Biochemical Studies on Carbohydrates.

LXXIV. Colorimetric Estimation of Minute Quantities of Protein-Hexosamine.

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The Hamasato and Akakura method¹⁾ is applicable to some proteins which involve only 1% hexosamine. Nevertheless, some other proteins with hexamine contents of even 2% are hardly analysed, because of the formation of products by hydrolysis, which develop yellow colour with the Ehrlich reagent and depress simultaneously the colour reaction of hexamine to a large extent similar to urea. We encountered such an obstacle in analysis of ichthulins.

Attempts were made to overcome the above difficulty :—

At first, decolorizing agents were tested if they are possible to remove the inhibitory products, but kaolin, charcoal and others were all found useless. In the next, glucosamine hydrochloride was added to the solution obtained by the preparatory operation for colour reaction. The colour developed could be easily compared with the standard, when 0.5 mgm. of glucosamine hydrochloride was added to 4 cc. of the solution, but its intensity did not keep pace with the quantity of protein analysed. In the third, the hydrolysate was precipitated with phosphotungstic acid and followingly the excess of this precipitant in the mother liquid was taken off by means of potassium hydroxide (cf. Masamune and Tanabe²⁾). In this case, however, orange colour was produced by the Ehrlich reagent due to the trace of phosphotungstic acid remaining in the solution.*

In the fourth process, which proved available, the effect of acetate was resorted to that accelerates the colour reaction of hexosamine.* The colour reaction was carried out after adding to the solution, which had been prepared

* Acetate depresses the reaction, when small quantities of it are used.

from the hydrolysate, as much glucosamine hydrochloride as that in the standard solution. On varying the amounts of a protein taken, the discrepancies of the colour depth from the standard gave hexosamine values coinciding in percent of the protein within analytical error. Cystine* and tryptophane* had no influence on the colour development of glucosamine by the present procedure. Glycine* intensified the colour by 12%, but it caused neither increase nor decrease of the colour depth in the co-presence of the two amino acids† above. By this modification of the method, protein hexosamine as little as 0.1 mgm. can be analysed, regardless of the nature of protein.

ANALYTICAL PROCEDURE.

Preparatory Operation.

It is carried out as did H. and A. A protein containing more than 0.1 mgm. of hexosamine is weighed for HCl-treatment.

Colorimetric Technique.

I. Reagents.

Reagents in the following were employed in addition to the acetylacetone and dimethylaminobenzaldehyde solutions of H. and A.‡

(1) Salt mixtures

12.86% NaCl solutions containing 0.0544 gm. (a), 0.07253 gm. (b) and 0.1632 gm. (c) or sodium acetate ($\text{NaC}_2\text{H}_3\text{O}_2 \cdot 3\text{H}_2\text{O}$) in 1 cc.

(2) Glucosamine solution A: Standard glucosamine solution.

A 12.86% NaCl solution which involves 0.5 mgm. of glucosamine hydrochloride in 1 cc.

(3) Glucosamine solution B.

In salt mixture a) is dissolved 0.5 mgm. of glucosamine hydrochloride per 1 cc. As glucosamine is quickly decomposed in an alkaline solution, this reagent should be prepared just before each use.

II. Processes.

2 cc. of the solution prepared by the preparatory operation are pipetted into a tube of Masamune and Nagazumi⁹⁾ and 1 cc. each of glucosamine

* 5 mgms. each of these amino acids were added separately per 0.5 mgm. of glucosamine hydrochloride.

† 3 mgms. each of the three amino acids were added together to 0.5 mgm. of glucosamine hydrochloride.

‡ In the information of H. and A., three words, "in reagent C" were left out between "Two solutions" and "containing", p. 160, line 18. H. Masamune.

solution B and salt mixture c) and 3 cc. of the acetylacetone solution are added. In another tube 1 cc. of the standard glucosamine solution and 3 cc. each of salt mixture b) and the acetylacetone solution are mixed together.

Following precesses are similar as those directed by H. and A., excepting that the colour comparison is made between the 5th and the 15th minute after pouring the dimethylaminobenzaldehyde solution into the tubes.

III. Calculation.

The amount of hexosamine is read as hydrochloride from a standardization curve plotted by the use of glucosamine hydrochloride and 0.5 mgm. is deducted therefrom. The difference multiplied by the factor, $\frac{179}{216} \times \frac{100}{\alpha}$, expresses the value of hexosamine in per cent of the protein. α denotes $\frac{2}{25}$ of the weight in mgm. of the protein analysed.

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References.

- (1) Hamasato and Akakura, J. Bioch., 1941, **34**, 159.
 - (2) Masamune and Tanabe, *ibid.*, 1938, **28**, 19.
 - (3) Masamune and Nagazumi, *ibid.*, 1937, **26**, 223.
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