been able to find in the literature the asymmetric dibenzoylhydrazine described with certainty and until it is prepared it is impossible to say whether or not we have it in the above product.

The first experiments made in the study of the action of hydrazine on ethyl mesoxalate were in alcoholic solutions. The effect of this solvent was to increase the polymerization products. Under certain exact conditions, a crystalline product was obtained.

0.17 gram (1 mol) of ethyl dihydroxymalonate was dissolved in 1.5 cc. of alcohol. This was slowly poured, without mixing, down the side of a test tube into 1.9 cc. of alcohol containing 0.4 cc. (2 mols) of a 22 per cent. hydrazine hydrate solution. If the liquids are mixed, gummy products form which it is impossible to remove. A turbidity forms at the line of contact of the two solutions. After about one hour at 10°, colorless needles form between the solutions, and in twenty-four hours the lower half is filled with beautiful clusters of crystals. 0.11 gram was filtered off and washed with ether. As the substance was quickly decomposed in all attempts to recrystallize it an analysis was made of the crude substance:

Calculated for $C_4H_{12}N_2O_6$:	C, 20.60;	H, 5.58;	N, 12.02.
Found:	C, 20.92, 20.54;	H, 5.67, 5.66;	N, 12.01.

The substance decomposes at 125°–130°, evolves a gas, and leaves a yellow solid, which melts at 170°, giving off a gas. It is easily soluble in warm water and potassium hydroxide solution, slightly soluble in ether, methyl alcohol, acetone, benzene, carbon disulphide, and 25 per cent. acetic acid, insoluble in chloroform, carbon tetrachloride, ligroin, and nitrobenzene. Its aqueous solution turns red when warmed (polymerization gums). Alcohol hastens this change.

Glacial acetic acid gives a white product, but slightly soluble in most solvents and turned blood-red by potassium hydroxide solution.

We have made no further attempt to determine the structure of this substance.

URBANA, ILL.

THE DETERMINATION OF UREA IN THE URINE.

BY CHARLES G. L. WOLF AND EMIL OSTERBERG.

Received January 12, 1909.

In the course of investigations in which one of us has been engaged for some years, dealing with the protein metabolism in normal and pathological conditions in man and animals,[1] it has been necessary to select a method

[1] Marriott and Wolf, *Amer. J. Med. Sci.*, Feb., 1906; *Biochem. Z.*, **7**, 213 (1907). Ewing and Wolf, *Amer. J. Med. Sci.*, **131**, 751 (1906): *Amer. J. Obstetrics.*, **55**, 1 (1907). Osterberg and Wolf, *J. Biol. Chem.*, **3**, 165 (1907); *Biochem. Z.*, **5**, 304 (1907). Wolf and Shaffer, *J. Biol. Chem.*, **4**, 439 (1908). Loewy and Wolf, *Biochem. Z.*, **8**, 132 (1908).

for the determination of urea which would be accurate, and at the same time allow of a large number of analyses being made simultaneously, with other work.

Of the methods which may be employed for this work there are:

1. Bunsen's method, with the modification of Pflüger, Bohland and Bleibtreu.[1]

2. The phosphoric acid method of Pflüger and Bleibtreu.[2]

3. The phosphoric acid method of Pflüger and Bleibtreu, as modified by Schöndorff.[3]

4. The Mörner-Sjöqvist method.[4]

5. The Folin method.[5]

6. The Folin method as modified by Mörner.[6]

All these methods were tried, and as a result, the Folin method was chosen. Since that time it has been used by other investigators, as Cathcart,[7] with success.

This method, as is well known, consists in heating solutions containing urea with boiling saturated magnesium chloride in the presence of hydrochloric acid.

Since the method was chosen, several thousand analyses all in duplicate have been made in this laboratory, and, when carefully used, it has never failed to give satisfactory results. It must be confessed, however, that the method requires care in the execution of the analyses, and has certain features, such as the employment of magnesium chloride, a salt which is rarely free from ammonia, and the necessity of using vaselin or paraffin in the distillation which render it somewhat inconvenient.

In a recent number of THIS JOURNAL, a method was described by Benedict and Gephart[8] which seemed to present many advantages over the method of Folin. The results of the analyses of urea solutions were exceedingly satisfactory, but comparisons with Folin's method showed that the latter gave uniformly lower results.

The method proposed by Benedict and Gephart was a revival of the original Bunsen method, in which solutions containing urea were heated to 150°. The heating was done in an autoclave in the presence of hydrochloric acid.

From the first it was assumed that the methods of Benedict and Gephart and of Folin were the same, but that the latter had devised a method of

[1] *Pflüger's Arch.*, **38**, 575 (1886); *Ibid.*, **43**, 30 (1888); *Ibid.*, **44**, 10 (1889).

[2] *Ibid.*, **44**, 78 (1889).

[3] *Ibid.*, **62**, 1 (1895).

[4] *Skand. Arch. Physiol.*, **2**, 438 (1891).

[5] *Z. physiol. Chem.*, **32**, 504 (1901); *Ibid.*, **36**, 333 (1903); *Ibid.*, **37**, 504 (1903).

[6] *Skand. Arch. Physiol.*, **14**, 297 (1903).

[7] *Biochem. Z.*, **6**, 109 (1907).

[8] THIS JOURNAL, **30**, 1760 (1908).

heating in which both the concentration of the hydrochloric acid and the regulation of the temperature were much more constant than in the former. It was therefore with much hope that we undertook an examination of this method, and to what extent it was fulfilled, the following results will show.

In order that a method for the determination of urea in the urine may be of value for accurate analysis, it must at least fulfil the following two conditions:

1. It must give quantitative results with pure urea solutions.

2. No decomposition may take place with the best known constituents of the urine.

In ordinary urines of individuals receiving a normal mixed diet, about 0.5 g. creatinine nitrogen are excreted in the twenty-four hours, and rather less uric acid nitrogen. As these two substances are the two best known constantly occurring nitrogenous constituents of the urine other than urea and ammonia, it is necessary that no decomposition should take place with these with the formation of ammonia in any method used for the determination of urea.

We therefore made a series of determinations of the amount of ammonia yielded by these substances. Of the pure compounds used in these tests, the urea was a sample of Kahlbaum. The uric acid came from the same source, and with the exception of one determination (No. 66) was recrystallized twice from boiling distilled water. The creatinine was prepared from urine, and on colorimetric analysis gave a creatinine content of 95 per cent.

The heating was done in Jena glass test-tubes, contained in an automatically regulated autoclave. The standard solutions used were a N/5 sulphuric acid and a N/10 sodium hydroxide. The former had been standardized by weighing the barium sulphate precipitated. The acid was delivered from an automatic pipette, and the alkali corresponded within 0.05 cc. Carminic acid was used as an indicator. Controls were made of all the reagents used in the work, and these controls were heated at the same time as the solutions to be tested.

It will be seen that the autoclave method, using solutions of urea, gives identical results with the Kjeldahl method and the Folin method, and therefore fulfils the first condition for a standard method.

On the other hand, uniformly higher results are given by the autoclave than by Folin's method when urines are tested. In both cases the duplicate analyses are perfectly concordant. The difference between the two methods is more apparent when concentrated urines are tested than in urines where the concentration of the organic constituents is low.

A reason for this difference was sought for, and is believed to be found in the difference in the behavior of the two methods to solutions of uric

acid and creatinine. As will be seen from Experiments 53–57, uric acid, when pure, yields absolutely no ammonia when heated in Folin's method. On the other hand the amount of ammonia given off when uric acid is heated in the method of Benedict and Gephart is very considerable, giving a nitrogen content for uric acid of 16.9 to 7.0 per cent. Similarly with solutions of creatinine, one finds that while Folin's method yields no ammonia nitrogen, Benedict and Gephart's method yields 8.4 and 4.2 per cent. of ammonia nitrogen depending on the concentration of the hydrochloric acid present in the hydrolyzing mixture.

It is therefore clear that the method of Benedict and Gephart, in the form in which these authors have presented it is unsuitable for the determination of urea in urine. It was possible that by lowering the temperature at which the hydrolysis took place, one might get a quantitative decomposition of urea, and at the same time the amount of uric acid and creatinine hydrolyzed would be insufficient to vitiate the results of the analysis. This attempt was made, and the hydrolysis was performed at 4 and 3 atmospheres. In experiment it is shown that while urea is quantitatively decomposed at these temperatures, both uric acid and creatinine are still hydrolyzed in too great amounts to allow the method to be used. It will be noted that the use of magnesium chloride in amounts sufficient to push back the dissociation of the hydrochloric acid and still not interfere with its solubility was not effectual in preventing the decomposition of uric acid (Experiment 57).

In some later experiments, which are not reported in this paper, hydrolyses of urine were attempted in saturated solutions of magnesium chloride. The results were not satisfactory, from control determinations by the Kjeldahl method.

Starting with the assumption that for the hydrolysis of urea it is only necessary to have a condition in which a low concentration of hydrogen ions is present, an attempt was made to perform the decomposition using other and less strongly dissociated acids, such as phosphoric, and certain organic acids, citric, lactic, tartaric and trichloracetic acids.

With the exception of trichloracetic acid, it will be seen that all the above acids effect a nearly quantitative hydrolysis of urea into carbon dioxide and ammonia, and with some of the acids, results are obtained surprisingly close to those obtained by Folin's method. The reason for this is evident when one takes into account the somewhat low results given with pure solutions of urea, and the decomposition which has taken place with solutions of uric acid and creatinine. Compensating errors have tended to equalize each other.

That the ammonia produced on hydrolysis is not due to the action of the alkali used in the subsequent distillation is shown by Experiments 52 and 68. No ammonia was given by this treatment.

No.	Solution.	cc. taken.	Method of heating.	Reagent.		Time. Min.	Per cent. N.	Per cent. H.
1	Urea, 2%	5	Kjeldahl	.....	....	..	46.08	46.08
2	"	5	Folin	.....	....	..	46.10	46.10
3	"	5	Autoclave	5 cc. 1–4 HCl	145°	90	46.11	46.11
4	"	5	"	10 cc 1–4 HCl	4 atm.	"	46.07	46.07
5	"	5	"	10 cc. 1–4 HCl	3 atm.	"	46.10	46.10
6	"	5	"	5 cc. phosphoric acid, 5%	145°	"	45.96	...
7	"	5	"	10 cc. phosphoric acid, 5%	"	"	45.96	...
8	"	5	"	5 cc. phosphoric acid, 5%, plus 5 cc. sat. $MgCl_2$	"	"	44.70	...
9	"	5	"	5 cc. citric acid, 10%	"	"	44.56	44.70
10	"	5	"	5 cc. citric acid, 20%	"	"	44.98	...
11	"	5	"	5 cc. trichloracetic acid, 10%	"	"	28.76	...
12	"	5	"	10 cc. phosphoric acid, 5%	4 atm.	"	46.10	46.10
13	"	5	"	10 cc. phosphoric acid, 5%	3 atm.	"	46.00	46.00
14	Urine No. 850	5	Folin	.....	....	..	1.113	1.113
15	" "	5	Autoclave	5 cc. 1: 4 HCl	145°	90	1.140	1.140
16	" No. 851	5	Folin	.....	....	..	0.976	0.976
17	" "	5	Autoclave	5 cc. 1: 4 HCl	145°	90	1.011	1.011
18	" No. 853	5	Folin	.....	....	..	0.660	0.660
19	" "	5	Autoclave	5 cc. 1: 4 HCl	145°	90	0.665	0.665
20	" No. 854	5	Folin	.....	....	..	0.642	0.642
21	" "	5	Autoclave	5 cc. 1: 4 HCl	145°	90	0.650	0.650
22	" No. 789	5	Folin	.....	....	..	0.424	0.424
23	" "	5	Autoclave	5 cc. 1: 4 HCl	145°	90	0.432	0.432
24	Urine, dog	5	Folin	.....	....	..	0.335	0.335
25	" "	5	Autoclave	5 cc. 1: 4 HCl	145°	90	0.340	0.340
26	" "	5	Folin	.....	....	..	0.310	0.310
27	" "	5	Autoclave	.....	145°	90	0.315	0.315
28	" No. 852	5	Folin	.....	....	..	0.982	0.982
29	" "	5	Autoclave	5 cc. 1: 4 HCl	145°	90	1.031	1.035

No.	Solution.	cc. taken.	Method of heating.	Reagent.	Temp.	Time. Min.	Per cent. N.	Per cent. H.
30	Urine No. 852	10	Autoclave	10 cc. 1:4 HCl	145°	90	1.035	1.035
31	" "	10	"	10 cc. 1:4 HCl distillate boiled before titration	"	"	1.033	1.033
32	" "	10	"	15 cc. 1:4 HCl	"	"	1.035	1.035
33	" "	5	"	5 cc. phosphoric acid, 5%	"	"	1.022	1.022
34	" "	7.5	"	5 cc. phosphoric acid, 5%	"	"	0.970	...
35	" "	7.5	"	10 cc. phosphoric acid, 5%	"	"	1.038	...
36	" "	10	"	5 cc. phosphoric acid, 5%	"	"	0.894	...
37	" "	10	"	10 cc. phosphoric acid, 5%	"	"	1.024	1.024
38	" "	10	"	10 cc. phosphoric acid, 10%	"	"	1.025	1.025
39	" "	5	"	5 cc. phosphoric acid, 10%, plus 5 cc. sat. $MgCl_2$	"	"	1.015	1.015
40	" "	5	"	10 cc. tartaric acid, 10%	"	"	1.026	...
41	" "	5	"	10 cc. lactic acid, 10%	"	"	1.032	...
42	" "	5	"	10 cc. citric acid, 10%	"	"	0.976	0.979
43	" "	5	"	5 cc. citric acid, 10%	"	"	0.948	...
44	" "	10	"	5 cc. citric acid, 10%	"	"	0.842	...
45	" "	10	"	15 cc. citric acid, 10%	"	"	0.984	...
46	" "	5	"	10 cc. citric acid, 20%	"	"	1.015	1.015
47	" "	5	"	10 cc. trichloracetic acid, 10%	"	"	0.632	0.657
48	" "	5	"	10 cc. HCl 1:4	4 atm.	"	1.038	1.038
49	" "	5	"	10 cc. phosphoric acid, 5%	4 atm.	"	1.032	1.032
50	" "	5	"	10 cc. HCl 1:4	3 atm.	"	1.032	1.032
51	" "	5	"	10 cc. phosphoric acid, 5%	"	"	1.038	...
52	Uric acid, pure	0.010	"	Distilled with 500 cc. water and 3 cc. sodium hydroxide, 10%	....	..	0.0	0.0
53	" "	"	Folin	.....	....	..	0.0	0.0
54	" "	"	Autoclave	5 cc. water plus 5 cc. HCl 1:4	145°	90	16.92	...
55	" "	"	"	10 cc. water plus 5 cc. phosphoric acid, 5%	"	"	7.00	...
56	" "	"	"	10 cc. water plus 10 cc. phosphoric acid, 5%	"	"	7.00	...

No.	Solution.	cc. taken.	Method of heating.	Reagent	Temp.	Time. Min.	Per cent. N.	Per cent. H.
57	Uric acid, pure	0.010	Autoclave	10 cc. water plus 5 cc. phosphoric acid, 5%, plus 5 cc. $MgCl_2$	145°	90	7.00	7.00
58	" "	"	"	5 cc. water plus 5 cc. citric acid, 10%	"	"	0.0	...
59	" "	"	"	5 cc. water plus 10 cc. citric acid, 10 %	"	"	0.0	...
60	" "	"	"	5 cc. water plus 10 cc. citric acid, 20%	"	"	0.0	...
61	" "	"	"	5 cc. water plus 10 cc. trichloracetic acid, 10%	145°	"	5.64	...
62	" "	"	"	5 cc. water plus 10 cc. HCl 1:4	4 atm.	"	14.10	14.10
63	" "	"	"	5 cc. water plus 10 cc. phosphoric acid, 5%	4 atm.	"	7.00	7.00
64	" "	"	"	5 cc. water plus 10 cc. HCl 1:4	3 atm.	"	20.44	20.44
65	" "	"	"	5 cc. water plus 10 cc. phosphoric acid, 5%	3 atm.	"	14.10	14.10
66	Uric acid, comm.	"	"	10 cc. water plus 10 cc. HCl 1:4	145°	"	26.80	...
67	Creatinine, 95%	"	Folin	.....	....	..	0.0	0.0
68	" "	"	Folin	Distilled with 500 cc. water and 3 cc. sodium hydroxide, 10%	....	..	0.0	0.0
69	" "	"	Autoclave	10 cc. water plus 5 cc. HCl 1:4	145°	90	8.46	8.46
70	" "	"	"	10 cc. water plus 5 cc. phosphoric acid, 10%	"	"	4.23	....
71	" "	"	"	10 cc. water plus 10 cc. phosphoric acid, 10%	"	"	7.05	....
72	" "	"	"	10 cc. water plus 10 cc. citric acid, 10%	"	"	2.12	2.12
73	" "	"	"	10 cc. water plus 10 cc. citric acid, 20%	"	"	7.05	....
74	" "	"	"	10 cc. water plus 10 cc. trichloracetic acid, 10%	"	"	14.10	....
75	" "	"	"	5 cc. water plus 10 cc. HCl 1:4	4 atm.	"	8.50	....
76	" "	"	"	5 cc. water plus 10 cc. phosphoric acid, 5 %	4 atm.	"	8.50	8.50
77	" "	"	"	5 cc. water plus 10 cc. HCl 1:4	3 atm.	"	9.16	9.16
78	" "	"	"	5 cc. water plus 10 cc. phosphoric acid, 5%	3 atm.	"	11.28	11.28
79	" 75%	"	"	10 cc. water plus 10 cc. HCl 1:4	145°	"	9.17	9.17

It is now necessary to inquire why Folin's method gives results with urine, which, so far as present knowledge goes, fulfil the conditions for the accurate analysis of urea in urine; while others, such as that of Benedict and Gephart, in which the composition of the hydrolyzing agent is apparently much more constant, and the temperature is kept accurately uniform, produce a decomposition of substances other than urea, while Folin's method attacks that compound alone.

In the mixture employed by Folin at 150°, one has a system containing a saturated solution of magnesium chloride. In this solution, both by reason of the temperature, and of the great concentration of the salt, one has the solubility of hydrochloric acid decreased to a minimum. Furthermore, and probably the most important factor in Folin's method is that at the same time any hydrochloric acid present in the mixture is also dissociated to its minimum extent. The system, however, is such that once the hydrogen ions are removed by the neutralization of the acid a sufficient amount of acid is present, either in the solution, or in the atmosphere of the reaction flask or in the condenser to still provide hydrogen ions for further neutralization by the ammonia formed. One has therefore a hydrolyzing agent which while actively little acid, with the shifting of the equilibrium due to the entrance into the system of alkali, is able to furnish more than sufficient acid for any neutralization and hydrolysis that may be needed. The magnesium chloride has also the very convenient property of keeping the solution at a temperature very favorable to the speedy hydrolysis of the urea.

Summary.

Benedict and Gephart's method cannot be used for the accurate determination of urea in the urine. Urea is quantitatively decomposed, but at the same time uric acid and creatinine yield ammonia by this method. To the decomposition of these substances is due the high results which these authors have obtained when their method has been compared with that of Folin.

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NEW BOOKS.

Quantitative Experiments in General Chemistry. By John Tappan Stoddard. New York: Longmans, Green and Co. 1908. pp. vii + 115. Price, $1.00.

The nature of this book is sufficiently indicated by its title. It contains almost all the quantitative experiments that have previously been found workable in the hands of beginners, and a great many additional ones. Where the experiments are old, the method is often more or less changed. A very wide choice of experiments illustrating the same point is usually given. Thus, under composition of gases, we have experiments with