XLIV. NOTE ON THE ESTIMATION OF UREA BY UREASE.

By GEORGE MACFEAT WISHART.

From the Institute of Physiology, University of Glasgow.

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IN the course of an investigation into the reason for a series of erroneous results obtained by the Soja bean-urease method for the estimation of urea, it was discovered that the estimation could be carried out much more rapidly and easily by doing the hydrolysis of the urea and the evacuation of the formed ammonia simultaneously. Curiously enough, if the bean be used in its crude powdered state, it is possible to add to the urea-containing solution the requisite quantity of sodium carbonate to liberate the formed ammonia without interfering with the hydrolytic action of the urease; the alkali would appear to be taken up by some constituent of the bean, possibly the proteins, and thus prevented from exerting its inhibitory action on the enzyme.

A large number of experiments were performed in which the vessel containing the $N/10$ H_2SO_4 for absorbing the liberated ammonia was connected with a burette, and thus the course of the evolution of the ammonia could be followed, minute by minute. Such experiments served for a comparison of various types of air-inlet tubes, and for the determination of the optimum temperature, and the optimum quantities of the reagents. In the course of these experiments it was found that the more rapid the aeration, the more rapidly could the estimation be completed; *e.g.* in one experiment with an air-current of 6 litres per minute, 75 % of the urea-ammonia had been liberated in 10 minutes, while with an air-current of 10 litres per minute, 92.4 % of the theoretical amount was liberated within the same time.

To provide for as rapid an air-current as possible, and at the same time to prevent loss of ammonia, and frothing of the solutions, the apparatus was modified as shown in the diagram.

Tube *A* and the absorbers are made from the tubes of an ordinary ammonia absorption apparatus. The clip at *X* allows of the saturated sodium carbonate solution being introduced after the whole apparatus is connected up and the air-current started, without any disjoining of the tubing, thus making loss of ammonia impossible.

The constrictions in all three tubes should be placed at such a level that the capacity of the lower bulb is about three times the volume of fluid to be placed within it. This arrangement, along with the use of glass beads in tube *A*, renders the use of any anti-frothing solution unnecessary in most cases.

To complete the estimation in the times given, the pump should be capable of drawing air through the apparatus at a rate of not less than $5\frac{1}{2}$ litres per minute. The water-bath temperature should be maintained at about 40° .

Method of Estimation.

In tube *A* place 20 cc. water, 5 cc. urine, and 3–5 g. Soja bean meal (varying amounts are required according to the condition of the bean; a slight excess will not vitiate the result). Mix the contents of the tube and insert the central tube and glass beads as shown, immerse bulb in water-bath, then connect up to collecting tubes containing a known amount of decinormal acid. Start the pump and introduce about 2 cc. of a saturated solution of sodium carbonate from a pipette by holding the nozzle of the pipette at *X* and opening the clip. Close the clip and aerate for 40 minutes.

A smaller apparatus with tubes of about 100 cc. capacity, and using $N/50$ acid, has been found useful for estimating small amounts of urea. The quantities for this apparatus are: 5 cc. of urea-containing fluid (content not more than 10 mgm. urea); 1 g. bean meal; and 0.5 cc. saturated sodium carbonate solution. Aeration with this smaller apparatus, and using these quantities, is complete in 20 minutes.

Since the time required for complete evacuation of the ammonia depends on the velocity of the air-current and the amount of bean meal used, it is well to determine that all conditions are correct by doing one estimation on a solution containing a known amount of pure urea. The bean meal liberates a certain small amount of ammonia itself, so that, for accurate work, it is necessary to estimate this by doing a blank experiment, using water in place of the urea solution.

As examples of the results obtained by this method the following may be given.

Large apparatus (N/50 acid and alkali used):

Experiment	Theoretical amount	Estimated amount
1	1 %	0.9948 %
2	1	0.9936
3	1	0.9912

Small apparatus:

1	0.004 %	0.00408 %
2	0.004	0.00396
3	0.004	0.00402

Experiments on the estimation of urea in urines with and without added quantities of urea gave equally satisfactory results. In estimating urea in urine by the larger apparatus, it is usually advisable to dilute the urine so that it will contain approximately 1 % of urea.