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APPENDIX

Kjeldahl Method.

A. Reagents :

1. Methyl red methylene blue indicator : Mixed 5.4 ml 1 % methylene blue with 44.6 ml methyl red, saturated solution in 95% ethanol.

2. Primary standard, o.1 N potassium hydrogen phthalate : 2.0423 g of potassium hydrogen phthalate was dissolved in 100 ml distilled water. Potassium hydrogen phthalate was tritrated with 0.1 N sodium hydroxide in the presence of phenolphthalein or thymol blue as an indicator. The actual normality of sodium hydroxide was calculated and used as secondary standard. 0.1 N hydrochloric acid was tritrated with secondary standard sodium hydroxide contained 1-2 drops of methyl orange as an indicator. The actual normality of hydrochloric acid was then calculated.

3. Standard ammonium sulphate : 0.5188 g of ammonium sulphate was dissolved in 100 ml distilled water containing 1.1 mg nitrogen/ml.

B. Method for protein estimation.

1. Each 1.0 ml of standard ammonium sulphate, sample and distilled water or buffer used as a blank was digested with 10 ml conc. sulphuric acid containing 0.05 g selenium as a catalyst in distilled tube at 350°C-400°C for 1 h, allowed to cool at room temperature and made up to 100 ml with distilled water.

2. The contents in distilled tube were transfered to Kjeldahl distilled unit and 45 ml of 40% sodium hydroxide were added. Ammonia gas was then readily liberated by heat.

3. Ammonia gas was passed into 25 ml 4% boric acid containing 1-2 drops of methyl red methylene blue as an indicator for 5 min.

4. Ammonia in 4% boric acid was tritrated with hydrochloric acid and the volume of hydrochloric acid used in this tritration was recorded.

Calculation

$$\text{Mole of HCL} = \frac{N \times V}{1000} = m$$

where N = Normality of HCL

V = Volume of HCL used for tritration

m = Mole of HCL

This value (m) is equivalent to Mole of NH_4^+ and the nitrogen content was calculated.

$$\text{Nitrogen content} = m \times 14 \quad g.$$

Except in some very special cases, total protein in various plant and animal products has been shown to contain close to 16% nitrogen. Accordingly, the percentage of protein present may be obtained by multiplying the percentage of nitrogen present by 6.25, the latter figure is often referred to as the protein factor.

Bolton-Hunter Method for Protein Iodination.

1. Bolton-Hunter reagent 100 μl was transfered to the bottom of iodination tube and evaporated off solvent (benzene) by blowing gentle stream of dry nitrogen onto the surface of solution. Evaporation was repeated until the contents of this reagent was completely evaporated

2. 5 μg bTSH was carefully added to the bottom of iodination tube mixed in an ice-bath for 20-25 min.

3. Iodination reaction was stopped by addition of cold solution of 500 μl, 0.2 M glycine, o.1 M borate buffer, pH 8.5. The reaction mixture was mixed, allowed to stand for 5 min at $4^{\circ}C$ and then applied to a Sephadex G-25 column (1x5 cm) which was equilibrated in 0.25 % gelatin, 0.05 M phosphate buffer, pH 7.4.

4. Few fractions in the ^{125}I-labelled bTSH peak were collected, pooled and re-purified by a Sephadex G-100-40 column in NaCL/Tris/BSA buffer.

5. The fractions of undamaged ^{125}I-labelled bTSH were pooled, aliquoted and kept frozen at $-20^{\circ}C$

Method for Protein Iodination by Chloramine-T.

The iodination of bTSH was performed inside the hood at room temperature ($23^{\circ}C$-$25^{\circ}C$) and the reagents were added in the following order:-

1. 25 μl, 0.5 M phosphate buffer, pH 7.5 was added to a tube containing 5 μg bTSH and mixed gentlely by bubbling air through the micropipette.

2. 1 mCi Na^{125}I (IMS 30) was added and mixed throughly

3. 50 µg of chloramine-T in 10 µl phosphate buffer pH 7.5 was added and mixed well for 25 sec.

4. The reaction was stopped by addition of 125 µg sodium metabisulphite in 25 µl phosphate buffer and the reaction mixture was mixed.

5. 100 µl 10% BSA in 0.5 M phosphate buffer, pH 7.5 was added and mixed.

6. The iodination mixture was carefully applied on a Sephadex G-75 column (1 x 20 cm) which was equilibrated in phosphate buffer, pH 7.5

7. Each fraction of 0.5 ml was collected in tubes containing 100 µl 10 % BSA in phosphate buffer.

8. The undamaged ^{125}I-bTSH fractions were aliquoted and kept frozen at -20°C.

BIOGRAPHY

Miss Utumma Maghanemi was born on November, 7th 1953, in Bangkok, Thailand. She graduated Bachelor of Science in Radiological Technology (Second Class Honor) from Faculty of Medical Technology, Mahidol University, in 1976.