CLXXVIII. A METHOD FOR THE DIRECT DETERMINATION OF UREA IN URINE.

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THE method introduced by Marshall [1913] is not used extensively, probably because of the length of time necessary for the completion of the action of the urease.

By using a stronger enzyme preparation and by maintaining the fluid under optimal conditions of reaction, it is possible to complete the estimation in about 15 minutes.

The principle of the method in brief is as follows. The urine is treated with warm water and some phenolphthalein, the urease preparation is added, and the mixture is titrated with 0.1 *N* HCl, at such a rate as just to discharge the red colour. This prevents the loss of ammonia and maintains a reaction of about p_H 8.2, which is about the optimum for urease acting on dilute solutions of urea. After about 10 minutes the mixture is treated with mercuric chloride and some methyl red. The addition of the HCl is continued until a red colour is obtained which matches that given by a mixture of the same amount of urine, mercuric chloride, enzyme preparation and methyl red which is titrated from a separate burette to give a red colour. In this case the mercuric chloride is added before the urease, which is unable to act in the presence of the mercury. A certain amount of ammonium chloride is added to the control to ensure uniform salt conditions for a correct adjustment of the final p_H . The difference between the amounts of HCl required for the two solutions is a measure of the urea in the urine taken.

SOLUTIONS REQUIRED.

- (a) 0.1 *N* HCl, in two burettes.
- (b) Mercuric chloride. 0.5 % made up to 100 cc.
- (c) Ammonium chloride, 10 %.
- (d) Phenolphthalein. A saturated solution in 50 % alcohol.
- (e) Methyl red. 0.02 % in 60 % alcohol.
- (f) Urease solution. This is best made from "Arlco" Jack Bean Meal¹.

The simplest method is to place 4 g. of the meal in a mortar and rub it up

¹ This is prepared by The Arlington Chemical Co., Yonkers, N.Y., and is stocked by Baird and Tatlock, 14 Cross St., E.C. 1.

into a cream with 10 cc. of distilled water. Wash this into a beaker with more water until 100 cc. have been added. Stir well and allow to stand for some minutes. Pour off from a deposit of starch and other insoluble material. Such a preparation gives a considerable precipitate at the final end-point of the titration, but this does not materially interfere with the accuracy. The following method gives an almost clear solution. A 4 % suspension of the meal is made as above with the exception that the last 50 cc. of water are warmed to about 60° before being added. The whole is treated with 4 cc. of 0.1 *N* HCl, mixed well and filtered through a pleated paper. A water-clear filtrate is obtained. This gradually deposits a slight precipitate, which should not be filtered off.

Method of estimation. Into two similar 100 cc. beakers, labelled *A* and *B*, measure 3 cc. of the filtered urine. To each add about 20 cc. of distilled water at about 60° and 5 drops of the phenolphthalein. To *A* add 10 cc. of the enzyme preparation. As soon as this goes red add some 0.1 *N* HCl from a burette, and continue to do this at intervals, so as just to prevent a red colour developing. Care should be taken not to add too much acid, as this inhibits the action of the enzyme. As a rule it will not be necessary to add any acid after about 5 minutes. It is, however, advisable to allow the mixture to stand for about 12 or 15 minutes, depending on the concentration of the urea. During the interval place 1 cc. of the mercuric chloride and 1 cc. of the ammonium chloride in *B*. Mix well and then add 10 cc. of the enzyme preparation. Now add 20 drops of the methyl red (measured with a dropping pipette). With most samples of urine the mixture is yellow. Add 0.1 *N* HCl from a second burette until a full red colour is obtained, enough water being added to make the volume slightly greater than that in *A*. At the end of 12 or 15 minutes' digestion the fluid in *A* is treated with 1 cc. of the mercuric chloride, and 20 drops of methyl red. The contents of *A* are then titrated with the HCl until the colours of the fluids in the two beakers match, the volumes being kept constant by the addition of water to one or the other. It will be found that the red colour produced in *A* by the addition of acid tends to fade a little at first, owing to the rather slow establishment of equilibrium of the carbonic acid, but the two colours can be matched with great exactitude if a little care is given to this point. It is advisable to add enough acid to *B* to produce a rather bright red tint, otherwise the result may be a trifle low, owing to the effect of the carbonic acid in *A*. But the effect of this is negligible if the end-point of the titration is sufficiently acid.

Calculation. As 1 cc. of 0.1 *N* HCl is equivalent to 3 mg. of urea, the number of cc. of the acid added to *A* less that added to *B* gives the urea in g./litre.

Accuracy of the results. Duplicate estimations on the same sample of urine agree to 1 % with the filtered extract, and to about 2 % with the untreated suspension.

The results agree with those obtained by estimating the ammonia and the ammonia- plus urea-nitrogen by Van Slyke's methods [Van Slyke and Cullen, 1914] with the same degree of accuracy.

Theoretical results are obtained with solutions of urea up to 5 %, and also with urines to which urea has been added to make a total of 5 %.

With urines obtained when making the urea concentration test of renal efficiency, it is advisable to use 2 cc., and to multiply the cc. of acid used for *A* less that used for *B* by 1.5 to give the urea in g./litre.

SUMMARY.

A method is described for the estimation of urea in urine which can be completed in 20 minutes, and which is accurate to within 2 %.

REFERENCES.

- Marshall (1913). *J. Biol. Chem.* **14**, 483.
Van Slyke and Cullen (1914). *J. Biol. Chem.* **19**, 141.