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Determination of the amino-acid nitrogen in the urine.

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Since the abandonment of the Pfaundler method for determination of the amino-acid nitrogen of the urine, the only direct methods proposed which have met with any favor, are the gasometric method of D. D. Van Slyke and the titration method of Henriques and Sörensen. In our hands the Van Slyke method as originally described[1] has not proved entirely satisfactory, (1) because of the difficulty of removing all the ammonia after conversion of urea, and (2) because poly-peptids and other condensation products of amino-acids, *e. g.*, hippuric acid, are estimated as well as free amino-acid nitrogen. The method, therefore, probably gives results which are too high.

The Henriques and Sörensen method as improved by the authors themselves[2] likewise presents some difficulties. For example, as objected by de Jager and since admitted by Henriques and Sörensen, in the presence of large quantities of ammonia the total titration is less than the sum of the ammonia N and amino-acid N done separately. It is therefore necessary first to remove the ammonia. Henriques and Sörensen recommend for this the method of Krieger and Reich slightly modified. This is essentially the method well known in this country by Shaffer's name. Since the method is based on distillation under diminished pressure, it is not adapted to rapid determinations in a series of urines simultaneously. Besides, as used by Henriques and Sörensen, we have not been able to obtain as high ammonia figures as by the Folin method. Use of the latter method for removal of ammonia, while perfectly satisfactory for small samples of urine (10 or 20 c.c.) is not satisfactory for a sample large enough to give a titration for amino-acid nitrogen (40–50 c.c.).

Another objection to the Henriques and Sörensen procedure is the difficulty of titration with phenolphthalein in a barium filtrate because of interference of carbon dioxide.

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[1] PROC. SOC. EXP. BIOL. AND MED., 1910, VII, p. 47.

[2] *Zeitschr. f. physiol. Chemie*, 1909, LXIV, p. 120.

These various difficulties have led us to return to the precipitation of ammonia by means of phosphotungstic acid. Gumlich[1] showed originally that the proper conditions for removal of ammonia by 10 per cent. phosphotongstic acid are a strongly acid urine and sedimentation for 24 hours. We have satisfied ourselves that this strength of phosphotungstic acid does not precipitate the mono-amino acids in the concentration usually found in urines. Instead, however, of removing the urea from the phosphotungstic filtrate and the determination of amino-acid nitrogen by difference, as in the original Schöndorff-Pfaundler method, or the modifications of it by Krieger and Schmidt, Van Leersum, and others, we remove the phosphotungstic acid by means of tribasic lead acetate and litharge, and titrate for mono-amino acids in the filtrate (after removal of lead) according to the procedure of Henriques and Sörensen. The filtrate is water-clear and free of any constituents which can interfere with the formalin titration.

COMPARISON OF THE HENRIQUES AND SÖRENSEN METHOD WITH MODIFIED METHOD.

Urine	No.	H. and S.	Modified Method.
Case 1. Pernicious vomiting.	Urine No. 1	0.215 gm. NH_2N	0.015 gm. NH_2N
	Urine No. 2	0.434 gm. NH_2N	0.048 gm. NH_2N
	Urine No. 4	0.268 gm. NH_2N	0.025 gm. NH_2N
	Urine No. –	0.245 gm. NH_2N	0.023 gm. NH_2N
Case 2. Eclampsia.	Urine No. 3	0.397 gm. NH_2N	0.120 gm. NH_2N
	Urine No. 7	0.157 gm. NH_2N	0.035 gm. NH_2N
	Urine No. 8	0.375 gm. NH_2N	0.148 gm. NH_2N
	Urine No. 11	0.210 gm. NH_2N	0.155 gm. NH_2N

All the urines contained a large amount of ammonia. It would seem from this comparison that the Henriques and Sörensen method like the Van Slyke method gives results (especially on pathological urines) even after the supposed removal of ammonia which may be quite misleading.

The following are a few of the results obtained with pure substances.

A. Pure Solutions:

1. 20 c.c. *N*/20 leucine, purity tested by Kjeldahl and formol titration, added to 5 c.c. *N*/10 NH_4Cl < 200 c.c.; 45 c.c. of final filtrate, titration 2.4, theory 2.28.

[1] *Zeitschr. f. physiol. Chemie*, 1893, XVII, p. 13.

2. 20 c.c. leucine + NH_4Cl < 180 c.c.; 50 c.c. final filtrate, titration 2.7, theory 2.77.
3. Mixture of urea, $(NH_4)_2SO_4$, uric acid, alanin, glycocoll, glutamic acid (all tested substances), containing 0.144 gm. amino-acid nitrogen, yielded by new method 0.132, 0.148, 0.143 gm.

B. Pure Substances added to urines.

1. 200 c.c. normal urine; 100 c.c. final filtrate, titration 9.6 c.c. *N*/10 NaOH.
   200 c.c. normal urine + 20 c.c. *N*/20 leucin; 100 c.c. filtrate, titration 11.8 NaOH
   Difference 2.2 c.c., theory 2.5 c.c.
2. 200 c.c. urine of puerperient woman; 100 c.c. filtrate, titration 9.7 *N*/10 NaOH.
   200 c.c. urine of puerperient woman + 20 c.c. *N*/10 alanin, titration 14.7 *N*/10 NaOH.
   Difference 5.0 c.c. *N*/10 NaOH, theory 5.0 c.c.

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Picrolonates of the monoamino acids.[1]

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Picrolonic acid, used by Steudel to precipitate the hexone bases, and later shown by Mayeda to form salts with the aromatic amino acids tryptophane and phenylalanine, also forms crystalline salts of normal composition with the other monoamino acids obtained on hydrolysis of proteins. The salts are made by dissolving molecular proportions of amino acid and picrolonic acid in a minimum amount of boiling water. The picrolonates crystallize from the cooling solutions, usually while they are still warm. In cold water many of them are very insoluble. In alcohol they are all more soluble than in water. Following is a list of the amino acids of

[1] After this title had been sent to the secretary an article by Abderhalden and Weil appeared describing picrolonates of glycocoll; d-alanine, and dl-leucine (*Ztschr. physiol. Chem.*, 78, 150). They were formed in alcoholic solution, which yielded products of abnormal composition in the cases of glycocoll and alanine.