formula its property of splitting up into formaldehyde and ammonia, its basic properties, as well as its action with nitric acid to form nitrosopentamethylentetramine are in accord. To the physician the property of decomposing into formaldehyde and ammonia is of chief interest and it is also this property upon which the estimation of hexamethylenamine is based.

Recently a pharmaceutical preparation of "urinary antiseptic, uric acid solvent and diuretic," masquerading under a name intended to hide its real identity, was examined in the chemical laboratory of the American Medical Association. Besides extract of couch grass and corn silk the preparation is said to contain a "lithium-formaldehyde" compound which slowly liberates formaldehyde in the bladder. From the claim that formaldehyde is liberated in the bladder and similar statements appearing in the advertising matter accompanying the preparation, the conclusion was drawn that the active ingredient was hexamethylenamine. In order to determine this, several tests were made according to the meager literature appearing on the subject of identification of hexamethylenamine. In view of the presence of other substances, the United States Pharmacopoeia identity tests were considered insufficient; they were also rejected because it might be claimed that these tests

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[1] *Chem. Z.*, **16**, 1408.

break up the compounds of formaldehyde and lithium, which are claimed to be present.

Identification of Hexamethylenamine.—The reactions of hexamethylenamine with the alkaloidal reagents are mentioned in the literature but the accompanying information did not promise that they could be advantageously used in this investigation. The reaction with bromine[1] and the reaction with mercuric chloride,[2] both of which have been used quantitatively, appeared more promising.

Hexamethylenamine is thrown down from an aqueous solution of bromine as an orange-colored precipitate, to which the formula $C_6H_{12}N_4Br_4$ has been assigned. This tetrabromide can be filtered, washed and dried and in this way be definitely isolated. The washed and dried tetrabromide when allowed to stand, especially over caustic potash, loses two atoms of bromine, going quantitatively over to the dibromide. This body then can be identified by its canary-yellow color and its melting point, which has been found to be 198–200°, at which temperature it decomposes. When boiled with water the dibromide is decomposed with liberation of formaldehyde, ammonia, bromine and hexamethylenamine.

From an aqueous solution hexamethylenamine is precipitated with mercuric chloride; a white amorphous powder forms which on standing becomes crystalline. This compound, although more soluble than the dibromide, can be quantitatively separated and determined. It is split by boiling dilute acid into formaldehyde, ammonia, hexamethylenamine and the mercury salt of the acid employed, all of which can be identified in the resulting solution. This with the bromine reaction constitutes the most satisfactory and convincing tests for hexamethylenamine, especially when combined with the property of splitting with acid into formaldehyde and ammonia.

The above tests were applied to the preparation under examination with very satisfactory results. It was found that bromine water precipitated an orange-colored body which was insoluble in water and could be washed and dried, and which on drying over potassium hydroxide gradually became a light yellow in color. This substance was then subjected to the boiling test and in every way indicated that it was the dibromide of hexamethylenamine. As a final test of identification the melting point was taken and found to be 196–200°; and when mixed with equal parts of dibromide made from pure hexamethylenamine it melted at 194–200°. This constancy of melting point when mixed with a dibromide of hexamethylenamine points conclusively to the identity

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[1] Horton, *Ber.*, 21, 2000.

[2] P. Dobriner, *Z. anal. Chem.*, 36, 44.

of the two bodies and to the fact of the presence of hexamethylenamine in the preparation.

As a confirmative test the preparation was treated with mercuric chloride, which produced a white precipitate that after standing became crystalline in character. This crystalline body when washed and filtered was boiled with dilute acid which caused an evolution of formaldehyde. The resulting solution was then examined and was found to contain an ammonium salt. As a further confirmation that the hexamethylenamine identified by the above tests, was not liberated by the action of bromine or mercuric chloride but was contained in the preparation as such, a portion was extracted with chloroform, it having previously been demonstrated that chloroform would extract hexamethylenamine from its aqueous solution.

The chloroform extract upon evaporation deposited white crystals which under the microscope had the same appearance as hexamethylenamine crystals, deposited from chloroform solution. When these crystals were dissolved in water the solution responded to the pharmacopoeial tests as well as those mentioned above for hexamethylenamine.

Estimation of Hexamethylenamine.—Kippenberger[1] and Wöhlk[2] demonstrated that hexamethylenamine can be boiled in alkali with practically no change but that in the presence of acids it is decomposed into formaldehyde and ammonia. This suggested the feasibility of estimating hexamethylenamine in a solution containing ammonia or ammonium compounds, simply by making the solution alkaline and boiling off the ammonia, without destroying the hexamethylenamine, than making acid and boiling sufficiently to completely decompose the hexamethylenamine and finally making alkaline and estimating the liberated ammonia by standard acid solution.

The following confirmatory experiments were made: 0.2813 gm. hexamethylenamine was treated with 250 cc. water and 5 gm. potassium hydroxide and the liquid submitted to distillation. The distillate by residual titration was found to have an alkalinity equivalent to 0.15 cc. of tenth-normal acid, and a second experiment indicated that no ammonia was liberated.

Experiments carried out to confirm Kippenberger's and Wöhlk's statements regarding the action of boiling acid upon hexamethylenamine, demonstrated that for complete decomposition of hexamethylenamine in acid solution, one and a half hours' boiling are required, and this time was given all the estimations.

0.2213 gm. hexamethylenamine yielded ammonia corresponding to 0.2212 gm. hexamethylenamine or 99.99 per cent.; 0.2079 gm. hexa-

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[1] *Z. anal. Chem.*, **42**, 691.

[2] *Ibid.*, **44**, 265.

methylenamine yielded ammonia equivalent to 0.2073 gm. hexamethylenamine or 99.74 per cent.

These results demonstrate that hexamethylenamine is stable toward boiling alkali but is quantitatively decomposed when boiled in acid into formaldehyde and ammonia, the latter of which can be estimated in the manner given above and calculated to hexamethylenamine.

The above method was then applied to the estimation of hexamethylenamine in the preparation under examination as follows:

5.0346 gm. of the preparation were diluted to 500 cc. and 10 gm. potassium hydroxide added. This was then boiled in an open flask for one-half hour to drive off all ammonia not combined as hexamethylenamine. The mixture was then made acid by the addition of 125 cc. of dilute sulphuric acid and boiled for one and one half hours; after cooling, 50 cc. of a 30 per cent. solution of potassium hydroxide were added to the mixture and then distilled into 97.44 cc. tenth-normal acid, until about two-thirds had been distilled over. The acid then required 23.01 cc. normal alkali for neutralization, indicating that an equivalent of 74.43 cc. of tenth-normal ammonia had been liberated. In a second determination carried out precisely as the preceding one 5.0197 gms. of the preparation yielded an equivalent of 74.32 cc. of tenth-normal ammonia. From the following formulas the factor 0.003479 is obtained, which multiplied by the number of cc. of tenth-normal ammonia, representing the ammonia liberated from hexamethylenamine, gives the weight of the latter.

$$(CH_2)_6N_4 + 6H_2O + 2H_2SO_4 = 6CH_2O + 2(NH_4)_2SO_4$$
$$2(NH_4)_2SO_4 + 4KOH = 2K_2SO_4 + 4NH_3 + 4H_2O.$$

From the average of the above results it was found that the preparation contains 1.6300 gm. hexamethylenamine per fluid ounce.

As indirect evidence that the formaldehyde is all in combination as hexamethylenamine and not in combination with lithium as claimed by the manufacturers the following is presented:

Qualitative tests having indicated the presence of lithium and benzoic acid, quantitative determinations were made: the lithium by the method of Gooch,[1] and benzoic acid by extracting with chloroform from an acid solution and titrating the residue left by the evaporation of the chloroform solution.

The results indicated that the lithium and benzoic acid were present in equivalent amounts and hence had been most likely added in the form of lithium benzoate.

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[1] *Am. Chem. J.*, 9, 33.