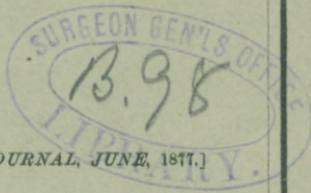


↓ by FOWLER (Geo. B.)

ON A
NEW AND SIMPLE METHOD
FOR THE
QUANTITATIVE ESTIMATION OF UREA.

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GEORGE B. FOWLER, M. D.,
INSTRUCTOR IN PHYSIOLOGY, COLLEGE OF PHYSICIANS AND SURGEONS, ETC.,
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ON A NEW AND SIMPLE METHOD FOR THE QUANTITATIVE ESTIMATION OF UREA.¹

UREA, being one of the most interesting and important constituents of the animal body, has occupied the attention of chemists and physiologists since its discovery in 1773, by Rouelle, Jr. Its properties becoming known, and its significance as a decomposition product appreciated, a certain stimulus has ever since attached to its study; and, as a result, various methods for its estimation have been devised, based, of course, upon its properties and chemical relations.

Other characters of urea than those directly connected with the most reliable quantitative methods, do not at present concern us; and a hasty survey of these, with a special view to judge of their simplicity, will not be out of place.

Mitscherlich² and Lacanu³ availed themselves of the striking reaction manifested by urea when treated with nitric acid, and estimated the quantity from the very stable and copious precipitate of *urea nitrate*. But with this method considerable time and chemical manipulation are necessary in order to insure accuracy.

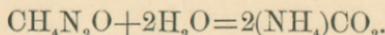
¹ Prize essay of the Alumni Association, College of Physicians and Surgeons, New York, 1877.

² Poggendorff's "Annalen," Bd. xxxi., S. 303.

³ *Jour. de Pharm.*, tome xvii., p. 651.

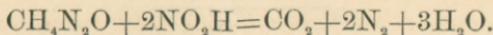
When urea is treated with strong *sulphuric acid*, and heated, it is decomposed into ammonia and carbonic acid. Ragsky¹ proposed to estimate the amount of urea originally present by the ammonia thus formed. But, in order to do so, a complicated and delicate process must be gone through with.

Urea, heated with water in sealed tubes to about 100° C., is resolved into ammonium carbonate by the appropriation of two equivalents of water, thus :



This product treated with barium hydrate gives us barium carbonate, from which a quantitative estimation of the urea can be made. This is the basis of Bunsen's² method.

Nitrous acid decomposes urea into water, carbonic acid, and nitrogen, as follows :



This reaction constitutes the basis of Millon's method, whereby he estimates the quantity of urea in a given solution by the weight of the gases given off. A modification of this process has been devised by Boymond,³ in which he employs a rather complicated flask, provided with separate divisions for each of the substances used in the analysis, viz., one for the urine, another for the reagent, and a third for the sulphuric acid, through which the gases are made to pass to rid them of moisture. Each apartment being supplied with the three fluids, the urine and nitrous acid are allowed to come in contact, and decomposition to continue until gas ceases to be evolved. Then the difference between the weight of the flask and its contents, before and after the reaction, will give the weight of nitrogen and carbonic acid lost; and having found that the relation of the gases to urea, by weight, is as 12 to 10, the subsequent calculation is very simple.

Dr. H. G. Piffard⁴ has still further modified this method, with a view to avoid the expensive apparatus of Boymond, and with exceedingly accurate results.

¹ "Ann. der Chem. und Pharm.," Bd. lvi., S. 29.

² Ibid., Bd. lxx., S. 29. ³ "De l'Urée," Paris, 1872.

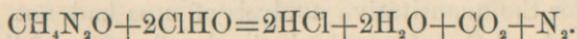
⁴ NEW YORK MEDICAL JOURNAL, December, 1874.

It will be seen that the foregoing methods involve the skillful use of delicate and expensive scales, and most of them necessitate a degree of technical skill and a supply of apparatus not generally possessed by physicians.

Liebig having discovered that *mercuric nitrate* added to a solution of urea would precipitate a white compound composed of one equivalent of urea with four of mercuric oxide, $U+4H_2O$, thereon based his well-known method for the volumetric analysis of urea, simply by the use of a graduated solution of the reagent.

This perhaps is the most reliable and popular procedure ever proposed, but, like all of those thus far mentioned, offers difficulties in chemical manipulation not readily overcome by a novice.

Urea is resolved into hydrochloric acid, water, carbonic acid, and nitrogen, by *hypochlorous acid*, formulated thus :



As far back as 1853 Wöhler determined the ammonia in guano by the hypochlorites; and in 1854 Dr. Davy¹ published his celebrated process for estimating urea by measuring the volume of nitrogen set free from it by solutions of the hypochlorites.

Davy's familiar process has been variously modified, first by Knop,² who, instead of using hypochlorous, employed *hypobromous acid*, which he found would effect the same result, his apparatus consisting of flasks with a complicated series of glass stopcocks.

Hüfner³ then proposed an improvement on Knop's method, consisting chiefly in the employment of moderate heat to hasten reaction. But by this procedure oxygen is evolved from the hypobromite solution, which has to be subsequently estimated.

Russell and West⁴ next came forward with a very much

¹ *Philosophical Magazine* (4), vii., p. 385.

² Fresenius's *Zeitschr. für analyt. Chem.*, ix., 2, S. 225.

³ *Journal of Chemical Society*, New Series, ix., p. 162.

⁴ *Practitioner*, February, 1875, p. 86, and *Journal of Chemical Society*, 1874, p. 749.

less complicated apparatus, adapted to the use of sodium hypobromite, and the collection and measuring of the nitrogen disengaged by it from urea. This method has deservedly become very popular.

Last of all, Blackley¹ has invented still another form of apparatus for the hypobromite method; but in my hands it has proved very inconvenient and difficult to manage.

As a general objection to the use of a standard solution of hypobromite, it may be said that it is very unstable, and therefore the preparation of a fresh supply becomes necessary at very short intervals. And, on account of the disgusting odor and injurious fumes of the bromine employed, this procedure itself is not one of the most agreeable.

Having experienced the difficulties in the way of technical details which most of these methods involve, it seemed to me that a simple and sufficiently accurate process for clinical purposes for the quantitative analysis of urea had not yet been published. I therefore submit the details of the following:

Every one who has been in the habit of making volumetric analyses of sugar in urine must have had his patience tried by the frequently sudden and unexpected behavior of the test, which necessitated a complete washing out and new beginning; and I know he must have been delighted when Dr. Roberts published the description of his simple plan, which consists in determining the amount of sugar in a solution, merely by *the difference in specific gravity before and after fermentation*. It occurred to me that *the difference in specific gravity before and after decomposition by the hypochlorites or hypobromites should bear a definite relation to the quantity of urea originally present in the solution*. I therefore instituted a series of experiments, of which the following is an account, and which proved my theory to be correct.

Now, between stable and fermenting *saccharine* solutions, there is obviously a wide margin as regards specific gravity; for, by the action of yeast, not only is there a loss of matter, CO₂, but a generation of a liquid lighter than water, alcohol. It was therefore a question at the outset whether we would have sufficient margin, with urine and the proposed reagents,

¹ *Journal of Chemical Society*, 1876, p. 466.

upon which to base a calculation, and whether small differences in the amount of urea would be indicated with sufficient accuracy. Theoretically it seemed that there should be a loss in gravity, after the mutual decomposition between urea and a hypochlorite or hypobromite, as shown in the preceding formula, where, besides the loss of nitrogen, there is a production of water. According to Dr. Davy, the carbonic acid does not escape from the solution, but is reabsorbed by the excess of caustic alkali of the reagent. My own observations support this statement, and therefore this product does not add to our margin.

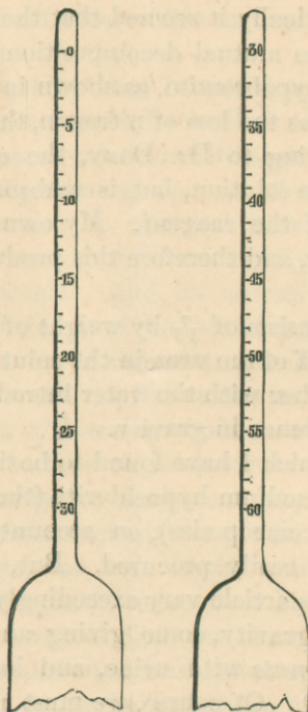
Again, urea consists of $\frac{7}{15}$ by weight of nitrogen. Therefore almost one-half of the urea in the solution will disappear; and this loss, together with the water introduced, should cause a perceptible difference in gravity.

The reagent which I have found to be the most convenient for this analysis is sodium hypochlorite (the *liquor sodæ chlorinatae* of the pharmacopœias), on account of its cleanliness, and because it is easily procured. But, unfortunately, the preparations of this article vary exceedingly as regards composition and specific gravity, some giving an insoluble precipitate of *lime carbonate* with urine, and having a gravity of from 1050 to 1100. Of course, we must not have a precipitate, and such a density would require an hydrometer of very long stem and large bulb, and would necessitate the use of a larger volume of fluid than would be desirable.

In the standard solution prepared by Dr. E. R. Squibb, of Brooklyn, however, these objections are overcome, as there is not the slightest opacity following its addition to urine; and he informs me that the density, when it is bottled, is about 1048. By exposure to air and light it is reduced.

The next important step is to procure a suitable hydrometer. The requirements are a stem which will indicate the gravity of the hypochlorite, and also that of the urine, that is, an index ranging from 1000 to 1050, and that each graduation should indicate *one degree*, and be about two millimetres (one-twelfth of an inch) apart, in order to allow of accuracy in the reading. To be able to work with smaller quantities of fluids, the instrument should be divided into two of equal

size, one reading from 1000 to 1025, the other from 1025 to 1050.¹



Dr. Davy found that about six parts of the hypochlorite were required to one of the urine, to decompose the urea ordinarily contained.

This accords with my experience, but, in order to insure complete decomposition, and to provide for an excess of urea, I employ seven parts of the reagent.

My preliminary experiments were performed with solutions of pure urea of known strength, and it was found that after decomposition *every degree of density lost indicates 7.791 milligrammes of urea per cubic centimetre of the solution (3.55 grains per ounce)*. Or every degree lost corresponds to .77 per cent.

I then took different specimens of urine and analyzed

¹ Such hydrometers will be made on application to John Tagliabue, 69 Fulton Street, New York.

them by this method, taking these figures as a basis of calculation, and compared the results with those obtained with the same specimens by Liebig's process.

Table showing comparative results obtained by Liebig's method and that described in this paper (calculated as milligrammes per cubic centimetre):

	LIEBIG'S.	NEW.
1	26.155	26.203
2	32.461	32.500
3	34.983	34.983
4	31.192	31.220

It will be seen that, with one exception, where there is an absolute agreement, the results by the new process are in excess of those by Liebig's by a few thousandths of a milligramme, which is probably due to the decomposition of nitrogenous ingredients other than urea, of which we will presently speak.

In order to use this method, proceed as follows: Procure two glass cylinder jars, one about nine inches in height by one and a half in diameter, the other about seven inches by one inch. Into the smaller pour a quantity of the urine, and ascertain its specific gravity. Take about fifteen cubic centimetres (one-half ounce) of it and pour it into the large jar. Now wash out the smaller jar thoroughly, and in it take the specific gravity of the hypochlorite solution. Then add to the urine in the large cylinder exactly seven times as much hypochlorite as there was urine taken.¹

When the two fluids mingle there will be a brisk efferves-

¹ It is not absolutely necessary to employ a special hydrometer except where great accuracy is desired. The instrument ordinarily possessed by physicians, if it is marked up to 1050, does very well for clinical work, a little care and patience enabling one to read the exact specific gravity after a few trials. With this short stem we have a small bulb, and gain the advantage of economy in the use of the fluids. The first thing to do is to procure a couple of jars (or large test-tubes with feet) whose calibre will freely accommodate the hydrometer, and then ascertain, by using water, what will be the smallest quantity of liquid which, in a particular jar, will float the hydrometer. One-eighth of this quantity will be the measure of urine to employ.

cence, and care must be taken that none of the mixture be lost. After several minutes the disengagement of gas is less rapid; and now, by vigorously shaking the jar at short intervals, I have found the process to be hastened, so that at the expiration of an hour the decomposition is complete. It then only remains to take the gravity of the quiescent solution, deduct from what it was before decomposition, and estimate the amount of urea on the basis already given; after this manner:

Suppose the specific gravity of the urine to be 1020, and that of the hypochlorite 1045. But it is impracticable to take the specific gravity of the mixture of these two on account of their immediate reaction; we therefore resort to the law of proportion, and estimate it as follows:

$$\begin{array}{r} 1 \text{ part at } 1020 = 1020 \\ 7 \text{ parts at } 1045 = 7315 \\ \hline 8 \qquad \qquad \qquad)8335 \end{array}$$

1041 $\frac{5}{8}$ sp. gr. of mixture.

From the gravity of the eight parts we subtract that which is obtained after decomposition, which, we will say, is 1038 $\frac{5}{8}$. There is then a loss of 3°. Every degree of loss equals 7.791 milligrammes per cubic centimetre of urine used. Therefore, the urine contained 23.373 milligrammes of urea per cubic centimetre, or 2.3 per cent.

It is, of course, highly important that the temperature of the fluids should all agree when their specific gravity is taken. This may be regulated by the use of a plain thermometer, or by the combination hydrometer and thermometer now so generally employed. But the simplest method is to mix the urine and reagent, and set the mixture aside with the pure urine and hypochlorite in the same locality, when the specific gravity of the three can be taken at a uniform temperature.

The presence of neither albumen nor sugar interferes with the accuracy of this method.

But it may be urged as an objection that other nitrogenous ingredients of the urine, such as uric acid and creatinine, suffer the same decomposition as urea, and therefore constitute elements of error. Yet, when we take into consideration the

facts regarding these substances, namely, that, of the nitrogen contained in uric acid, thirty-five per cent., in hippuric acid, eighty-two and a half per cent., and in creatinine twenty-five per cent., *is not disengaged* by solutions of the hypochlorites, and when we remember how insignificant are the quantities of these substances in the urine compared to urea—the excretion per day being respectively about 0.7, 0.35, and one gramme, a total of 2.05 grammes—it is obvious that their presence does not establish a serious fallacy. Ammonia, however, would interfere, and give an erroneous result.

When the urine contains a great excess of urea, more than would be decomposed by seven parts of hypochlorite, it is simply necessary to dilute it with an equal volume of water, use one part of the dilution to seven of the reagent, and multiply the result obtained by two.

It is evident that with this method we must be able to fix the point at which the hydrometer floats in the several liquids with nicety. When these liquids are perfectly transparent there is no difficulty, the proper way being to read from the under surface of the fluid, bringing the eye on a level with that surface. But, when the urine from any cause is opaque, this process is not practicable, and, under these circumstances, I resort to the following simple device :

Take a piece of soft pine, and shape it into a circular disk somewhat smaller than the calibre of the smallest jar to be used. Make it exceedingly thin and uniform, and perforate it in the centre with a round hole which will freely accommodate the stem of the hydrometer. Having put the stem of the instrument through this opening and immersed it in the fluid, the bit of wood will float on the surface, and the specific gravity can be readily taken by noting the point at which the float comes in contact with the stem. The weight of the wood will cause a slight increase in density, but, when used, in every case the error will be the same, and therefore corrects itself.

