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A method for the determination of amino nitrogen and its applications.

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It has long been known that aliphatic amines react with nitrous acid according to the equation $RNH_2+HNO_2=ROH+H_2O+N_2$, and several methods have been devised for estimating amino groups by measuring the nitrogen gas evolved by this reaction.[1] None of them has been sufficiently simple or accurate to attain general use, however. The method proposed requires but little apparatus, but a few minutes for completion, and is as accurate as a Dumas or Kjeldahl determination. The reaction is carried out in a bottle with a capacity of about 35 cubic centimeters, fitted with a three hole No. 4 rubber stopper. Through the stopper pass: (1) the stem of a 10 c.c. burette; (2) the thick-walled capillary inlet from a cylindrical dropping funnel of 25 c.c. capacity; the capillary is of 2 mm. internal diameter and reaches nearly to the bottom of the bottle; (3) an outlet tube for gas. This is a capillary 25 to 30 cm. high, 1 mm. internal, 5 to 6 mm. external diameter. The lower end is flush with the bottom of the stopper, the upper end is bent in a semi-circle to meet the inlet of a gas burette. The tube has a stopcock near the middle.[2]

The amino solution for analysis is placed in the burette, and a few cubic centimeters of water in the dropping funnel. Into the bottle are poured 27 c.c. of a 10 to 3 solution of sodium nitrite followed by 7 c.c. of glacial acetic acid. The stopper is placed in position and the slight air volume left in the bottle displaced by water from the dropping funnel. The outlet tube is then closed, whereupon the nitric oxide gas formed by decomposition of the nitrous acid fills the upper part of the bottle, forcing the solution back into the funnel. When from 5 to 10 c.c. of gas are thus gathered, which requires but a few seconds, the outlet is opened and the gas driven out again, washing out the remaining traces of

[1] Hans Meyer: Anleitung zur quant. Best. organischen Atomgruppen, p. 81. Fischer and Koelker, *Annalen d. Chem.*, 1905, cccxl, 177.

[2] The apparatus can be obtained from Eimer and Amend, New York.

air. This is repeated to make absolutely certain that no air remains; then the outlet is closed until a gas space of from 15 to 18 c.c. has formed. The stopcock of the dropping funnel is then closed and the outlet connected with a gas burette. The amino solution is run in from the 10 c.c. burette, and the bottle shaken at short intervals to hasten the evolution of gas. The latter is continued until 30 to 40 c.c. more gas, than the volume of nitrogen expected, is in the gas burette. The cock of the dropping funnel is then opened, and all the gas from the bottle and outlet tube displaced into the gas burette. This mixture of nitric oxide and nitrogen is now run into a Hempel pipette containing a 5 per cent. potassium permanganate 2.5 per cent. potassium hydroxide solution, which absorbs the nitrous oxide. The pure nitrogen is then measured in the burette.

Alanin, valin, leucin, glycocoll, aspartic acid, glutaminic acid, phenylalanine, serine, oxyprolin, tyrosin, arginin, histidin, tryptophan, and guanin yield one molecule each of nitrogen. Lysin yields 2 molecules of nitrogen. Prolin, being an imino substance, does not react at all. Guanidine and its derivatives also fail entirely to react.

This method will be of value for convenient analysis in identifying the amino acids, also for the estimation of the amount of amino nitrogen in unknown substances, and in mixtures such as hydrolyzed protein. It further renders possible an accurate determination of the prolin obtained by the ester method. The alcoholic extract of the amino acids, whose esters distill below 100°, contains prolin with a hitherto undeterminable amount of the other acids as impurities. The amount of these impurities can be determined by amino nitrogen estimation, and this nitrogen subtracted from the total, gives the prolin nitrogen. Histidin and arginin, as obtained in solution by the Kossel and Patten method, can be analyzed without isolation. The ratio, total N : amino N, in the case of histidin is 1 : 3, of arginin 1 : 4, and as these ratios are characteristic, amino and Kjeldahl determinations on their separate solutions are sufficient to identify these bases.

The amino nitrogen method has been made the basis of a quantitative determination of amino nitrogen (amino acids) in the urine. Urea and ammonia react slowly with nitrous acid so must

be removed; 75 c.c. of urine plus 2.5 c.c. concentrated sulphuric acid are heated in an autoclave, for one and one half hours at 175°. The urea is entirely changed to ammonia, which is boiled off after adding 10 gm. of calcium oxide and a piece of paraffin. The ammonia-free filtrate is concentrated to a small volume, diluted to 25 c.c., and amino nitrogen determinations made on 10 c.c. portions. In blank determinations, 11.55 mg. nitrogen as alanine were added to a normal urine. The increase in amino nitrogen was 11.47 and 11.58 mg. n duplicates. Determinations on a number of normal urines indicate a normal amino nitrogen of 2.0 per cent. ± 0.5 per cent. for a normal urine. The study of pathological urines is being undertaken, as the amino determination may be of value in indicating conditions where physiological oxidation of protein nitrogen is incomplete.

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Note on the production of glycosuria by parathyroids, pancreas and the infundibular extract of the pituitary.

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In experiments upon cats we have found that injection per jugular of filtered watery solution of the parathyroid or the pancreas produced 3 to 4 per cent. of sugar in the urine. Injections into abdominal cavity of the pancreas also produced glycosuria. Borchardt has shown that the pituitary gland as a whole causes sugar to appear in the urine. We have found 1 c.c. of the 20 per cent. extract of the infundibular part (Burroughs, Wellcome and Co.) of the pituitary by the jugular causes about 3 per cent. of sugar to appear in the urine. We took care that the binding down and etherization did not cause any sugar to be present in the urine of our animals. Falta and Priestly did not find any increase of the sugar in the blood of the rabbit by relatively large doses of infundibulin. The presence of glucose was always determined by Fehling's, fermentation, and phenylhydrazine tests.