process, throws doubt upon the accuracy of results heretofore announced with reference to the iodine content of protein substances.

The modifications of Baumann's process suggested in this paper still leave much to be desired. It is therefore my intention to continue the study of the problem, especially the fusion and reduction features.

Summary.

Ten-cubic-centimeter Nessler tubes of clear *white* glass, and giving a column of liquid 10 cm. in length, yield more delicate readings with dilute solutions of iodine in carbon tetrachloride than larger-sized tubes or a Duboscq colorimeter.

A portion of the iodine is oxidized to iodate during the fusion and may be lost unless subsequently reduced. Devarda's alloy was used as the reducing agent. The reduction is particularly necessary in the analysis of proteins containing but a small proportion of iodine.

Excess of nitrous acid fails to reduce iodates so that the iodine can be estimated colorimetrically in carbon tetrachloride solution, and a sufficient excess of nitrous acid will modify or discharge the color of a carbon tetrachloride solution of iodine. Too great a quantity of sodium nitrate must not be added during the fusion, or an excess of nitrous acid will be formed upon acidifying.

Mixtures of protein substances and potassium iodide subjected to analysis by the foregoing process do not give results comparable with those obtained from the analysis of a protein substance containing *combined* iodine, such as thyroid gland tissue.

Extreme care must be used to make the conditions under which standards are prepared and read parallel to those to which the substance under examination is subjected. Reductions and colorimetric readings should be made in duplicate or triplicate, and repeated, if necessary, until concordant results are obtained.

The writer wishes to express his thanks to Dr. S. P. Beebe for much of the material upon which this investigation was made, and for his hearty encouragement throughout the work.

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THE DETERMINATION OF UREA IN URINES.

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Recent work on protein metabolism has established the fact that the ratio of urea nitrogen to total nitrogen in urine is a variable, depending on many physiological and pathological conditions and, furthermore,

that given conditions exert a constant influence on the ratio. Thus Folin[1] has demonstrated that an increased protein intake caused a rise in the ratio, while in fasting the ratio fell to its lowest value. Levene and Kober[2] have demonstrated that in dogs all the nitrogen intake above the starvation requirement is removed in the form of urea. It has, therefore, become important to be able to estimate the urea output with absolute accuracy. To this end several methods have been recommended in recent years.

Of all recent methods the one of Folin[3] has proven the most satisfactory. But even this method is not free of inconveniences and of certain sources of error. This was very correctly pointed out by C. G. L. Wolf and E. Osterberg[4] and also by Ronchèse.[5] However, we wish to make clear that with care, patience and experience it is possible to obtain by Folin's method uniform and satisfactory urea values.

In order to obviate the inconveniences of Folin's process, Benedict and Gephart[6] have proposed a new method for urea estimation. This process was based on the hydrolysis of urea by hydrochloric acid at the temperature of 150°. This process has actually removed the objectionable features of the Folin method, but, on the other hand, it has accentuated the possible sources of error. It was proven by Wolf and Osterberg and also found in our laboratory that under the conditions indicated by Benedict and Gephart, not only urea, but also uric acid and creatinine suffer hydrolysis, though in minor degree than urea. It was, however, found in our laboratory that results obtained by the method of Benedict and Gephart were satisfactory for urines with a minimal content of uric acid and creatinine, such as dog's urine. On urines of normal man and of dogs in pathological conditions, the method could not be applied without further modification.

Appreciating the great convenience of the method of Benedict and Gephart and at the same time realizing that the principal source of error lay in the uric acid and creatinine, it was natural to attempt a modification of the method consisting in removing from the urine the uric acid and creatinine. We proposed to accomplish this by removing the basic substances of the urine by means of phosphotungstic acid and estimating the urea in the filtrate. This process offers also the advantage that the ammonia value does not enter into the urea value and that therefore the figures obtained for the urea do not depend on possible sources of error in the ammonia determinations.

[1] *Am. J. Physiol.*, 13, 66 (1905).
[2] *Ibid.*, 23, 324 (1909).
[3] *Z. physiol. Chem.*, 36, 333 (1902).
[4] THIS JOURNAL, 31, 421 (1909).
[5] *Bull. soc. chim.* [4], 3, 1138 (1908).
[6] THIS JOURNAL, 30, 1760 (1908)

Doubts have been expressed as to the possibility of removing the ammonia completely from the urine by means of phosphotungstic acid.[1] It can be seen from experiments recorded in this paper that this criticism lacks experimental foundation.

In order to test the process the following series of experiments were performed:

1. The estimation of urea in pure urea solutions (Table I).

2. In solutions of urea, uric acid and creatinine in proportion analogous to their occurrence in urine (Table I).

3. In urines to which varying quantities of uric acid were added (Tables II and III).

4. In urines to which varying quantities of ammonia were added (Table IV).

5. A comparative estimation of urea was accomplished by means of the methods of Folin, Benedict and Gephart and by the modification of the latter process (Table V).

TABLE I.—ANALYSIS OF SOLUTIONS OF PURE UREA IN WATER ALONE AND WITH THE ADDITION OF URIC ACID AND CREATININE.

Solution.	Method.	Per cent. nitrogen.
A. Uric acid and creatinine.....	Kjeldahl	0.0392
	B. and G.	0.0070
	P. T.	0.0028
	Kjeldahl on filtrate	0.0056
B. Urea.....	Kjeldahl	0.9120
	B. and G.	0.9156
	P. T.	0.9150
	Kjeldahl on filtrate	0.9160
C. Urea, uric acid and creatinine...	Kjeldahl	0.9580
	B. and G.	0.9240
	P. T.	0.9160
	Kjeldahl on filtrate	0.9160

TABLE II.—ANALYSIS OF URINE TO WHICH VARYING AMOUNTS OF URIC ACID AND UREA WERE ADDED.

Solution.	Method.	Per cent. nitrogen.	Calculated value.
A. Uric acid.....	Kjeldahl	0.031	...
B. Urea.....	Kjeldahl	1.795	...
1. Urine, 50 cc	Kjeldahl	0.475	...
Water, 50 cc.....	P. T.	0.384	...
2. Urine, 25 cc.....	Kjeldahl	0.492	...
A, 25 cc.....	P. T.	0.385	0.383
3. Urine, 25 cc.....	Kjeldahl	0.480	...
A, 12.5 cc.; water, 12.5 cc.	P. T.	0.384	0.383
4. Urine, 25 cc.....	Kjeldahl	1.374	...
B, 25 cc.....	P. T.	1.288	1.283
5. Urine, 25 cc.....	Kjeldahl	0.926	...
B, 12.5 cc.; water, 12.5 cc.	P. T.	0.832	0.835

[1] *Pflüger's Archiv.*, **43**, 31 (1888); *Ibid.*, **44**, 77 and 97 (1889).

TABLE III.—ANALYSIS OF URINE TO WHICH VARYING AMOUNTS OF URIC ACID AND UREA WERE ADDED.

	Solution.	Method.	Per cent. nitrogen.	Calculated value.
A.	Uric acid	Kjeldahl	0.051	...
B.	Urea	Kjeldahl	1.856	...
1.	Urine, 50 cc.	Kjeldahl	0.440	...
	Water, 50 cc.	P. T.	0.368	...
2.	Urine, 50 cc.	Kjeldahl	0.448	...
	A, 12.5 cc.; water, 37.5 cc.	P. T.	0.368	0.368
3.	Urine, 50 cc.	Kjeldahl	0.454	...
	A, 25 cc.; water, 25 cc.	P. T.	0.368	0.368
4.	Urine, 50 cc.	Kjeldahl	0.461	...
	Water, 50 cc.	P. T.	0.368	0.368
5.	Urine, 50 cc.	Kjeldahl	0.810	...
	B, 12.5 cc.; water, 37.5 cc.	P. T.	0.726	0.738
6.	Urine, 50 cc.	Kjeldahl	1.011	...
	B, 25 cc.; water, 25 cc.	P. T.	0.901	0.939
7.	Urine, 50 cc.	Kjeldahl	1.358	...
	B, 50 cc.	P. T.	1.246	1.286

TABLE IV.—ANALYSIS OF URINES TO WHICH VARYING AMOUNTS OF AMMONIUM CHLORIDE WERE ADDED.

	Solution.	Method.	Per cent. nitrogen.
A.	Ammonium chloride	Kjeldahl	0.084
1.	Urine, 50 cc.	Kjeldahl	0.440
	Water, 50 cc.	Ammonia N, Folin	0.018
		P. T.	0.368
2.	Urine, 50 cc.	Kjeldahl	0.484
	A, 50 cc.	Ammonia N	0.056
		P. T.	0.368
3.	Urine, 50 cc.	Kjeldahl	0.473
	A, 25 cc.	Ammonia N	0.037
	Water, 25 cc.	P. T.	0.368
4.	Urine, 50 cc.	Kjeldahl	0.451
	A, 12.5 cc.	Ammonia N	0.028
	Water, 37.5 cc.	P. T.	0.368

Before recording the results we wish to make clear that we do not claim originality for any one step in the proposed method but desire to call the attention of workers to it, for the reason of its convenience and accuracy.

Experimental.—The method finally adopted was as follows: 12.5 cc. of urine were pipetted into a 50 cc. graduated flask and 10 per cent. solution of phosphotungstic acid in 10 per cent. sulphuric acid was added slightly in excess of that required to completely precipitate the basic substances. This amount was best determined on a separate sample of urine as recommended by Pflüger and Bohland.[1] Too large an excess

[1] *Pflüger's Archiv.*, 38, 622 (1886).

of phosphotungstic acid should be avoided as is evident from the results recorded in Table IV. After allowing the mixture to stand for 24 hours, 10 per cent. sulphuric acid were added to complete the volume of 50 cc. and the content filtered through a dry filter paper into a dry flask. Two portions of 20 cc. each were measured into Jena glass test tubes and subjected to heating in an autoclave as described by Benedict and Gephart. The contents of each test tube are equivalent to 5 cc. of the urine employed.

TABLE V.—UREA DETERMINATIONS ON THE SAME SAMPLE OF HUMAN URINE BY THE METHODS OF FOLIN, BENEDICT AND GEPHART AND P. T.

Method.	Per cent. nitrogen.	Ratio of total N. to Urea N.
Total N, Kjeldahl	0.859	....
Urea, Folin	0.726	
	0.725	
	0.725	84.5
Urea, B. and G.	0.722	
	0.723	84.2
Urea, P. T.	0.682	
	0.681	
	0.681	
	0.679	
	0.681	
	0.681	79.5

It was invariably noticed that a granular precipitate was present in the test tubes after heating, probably due to the formation of an insoluble ammonium salt of phosphotungstic acid. The contents of the test tube were washed as completely as possible into a distilling flask. The granular precipitate which adhered persistently to the glass was dissolved in a very small quantity (5 to 10 cc.) of 10 per cent. sodium hydroxide and together with the further rinsings immediately added to the main bulk, still acid in the flask. An excess of alkali was now added (40 cc. of 10 per cent. sodium hydroxide) and the ammonia distilled into decinormal acid.

The above method was used in all the experiments about to be described and designated in the tables by the letters P. T.

In Table I are summarized the results of the experiments on the estimation of urea in solutions of pure urea in water and the influence of added uric acid and creatinine on such determinations. Solutions of uric acid and creatinine (uric acid 0.184 gram, creatinine 0.200 gram, water 100 cc.) and of urea (urea 4 grams, water 100 cc.) were made of approximately twice the strength as occur in the urine. From these solutions the following mixtures were prepared for analysis:

A. 50 cc. uric acid and creatinine solution + 50 cc. water.

B. 50 cc. urea solution + 50 cc. water.

C. 50 cc. uric acid and creatinine solution + 50 cc. urea solution.

Analysis of total nitrogen according to Kjeldahl-Gunning, and for urea nitrogen by the Benedict and Gephart and the P. T. methods were carried out in each mixture. Besides these the total nitrogen in the filtrate from the phosphotungstic acid precipitation was determined. The Benedict and Gephart method was modified insofar as 10 per cent. sulphuric acid was substituted for hydrochloric acid in the hydrolysis.

The results obtained indicate that the accuracy of the urea estimation is not impaired by the addition of phosphotungstic acid. Uric acid and creatinine are practically completely precipitated by phosphotungstic acid, and that which remains in solution is not sufficient to appreciably affect the urea values.

In Tables II and III are recorded figures on a mixed sample of human urines to which varying amounts of uric acid and urea had been added. To make the results comparative the urine was diluted one-half with water previous to its analysis and the mixtures with urea and uric acid diluted in the same proportion. It is evident that if the addition of uric acid does not affect the method then the values of urea nitrogen for the mixtures should coincide with the figure obtained on the urine diluted one-half with water. The calculated values in the last column are such as would be expected, considering that the increase of total nitrogen is due entirely to added urea when such was added.

The influence of added ammonium chloride is shown in the figures of Table IV. The same urine was used for this experiment as in the previous series reported in Table III. It is evident from the figures that there is no reason to believe that under these conditions ammonia is not completely precipitated by phosphotungstic acid.

Finally the comparative results of urea estimation on one sample of mixed human urine as obtained by the Folin, the Benedict and Gephart and by the P. T. methods are compiled in Table V. We desire to emphasize the fact that the figures of urea by the P. T. method are duplicates of six distinct analyses of the same urine and not six duplicates of one filtrate.

[CONTRIBUTIONS FROM THE HAVEMEYER LABORATORIES OF COLUMBIA UNIVERSITY, No. 166.]

THE CONDENSATION OF ACETONE BY MEANS OF CALCIUM OXIDE.

BY ALFRED HOFFMAN.[1]

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The formation of mesityl oxide from two, and of isophorone and the xylitones from three and four molecules of acetone under the influence of calcium oxide has been studied and the constitution of the products

[1] Read at the General Meeting of the Am. Chem. Soc., Baltimore, Dec. 1908.