

210° for six hours. There was considerable pressure after the first period but none after the second. The contents were almost wholly crystalline. The quinazolone was extracted and purified by the same method as followed above, and proved to be the isoamyl derivative. Its melting-point was 183°-184°.

Action of Fatty Acids and Anhydrides on Anthranilic Acid.

These experiments were performed for the purpose already stated on page 614. Anthranilic acid was heated with normal butyric and isocaproic acids respectively, both in sealed tubes and open, at temperatures varying from but slightly above 100° to the boiling-point of the fatty acid, without any action being discernible. In contradistinction to this it was found that both normal butyric and isovaleric anhydrides reacted quite smoothly to form the corresponding acylanthranilic acids. Merely heating the acid and anhydride together on the water-bath for three to four hours was sufficient to give a yield of about 30 per cent.

Only the isovaleryl anthranilic acid was closely examined. It crystallizes from water in needles melting at 110°-111°.

I. 0.1816 gram gave 10.6 cc. nitrogen (26°, 755 mm.).

II. 0.2016 gram gave 11.7 cc. nitrogen (23°, 754 mm.).

	Calculated for $C_{12}H_{15}NO_3$	Found.	
		I.	II.
Nitrogen.....	6.33	6.43	6.49

This work is being continued and extended, and other papers will appear shortly.

ORGANIC LABORATORY, HAVEMEYER HALL,
COLUMBIA UNIVERSITY, June 1, 1901.

ON THE ESTIMATION OF UREA IN URINE.

By J. H. LONG.

Received July 23, 1901.

INASMUCH as about 90 per cent. of the total assimilated nitrogen leaving the human body is excreted in the form of urea the accurate determination of this substance in the urine must remain among the most important of physiological-chemical problems. The interest attaching to the question is fully shown by the great number of contributions to the subject appearing in the chemical literature, yet, notwithstanding all that has been written, the problem is still far from satisfactory solution. In the older text-books of urine analysis the Liebig method by use of mercuric

nitrate held long the first place, which was later taken by that of Knop and Hüfner, depending on the reaction between a hypochlorite or hypobromite and urea.

The original Liebig method was found to give results which were too high because of the precipitation of other substances than urea by the mercuric nitrate, and as the extent of the possible error here became more and more apparent, the method fell into disfavor and was finally practically discarded. In the later editions of the well-known manual of urine analysis by Neubauer and Vogel, revised by Huppert, the Liebig process is no longer described among the quantitative methods.

The fate of the hypobromite processes, as scientific methods, is almost as interesting. In these, plus and minus errors were long supposed to nearly balance each other. A part of the urea fails to decompose according to the simple reaction,



while, on the other hand, many other nitrogenous bodies in the urine give up a large but variable part of their nitrogen in the free form under the influence of the same reagent. The modifications of this general method are almost beyond number, but in the final results there is probably even less constancy than in the older mercury method. In the editions of the Neubauer and Vogel work referred to above the Knop-Hüfner method is described only among the qualitative reactions.

From the standpoint of those interested in the practical determination of urea this omission must be looked upon as a mistake since the supposedly more accurate processes which are now described are not at all suited for quick comparative determinations in cases where many tests have to be made. It is possible that the mercury method, under special circumstances, may still render service here, and it is the object of this communication to point out how certain corrections may be applied, where comparatively full analyses are being made, with fresh urine.

The main sources of error in titrations of urea in urine by the standard solution of mercuric nitrate are these :

a. The chloride present retards the reaction, but as this effect has long been accurately known and easily corrected it will not be discussed in this place.

b. Ammonia which is always normally present, even in fresh

urine, combines likewise with the mercury solution, and prevents precipitation until a certain excess of the latter is added.

c. Uric acid, which amounts to 800 mg., or more sometimes, in the day's urine combines with the reagent to form a definite compound. The effect of this is, however, relatively small.

d. Creatinin, after urea the most abundant of the nitrogenous urinary products, forms several crystalline combinations with mercuric nitrate. The composition of these is variable with the concentration of the reacting solutions, but in any event the error in the titration of the urea in presence of this body is quite appreciable.

e. In addition to the above, several other nitrogenous compounds, present in small amount in the urine, are known to combine with mercuric nitrate, but their effect is extremely small and may usually be neglected.

I have undertaken to estimate the effect of the main disturbing factors referred to and present below some experimental data.

AMMONIA.

In fresh normal urine the amount of ammonia present is about 600 mg. for each 1000 cc. of excretion. In urine which is allowed to stand, the amount rapidly increases from decomposition of the urea. To determine the influence of this small weight of ammonia in urea titrations, tests were carried out on solutions containing ammonia alone, in the form of nitrate, and on solutions containing ammonia and urea. The ammonia was used in the form of nitrate because in the usual method of preparing urine for titration it would be left in that combination.

Experiment 1.—A solution was made which contained in 500 cc. 2.5 grams of pure ammonium nitrate. This corresponds to 1.06 gram of NH_3 to the liter; 20 cc. of this solution were measured into a beaker and standard mercuric nitrate solution added. No precipitate appeared but at the same time no reaction immediately followed on bringing a drop of the liquid in the beaker in contact with a paste of sodium bicarbonate and water. It was found that about 2.5 cc. of the usual standard mercuric nitrate solution must be used before a reaction appeared, indicating the presence of an excess of the mercury. Neutralization of the liquid in the beaker with sodium carbonate made no appreciable difference in the result.

Experiment 2.—Tests were made with a weaker ammonia solution containing 0.6 gram to the liter. The results were nearly the same as before, the volume of the mercury solution required being, however, relatively a little greater. As the end reaction is by no means as sharp as with pure urea solutions it is easy to pass the right end-point in titration. Many single titrations were made and as a mean of the closely agreeing results it may be said that with the solutions used, 20 mg. $\text{NH}_3 = 2.5$ cc. of the mercury solution.

This corresponds to 17 NH_3 to 2.12 cc. mercury solution, 17 NH_3 to 164 mg. HgO , 1 mol. NH_3 to 0.75 mol. HgO , or 4 NH_3 to 3 HgO , approximately.

Experiment 3.—Here solutions containing both urea and ammonia were titrated. The urea solution used contained exactly 2 grams in 100 cc. In a series of experiments it was found that mixtures containing 10 cc. of this urea solution, and 10 cc. of the first described ammonia solution required 21.6 cc. of the mercury solution instead of 20 cc. This makes the effect of the ammonia a little greater than in the above experiments, 1 and 2. But as intimated, the reaction is not as clear as in absence of ammonia, and a trifling excess must always be added from the mercuric nitrate burette. The end-point seems to vary also slightly with the temperature.

As a mean result the presence of 10 mg. of ammonia in the 20 cc. of mixed liquid titrated increases the amount of mercury solution used by about 1.6 cc. This corresponds closely to 1 cc. of the mercury solution for 6 mg. of ammonia, and this is the average amount present in 10 cc. of urine, the volume usually taken for titration; this is much lower than is a result given by Feder¹ according to whom 10 mg. of ammonia require 2.6 cc. of the mercury solution. This result is probably too high, and was not approached in any one of the numerous single titrations I made.

URIC ACID.

The amount of uric acid in the urine was formerly much underestimated, but since the introduction of better methods of determination higher, and probably pretty accurate, results may be easily obtained. In a large number of urines studied in this laboratory, the uric acid has been found to amount to about 650 mg.

¹ See Neubauer and Vogel: "Urine Analysis," 8th German Edition, p. 272.

for 1000 cc. of urine. In 10 cc. of urine we have therefore about 6.5 mg. of uric acid normally, and it is the interference of this that we have to consider in the urea titration. For the experiments I made up a test solution containing 250 mg. of pure uric acid dissolved by the aid of a little alkali to make 250 cc. The behavior of this with the mercury solution is regular and the same slight increase is found whether we use the simple uric acid solution or one containing uric acid and urea.

For a solution containing in 20 cc. 200 mg. of urea, and 5 mg. of uric acid the amount of mercury solution required was just 0.1 cc. more than for the urea solution alone, and by increasing the uric acid the increase in the required mercuric nitrate was found to be in the same proportion. The correction for the presence of uric acid is therefore very small, and in any case would not exceed 0.15 cc. of the mercury solution for the volume of 10 cc. of urine ordinarily taken for the titration.

CREATININ.

This is present in urine to the extent of 1 gram to 1.6 grams or more in the volume of 1000 cc. In a large number of tests I found an average of 1.4 grams by the method of precipitation with zinc chloride. On this basis 10 cc. of urine would contain 14 mg. of creatinin.

To study the behavior of this body I first prepared it in pure condition from urine by decomposing the zinc chloride precipitate with lead hydroxide. Later much larger quantities were made by separating first pure creatin from extract of beef and then decomposing the product obtained by long heating of this with strong hydrochloric or 25 per cent. sulphuric acid, by means of lead hydroxide or barium carbonate.

As was long ago pointed out by Neubauer¹ mercuric nitrate added to a creatinin solution produces no immediate precipitate; a combination is formed in which acid is liberated and this interferes with precipitation. On neutralizing the liquid with sodium carbonate a point is reached, however, when a precipitate soon forms and settles out. Under certain conditions a precipitate with the composition $(C_4H_7N_3O)_2Hg(NO_3)_2HgO$ may be obtained in this way. But with variations in the concentration of the reacting bodies the composition of the precipitate varies, as I have found by experiment. In the titration of weak creatinin solutions

¹ See Neubauer and Vogel: "Urine Analysis."

similar results are found ; the solution must be neutralized with sodium carbonate to secure precipitation. In tests made I employed a creatinin solution containing 1 gram in 100 cc.

Experiment 1.—Of this solution I took 10 cc. and added nearly 5 cc. of the mercuric nitrate solution before any appreciable reaction appeared, on testing as usual with sodium bicarbonate. The solution in the beaker was still quite clear ; a little methyl orange was added and then 3 cc. of normal sodium carbonate solution to change of color. This is a rough measure of the liberated acid.

Experiment 2.—In a second experiment with 20 cc. of the creatinin solution, 9 cc. of the mercuric nitrate were added before securing a trace of a reaction in the sodium bicarbonate. With 10 cc. of the mercury solution the reaction was sharp and 1 cc. of normal sodium carbonate was added to produce haziness in the liquid in the beaker. It appears, therefore, that about 9.5 cc. of the standard mercuric nitrate solution may be taken as equivalent to 20 cc. of the creatinin solution. For this concentration this corresponds very closely to a relation of 1 molecule of creatinin to 2 molecules of mercuric oxide. The experiments were many times repeated.

Experiment 3.—Numerous tests were made with weaker creatinin solutions containing amounts corresponding with those in urine. Here the volume of mercury solution used was always relatively a little greater, suggesting a combination of 1 molecule of creatinin with $2\frac{1}{2}$ molecules of mercuric oxide, but the difference is doubtless due mainly to the slight excess required to give a reaction in dilute solution.

Experiment 4.—In titrating mixtures of urea and creatinin the same general difference was found as last shown, the reaction with urea being apparently slightly retarded by the presence of the creatinin. Tests of mixtures were made in large number so that the result may be looked upon as well established.

The final value of all these reactions may then be expressed as follows, the corrections being those necessary for average normal urine :

6 mg. NH_3	in 20 cc. (10 cc. urine, 10 cc. water)	require 1.0 cc. Hg sol.
7.5 " $\text{C}_5\text{H}_4\text{N}_4\text{O}_3$	" 20 " 10 " " 10 " " "	0.15 " " "
14.0 " $\text{C}_4\text{H}_7\text{N}_3\text{O}$	" 20 " 10 " " 10 " " "	0.85 " " "
		2.00

The average correction to be made then on the Liebig titration is 2 cc. of the mercuric solution, but as readily seen this can be applied to fresh urine only since in old urine the effect of the ammonia of decomposition becomes too great to be easily corrected. In the clinical examination of fresh urine the correction may be applied with only a small margin of error, while in fuller analyses, where the disturbing factors are also estimated, it may be used with considerable accuracy. My thanks are due to Mr. Grulee, who made many of the experiments above.

NORTHWESTERN UNIVERSITY, CHICAGO.
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ON THE DETERMINATION OF FORMALDEHYDE.

BY A. G. CRAIG.

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THE methods for the determination of formaldehyde may be classed, by their reactions, in three groups, as follows :

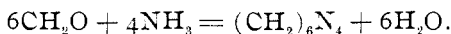
Group 1.—Depending on a specific reaction.

Group 2.—Formation of addition products with elimination of the elements of water.

Group 3.—Oxidation and reduction.

Group 1 contains :

(a) The ammonia method.¹



(b) The potassium cyanide method.²

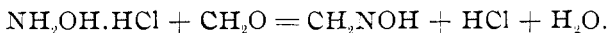


(c) The fixed alkali method.³

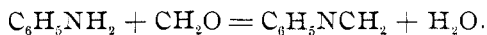


Group 2 contains :

(a) The hydroxylamine method.⁴



(b) The aniline method.⁵



The precipitate is weighed.

¹ L. Legler: *Ber. d. chem. Ges.*, 16, 1333.

² G. Romijn: *Ztschr. anal. Chem.*, 36, 15.

³ Legler: *Ber. d. chem. Ges.*, 16, 1333.

⁴ Brochet and Cambier: *Compt. rend.*, 120, 449.

⁵ Trillat: *Bull. Soc. Chim.*, [3], 9, 395.