A METHOD FOR THE ESTIMATION OF THE UREA, ALLANTOIN, AND AMINO ACIDS IN THE URINE

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In investigations involving the determination of the distribution of nitrogen in the urine, the separation of the various nitrogenous constituents is a matter of no little difficulty, and involves so much time that it is almost impossible to carry through any prolonged series of observations.

The object of the present investigation is to determine how far the distribution of nitrogen can be more rapidly determined indirectly by taking advantage of the differences in solubility and stability to reagents of the various substances.

The chief substances in which nitrogen occurs in the urine are:—Urea, purin bodies including uric acid, creatinin (with or without creatin), ammonia, allantoin, amino acids (including hippuric acid), sulphur-containing bodies and bodies of unknown composition.

The present investigation is confined to the determination of urea, allantoin and amino acid nitrogen.

For the determination of *Urea* Bohland (1) recommended the precipitation of the other nitrogen-containing substances by phosphotungstic and hydrochloric acids. The urea was then estimated in the filtrate. He claimed that the results got by this method gave only urea nitrogen and ammonia nitrogen, all other nitrogen-containing bodies being precipitated.

Later Schöndorff (2) showed that the amino acids (glycocoll, leucin, &c.) are not precipitated by phosphotungstic and hydrochloric acids. He also showed that while creatinin is precipitated, as Bohland had already found, creatin is not. Allantoin also is not precipitated; a statement later confirmed by Mörner (3).

Pfaundler (4) experimented with various samples of phosphotungstic acid and found that, if Merck's preparation is used, ammonia is precipitated.

The nitrogen obtained by this method therefore includes urea, amino acids (with hippuric acid), creatin and allantoin nitrogen.

Mörner and Sjöqvist (5) recommended a method for the estimation of urea in which the urine is precipitated with a saturated solution of barium chloride in which five per cent. of barium hydrate is dissolved, and an alcohol ether mixture containing two parts alcohol to one of ether. After twenty-four hours it is filtered, the filtrate evaporated at 50° to small bulk, after the addition of a pinch of magnesium oxide to drive off the ammonia, and the nitrogen in it estimated by Kjeldahl's method. Creatinin, hippuric acid, and also some amino acids (leucin, bile acids) are not precipitated, and their nitrogen is thus included in the amount obtained by this method.

Folin (6) proposed a method of heating the urine with crystallised magnesium chloride and hydrochloric acid. At the temperature thus employed—150° C.—urea and allantoin alone are decomposed to give off ammonia. Thus the nitrogen obtained by this method includes allantoin nitrogen in addition to urea nitrogen and ammonia nitrogen.

Mörner, in a more recent paper (7), develops what may be called the Mörner-Folin method by which urea alone yields its nitrogen. The bulk of the nitrogen-containing substances are precipitated as described above, with barium chloride, barium hydrate and alcohol ether solution, but the evaporated filtrate is heated with magnesium chloride and hydrochloric acid as in Folin's method. Allantoin is by this procedure almost entirely removed before heating with magnesium chloride, thus avoiding Folin's error. The creatinin, &c., which are not precipitated in the Mörner method are not decomposed in the Folin method. Thus the Mörner-Folin method gives only the nitrogen which is contained as urea.

In the present investigation a slight modification of this method was adopted, a modification first employed by Underhill and Kleiner (8), viz., the use of an alcohol ether solution containing equal parts of alcohol and ether, since as Haskins (9) had previously observed, allantoin is more insoluble in such a solution than in the one employed by Mörner. In addition a few drops of hydrochloric acid were at once added before the evaporation of the alcohol ether filtrate, thus preventing the escape of ammonia, the nitrogen in which is thus included in the results. This addition of acid was also recommended by Folin, instead of driving off the ammonia by means of magnesium oxide as practised by Mörner.

The method in detail as employed by me is, 5 c.c. of the urine are mixed with 5 c.c. barium chloride, barium hydrate solution, and 100 c.c. alcohol-ether (50 c.c. absolute alcohol, 50 c.c. ether). After twenty-four hours it is filtered, an aliquot portion of the filtrate is taken, and a few drops of hydrochloric acid immediately added. This is then evaporated almost to dryness at a temperature of 50° C. in an apparatus described by Haskin; 20 grs. magnesium chloride are then added and a small piece of paraffin wax to prevent frothing, and the whole is heated for three hours on an electric heater described by Cathcart (10).

The direct determination of *Allantoin* has always proved difficult and unsatisfactory. The similarity between its properties and those of urea renders complete separation extremely difficult. Poduschka's method (11), in which allantoin is precipitated by silver nitrate in alkaline solution, is comparatively simple, though somewhat lengthy, but, as Salkowski says, the precipitation of the silver compound with ammonia is one of the most difficult of chemical procedures.

Wiechowski (12) proposed using mercuric acetate, which precipitates allantoin but not urea, instead of the usual mercuric nitrate, by which urea too is precipitated. Allantoin when thus precipitated shows a strong tendency to crystallise. The details of the method are, however, long and troublesome, though giving accurate results, and it is impracticable for a long series of daily observations.

For the estimation of the *Amino acids* various methods have been suggested.

Fischer and Bergell (13) proposed a direct method which depends on the power of naphthol-sulphonic acid to combine with amino acids. The urine was shaken with an ethereal solution of β naphthalene sulpho chloride and the amino acid compound subsequently precipitated with hydrochloric acid. This method was later modified by other workers. Glaessner (14), in criticising the method, states that he, as others also, did not recover on an average more than 60 per cent. of the amino acids.

Neuberg and Manasse's direct method (15) depends on the fact that on shaking a urine with a strongly alkaline solution of α -naphthol isocyanate, hydantoic acid is formed, which is precipitated after acidification. Glaessner found this method not at all constant. Some good results were obtained, but in general they were quite unreliable.

Pfaundler (16) endeavoured to devise an indirect method for the estimation of amino acids. His method was to precipitate the urine first with phosphotungstic acid. The precipitate and filtrate were then each

heated with phosphoric acid. The urine nitrogen was thus divided into four fractions.

- I. Substances precipitated by phosphotungstic acid.
 - (a) Nitrogen easily removed by heating with phosphoric acid.
 - (b) Nitrogen not thus easily liberated.
- II. Substances not precipitated by phosphotungstic acid.
 - (a) Nitrogen easily removed by heating with phosphoric acid.
 - (b) Nitrogen not thus easily liberated.

Of these four fractions, II (a) consists mainly, if not entirely, of amino acids.

Kruger and Schmid's method (17), also an indirect one, was based on the fact that amino acids do not give off ammonia when heated with concentrated sulphuric acid at 160° to 180° C. They estimated the nitrogen in the filtrate from phosphotungstic acid and also the nitrogen which was obtained after heating with half the volume of concentrated sulphuric acid. The difference between these two should correspond to the amino acid nitrogen.

Glaessner (18) proposed a somewhat similar method for the estimation of amino acids. He precipitated with phosphotungstic acid and evaporated the filtrate to dryness at a low temperature. The residue was freed from water and then extracted with alcohol-amyl alcohol for six hours. It was then filtered and a Kjeldahl nitrogen estimation done on the residue. This gave the amino acid nitrogen. The difficulty of this method is to ensure that the urea is completely dissolved.

PRESENT METHODS

It appeared possible that the combination of certain of these methods—Bohland, Folin, Mörner-Folin—might yield definite results as to the distribution of nitrogen, and the method which I employ is as follows:—

Estimations of the nitrogen present are made by these three methods, and by the differences between them the nitrogen in urea, allantoin and amino acids is determined.

(a) Bohland nitrogen, using Merck's phosphotungstic acid, which leaves unprecipitated amino acids, hippuric acid, creatin, allantoin and urea.

(b) Folin nitrogen, which includes urea, allantoin and ammonia nitrogen.

(c) Mörner-Folin nitrogen, which includes urea and ammonia nitrogen.

The ammonia nitrogen was separately estimated by Folin's method and subtracted from (b) and (c).

The difference then between the nitrogen of (a) and (b) should give the amount of amino acid nitrogen and creatin nitrogen present, hippuric acid being included as an amino acid. The creatin can be determined separately by Folin's method.

The difference between the nitrogen of (b) and (c) should give the amount of allantoin nitrogen present.

To verify the method estimations were made on solutions containing varying amounts of urea, allantoin and amino acids. The nitrogen in each solution was determined by Kjeldahl's method.

I. A solution was made consisting of 25 c.c. of a 2 per cent. solution of urea, 25 c.c. of a 0.5 per cent. solution of allantoin, and 10 c.c. of a 0.5 per cent. solution of alanin. 5 c.c. of this solution contained 0.0016 grs. nitrogen as allantoin and 0.0022 grs. nitrogen as alanin.

Bohland	nitrogen required	9 c.c.	$\frac{N}{10}$ -acid	=	0.0126 grs. nitrogen
Folin	"	7.4 c.c.	"	=	0.0104 "
Mörner-Folin	"	5.05 c.c.	"	=	0.0088 "
B. - F.	= 0.0022 grs. nitrogen		= 100 p.c. alanin nitrogen		
F. - M.-F.	= 0.0016 "		= 100 p.c. allantoin "		

II. Solution consisted of 25 c.c. of a 0.5 per cent. solution of allantoin, 25 c.c. of a 1 per cent. solution of glycocoll, 25 c.c. of a 2 per cent. solution of urea, and contained 0.042 grs. nitrogen as allantoin and 0.044 grs. nitrogen as glycocoll.

Folin	nitrogen required	13.1 c.c.	$\frac{N}{10}$ -acid	=	0.275 grs. nitrogen
Mörner-Folin	"	8.9 c.c.	"	=	0.234 "
F. - M.-F.	= 0.041 grs. nitrogen		= 97.6 p.c. allantoin nitrogen		

III. Solution consisted of a mixture of a 2 per cent. solution of urea and a 0.5 per cent. solution of allantoin, 5 c.c. of which contained 0.0034 grs. nitrogen as allantoin.

Folin	nitrogen required	15.25 c.c.	$\frac{N}{10}$ -acid	=	0.02135 grs. nitrogen
Mörner-Folin	"	12.9 c.c.	"	=	0.0181 "
F. - M.-F.	= 0.00325 grs. nitrogen		= 95.5 p.c. allantoin nitrogen		

IV. Solution consisted of a mixture of a 2 per cent. solution of urea and a 1 per cent. solution of glycocoll, 5 c.c. of which contained 0.0035 grs. nitrogen as glycocoll.

$$\text{Bohland nitrogen} = 15.2 \text{ c.c. } \frac{N}{10} \text{ acid} = 0.0212 \text{ grs. nitrogen}$$

$$\text{Folin} \quad \quad \quad \text{,,} \quad = 12.7 \text{ c.c.} \quad \quad \quad \text{,,} \quad = 0.0178 \quad \quad \quad \text{,,}$$

$$B. - F. = 0.0034 \text{ grs. nitrogen} = 97 \text{ p.c. glycocoll nitrogen}$$

V. Solution consisted of a mixture of a 0.25 per cent. solution of alanin and a 1 per cent. solution of urea, containing 0.45 grs. nitrogen as urea, and 0.037 grs. nitrogen as alanin.

$$\text{Bohland nitrogen} = 34.15 \text{ c.c. } \frac{N}{10} \text{ acid} = 0.478 \text{ grs. nitrogen}$$

$$\text{Folin} \quad \quad \quad \text{,,} \quad = 31.6 \text{ c.c.} \quad \quad \quad \text{,,} \quad = 0.442 \quad \quad \quad \text{,,}$$

$$B. - F. = 0.036 \text{ grs. nitrogen} = 97 \text{ p.c. alanin nitrogen}$$

These results are summarised in Tables I and II.

TABLE I

No. of solution	Grs. allantoin nitrogen	F. - M.-F.	P.c. allantoin]	Solution contained
I.	0.0016	0.0016	100	Urea, alanin, allantoin
II.	0.042	0.041	97.6	Urea, allantoin, glycocoll
III.	0.0034	0.00325	95.5	Urea, allantoin
No. of solution	Grs. amino acid nitrogen	B. - F.	P.c. amino acid	Solution contained
I.	0.0022	0.0022	100	Urea, allantoin, alanin
IV.	0.0035	0.0034	97	Urea, glycocoll]
V.	0.037	0.036	97	Urea, alanin

In order to ascertain whether these methods held when applied to urines, estimations were made on urines to portions of which known amounts of allantoin and amino acids had been added.

I. Setter's urine.

$$\begin{aligned} \text{Folin} \quad \quad \quad \text{nitrogen} &= 0.116 \text{ grs. nitrogen} \\ \text{Mörner-Folin} \quad \quad \quad \text{,,} &= 0.116 \quad \quad \quad \text{,,} \\ \text{F.} - \text{M.-F.} &= 0 \quad \quad \quad \text{,,} \\ \text{An allantoin solution containing 0.016 grs. nitrogen was added} & \\ \text{Folin} \quad \quad \quad \text{nitrogen} &= 0.1204 \text{ grs. nitrogen} \\ \text{Mörner-Folin} \quad \quad \quad \text{,,} &= 0.1036 \quad \quad \quad \text{,,} \\ \text{F.} - \text{M.-F.} &= 0.0168 \\ \text{Increase of difference} &= 0.0168 \text{ grs. nitrogen} = 105 \text{ p.c. allantoin nitrogen} \end{aligned}$$

II. Goose's urine.

$$\begin{aligned} \text{Folin} \quad \quad \quad \text{nitrogen} &= 0.00266 \text{ grs. nitrogen} \\ \text{Mörner-Folin} \quad \quad \quad \text{,,} &= 0.0021 \quad \quad \quad \text{,,} \\ \text{An allantoin solution containing 0.0096 grs. nitrogen was added.} & \\ \text{Folin} \quad \quad \quad \text{nitrogen} &= 0.0116 \text{ grs. nitrogen} \\ \text{Mörner-Folin} \quad \quad \quad \text{,,} &= 0.0021 \quad \quad \quad \text{,,} \\ \text{F.} - \text{M.-F.} &= 0.0095 = 98.9 \text{ p.c. allantoin nitrogen} \end{aligned}$$

III. Goose's urine.

10 c.c. urine + 10 c.c. water.

Folin nitrogen = 0.00532 grs. nitrogen

10 c.c. urine + 5 c.c. water + 5 c.c. allantoin solution containing
0.0096 grs. nitrogen

Folin nitrogen = 0.01456 grs. nitrogen

Difference = 0.00924 grs. nitrogen = 95.6 p.c. allantoin nitrogen.

IV. Goose's urine.

15 c.c. urine + 15 c.c. water.

Bohland nitrogen = 0.0042 grs. nitrogen

Folin " = 0.00798 " "

B. - F. = - 0.00378 grs. nitrogen

15 c.c. urine + 10 c.c. water + 5 c.c. glycocoll solution
containing 0.0091 grs. nitrogen

Bohland nitrogen = 0.01344 grs. nitrogen

Folin " = 0.00798 " "

B. - F. = 0.00546 grs. nitrogen

Increase of difference = 0.00924 grs. nitrogen = 101.5 p.c. glycocoll
nitrogen

The results are summarised in Table III.

TABLE III.

No.	Grs. nitrogen added. to urine	Grs. nitrogen determined	P.c. nitrogen	Remarks
I.	0.016	0.0168	105	Urine + allantoin
II.	0.0096	0.0095	98.9	Urine + allantoin
III.	0.0096	0.00924	95.6	Urine + allantoin
IV.	0.0091	0.00924	101.5	Urine + glycocoll

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