PAPERS READ BEFORE THE CHEMICAL SOCIETY.

XIV.—*On the Estimation of Urea by means of Hypobromite.*

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SINCE Knop in 1870 proposed a strongly alkaline solution of hypobromite of sodium for the estimation of urea in urine, the process has been worked at and modified by several chemists. Up to the present, however, neither of the methods proposed has found its way into general use, notwithstanding the rapidity and sharpness of the reaction on which they are based. This is due, I believe, to two circumstances mainly—firstly, no apparatus as yet described seems to combine ease of working with accuracy in a sufficiently high degree; and, secondly, the somewhat rapid deterioration of the hypobromite solution renders its frequent preparation a matter of necessity, and this preparation, owing to the highly pungent nature of the vapour of bromine, has been an extremely disagreeable task anywhere but in a regular laboratory. I therefore offer no excuse for making one more attempt to render the process really available for medical purposes.

*The Apparatus.**—This will be readily understood from the accompanying illustration. A burette (*a*, Fig. 1) having a lateral supply tube, put upside down into a cylinder with water, constitutes the part for measuring the gas evolved. The burette can be raised or lowered and fixed at any height by means of the clamp and rod, *b b*. The reaction between the urine and hypobromite is effected in a small flask or bottle, fitted up as for the estimation of carbonic anhydride in carbonates. The two forms which I have used, and find equally convenient, are shown in *c*, Fig. 1 and in Fig. 2. To work the apparatus, the pinch-cock, *d*, is taken off, and the burette is let down to the zero point of the division. 25 c.c. of the hypobromite solution are next put into the bottle, and the pipette (*e*) is filled with the urine in the usual manner, detaching the india-rubber tube at *f* for this purpose. Before putting the stopper back into the flask, it is advisable slightly to suck up the

* After having given directions for the manufacture of this apparatus to Mr. G. Smith, of the firm of How and Co., he informed me that they had made a very similar apparatus, some years since, for Mr. R. Apjohn of Cambridge. On referring to the *Chemical News* of January 22, 1875, I find that Mr. Apjohn's apparatus is indeed very similar to mine, and has two years' priority. It is, however, not identical, and mine will, I think, be found more complete, and much more readily worked.

urine, so that the extreme point of the pipette becomes empty, and there is no danger of a drop of urine falling into the hypobromite prematurely. This is best done by compressing the india-rubber tube attached to the pipette between the finger and thumb of one hand, above the pinchcock of course, and drawing the tube so compressed through the fingers for an inch or so. If now, before relaxing the pressure on the tube, the pinchcock is opened for a moment, the urine will be sucked up to the desired extent. If previous to use the point has been slightly greased inside, the urine will show no tendency to run back, and the pipette can be moved about without fear. The stopper with the pipette is now firmly inserted into the bottle, the india-rubber tube is slipped back over *f*, and the bottle put into a vessel with cold water. The pinchcock, *d*, is fixed on, and the burette is raised several inches, when, if all is tight, the column of water raised in the burette will remain stationary. The urine is now allowed to run into the hypobromite as rapidly as may be convenient, and a thorough mixing is effected by a gentle rotary motion of the bottle. According to Hüfner, the reaction is the more complete the more rapidly the two fluids are mixed. In from one to two minutes the reaction is almost over. The bottle should now be shaken briskly several times. This shaking causes an appreciable amount of effervescence to take place, and is the means of bringing the reaction sharply to the same point each time. In five minutes from the beginning of the experiment, the bottle is put into the cold water, the burette is let down into the cylinder for a few moments, then raised until the water inside and out is on a level, and the amount of gas collected is read off. If the arrangement Fig. 2 is preferred, the 5 c.c. urine are put into the little test tube fixed to the glass rod, the stopper

FIG. 2.

FIG. 1.

Scale $\frac{1}{2}$.

is replaced, and the apparatus arranged and tested as before. By now inclining the bottle in one direction the little tube can be gradually emptied; by inclining it in another direction it can be filled with the hypobromite. It is thus easy alternately to empty or fill the little tube, and thus effectually to wash out all the urine. The rest is done as before.

As before stated, at ordinary temperature the reaction is virtually over in five minutes. If now the hypobromite is heated for ten minutes to a temperature of 70°C ., a further evolution of gas takes place, equivalent, when 5 c.c. of a 2 per cent. urea solution have been taken, to about 0.04 per cent. of urea. Since, however, the reaction comes to an end almost equally sharply at either temperature, and in neither case do we get the whole amount of nitrogen, the heating may be entirely omitted, provided that the graduation of the burette is made accordingly. At a temperature of 65°F ., and a pressure of 760 mm. I found that 5 c.c. of a 2 per cent. urea solution evolved. when the reaction was finished by heating the hypobromite to 70°C .

37.26 c.c.

37.08 „

37.08 „

Mean.. 37.14 „

or almost exactly the amount found by Messrs. Russell and West under like conditions. When the heating was omitted, 5 c.c. of the same urea solution yielded—

36.73 c.c.

36.92 „

36.73 „

36.73 „

36.73 „

36.54 „

36.73 „

36.54 „

Mean.. 36.70 „

In the before quoted paper Mr. Apjohn gives the results of nine experiments, made apparently at a temperature of 60°F ., and a pressure of 760 mm., the mean of which when corrected for a temperature of 65°F . would give 36.70 c.c. gas evolved from 5 c.c. of a 2 per cent. urea solution, a figure identical with mine. If then we wish to work with 5 c.c. of urine, and at ordinary temperature, the graduations of the burette should be such that 36.70 c.c. indicate 2 per cent. of urea.

Under ordinary conditions variations of temperature and pressure may perhaps be neglected, although the error caused by such variations is not so small as the figures given by Messrs. Russell and West seem to indicate. Thus in a urine containing 3 per cent. of urea, a variation of 1 inch in the height of the barometer produces an error of 0.1 per cent. in the estimation. Now bearing in mind that at the beginning of January this year the barometer varied very nearly 2 inches in the course of a few days, it is evident that the barometer cannot always be safely neglected even for rough experiments. An error of similar amount would be produced by a rise of about 11° F., or a fall of about 13° F. In urine containing more or less of urea the errors would of course be in proportion.

Preparation of the Hypobromite.—For this I have retained the strength recommended by Knop in 1870, namely 100 grams of caustic soda dissolved in 250 c.c. water, and adding 25 c.c. bromine to the solution so produced. In order, however, to render the preparation easy, even to people unprovided with a laboratory, I have instructed Messrs. How to supply the caustic soda in bottles containing 100 grams each, and the bromine in hermetically sealed glass tubes holding 2.2 and 4.4 c.c., or enough for one and two experiments respectively. They also supply an ordinary glass stoppered bottle holding about 700 c.c. with marks at 250 and 500 c.c. capacity, and a small stoppered cylinder holding about 80 c.c., with marks at 23 and 46 c.c. capacity. If then the large bottle is filled with water up to say the 250 c.c. mark, and the contents of one bottle of caustic soda introduced, a soda-solution of proper strength is produced. When the hypobromite is wanted, say for one experiment, 23 c.c. soda-solution are put into the cylinder, and one of the smaller bromine tubes is dropped in. The cylinder is closed, and the bromine tube broken by a smart up and down shake, so as to bring the ends of the tube into sharp contact with the bottom and stopper of the cylinder. In a few moments the bromine is dissolved, and the solution has only to be cooled a little to be ready for immediate use, not a trace of bromine having escaped into the room. This plan has the further advantage that the hypobromite solution when used has always as nearly as possible the same composition, and is therefore likely to yield more uniform results. It now only remains to show by a few examples that the method as applied to urine is capable of yielding results sufficiently accurate for all practical purposes.

In every case 5 c.c. of urine or mixture were taken.

1st sample of urine, 1st experiment, 2.44 per cent. urea found.

2nd " 2.40 " "

2nd sample of urine, 1st experiment, 2.45 per cent. urea found.

2nd " 2.44 " "

The same urine diluted with various proportions of water gave—

1 part urine, 1 part water, 1·21 per cent. urea, or in original urine
2·42 per cent.

1 part urine, 3 parts water, 0·61 per cent. urea, or in original urine
2·44 per cent.

3rd sample of urine, 1st experiment, 1·77 per cent. urea.

		2nd	„	1·78	„
4th	„	1st	„	2·78	„
		2nd	„	2·80	„

The apparatus, therefore, leaves little to be desired on the score of accuracy, and is, I believe, worked with ease even by people comparatively little skilled in experimental work. A further advantage of this form of apparatus for clinical work will be found in the fact that as 25 c.c. of freshly made hypobromite are sufficient to decompose 15 to 20 c.c. of urine, two experiments may be made one after the other without changing the hypobromite. It is therefore simply necessary to wash and refill the little test tube, or refill the pipette, when the second experiment may at once be proceeded with.

It has been thought by some that it might be urged as an objection against the process that, besides the urea, there are other nitrogenised substances found in urine which evolve part of their nitrogen when treated with the hypobromite solution. It seems to me that so far from this being an objection it is rather an advantage which may be claimed for this method. The estimation of the urea in urine is of value chiefly, if not exclusively, as a measure of the amount of nitrogen eliminated. A process, therefore, which gives some portion at least of the nitrogen eliminated otherwise than in the form of urea, is for clinical purposes more valuable than a process which gives us nothing but the urea.

The apparatus and accessories may be obtained of Messrs. James How and Co., 5, St. Bride Street, Ludgate Circus, E.C.