VIII. THE QUANTITATIVE ESTIMATION OF UREA, AND INDIRECTLY OF ALLANTOIN, IN URINE BY MEANS OF UREASE.

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For the rapid routine analysis of urea in urine Folin's magnesium chloride method [1901; 1902, 1; 1903; 1905] is probably the one most usually adopted, but it is not an easy method to acquire and it requires a considerable amount of attention. A good substitute might soon displace it. Folin himself has devised other methods for the estimation of urea in small quantities. We believe we have found a substitute for the first method by using the urease of the soy-bean [Takeuchi, 1909]. Marshall [1913, 1] has already published a method for the rapid clinical estimation of urea in urine by this means, but by his procedure the results are not sufficiently accurate, being only within about two per cent.; an extract of the soy-bean is required and results are only obtained after at least three hours. Marshall [1913, 2] has since modified his method by removing the ammonia after the conversion of the urea into ammonium carbonate by means of an air current.

Our procedure is similar to this latter one of Marshall, but it is much simpler; accurate results are obtained in a little over two hours, and it is not necessary to use an extract of soy-beans.

In its essential features the method is no more than Folin's method [1902, 2] of estimating ammonia in urine. By fitting together three or four cylinders and Allihn bottles in series with a sulphuric acid bottle at the end, duplicate estimations of ammonia and urea in urine can be carried out simultaneously. In the cylinders for the urea estimations are put 50 to 60 cc. of water, 1 g. of finely ground soy-bean and 5 (or 10) cc. of urine. These

cylinders are kept in a water bath at a temperature of 35–40° and an air current is drawn through the series. After about an hour the rubber connections between the cylinders and bottles are disjointed and 1 g. of anhydrous sodium carbonate is dropped into the cylinders; they are then connected together again and the air current drawn through for another hour. To prevent frothing liquid paraffin B.P. has been used; it is superior to petroleum or toluene as it does not evaporate and it obviates the necessity of using a tube containing cotton wool between the cylinder and Allihn bottle. It is not necessary to carry out a blank experiment with soy-bean alone, since no ammonia was evolved by two different samples of the bean which were tested several times. The Allihn bottles are charged with excess of 0.1 N sulphuric acid (25 or 50 cc.) which is titrated with 0.1 N alkali using alizarin red as indicator.

The ammonia estimations are carried out exactly as described by Folin, except that liquid paraffin is used to prevent frothing.

The following are some comparative data obtained with 5 cc. of human urine by the urease method and Folin's magnesium chloride method:—

Urease		Folin	
cc. 0.1 N acid		cc. 0.1 N acid	
18.3	} 18.25	18.6	} 18.55
18.2		18.5	
16.1	} 16.1	16.1	} 16.15
16.1		16.2	
14.2	} 14.2	14.2	} 14.25
14.2		14.3	
19.0	} 19.1	19.2	} 19.25
19.2		19.3	

The urease method has been used daily for some weeks and the results are all of the same order, the two data always coming out together or differing at most by 0.4 cc.; in fact all our latest determinations of urea in urine have been made by the urease method as we have become convinced of its accuracy¹. The values are very slightly lower than by the magnesium chloride method, in which a decomposition of other urinary constituents, e.g. allantoin, or possibly urochrome as suggested by Haskins [1906], may occur.

The method has been tested on solutions containing different amounts of pure urea and the results are correct against a nitrogen determination by Kjeldahl's method; thus

¹ It is advisable to cleanse the cylinders immediately after use and occasionally with formalin, as the residues of protein, if left, form a suitable medium for the growth of bacteria, which may produce ammonia from amino-acids and other compounds.

Urea solution
taken

2 cc.	19.5 19.2	19.35 cc. 0.1 N acid = 9.68 for 1 cc.	{ N by Kjeldahl's method 19.5 cc. = 9.75 cc. for 1 cc. of solution.
4 cc.	38.5 38.8	38.65 ,, ,, 9.66 ,,	
6 cc.	57.6 57.9	57.75 ,, ,, 9.63 ,,	
8 cc.	77.2 77.4	77.3 ,, ,, 9.66 ,,	

The urease of the soy-bean was shown to be specific by Takeuchi [1909] and its specificity has been more fully emphasised by Armstrong and Horton [1912], who also showed that its action is inhibited by ammonia; the removal of ammonia, as it is formed, by means of the air current will thus favour the completion of the reaction. Our method is in this way again more advantageous than Marshall's.

Not only urea, but also allantoin, is decomposed by the magnesium chloride method of Folin, as is stated by Cathcart [1906] and Haskins [1906]. Since urease has no action upon allantoin the two substances can therefore be readily estimated in urines which contain both compounds; the difference between the two data will give the amount of allantoin. The two compounds and mixtures of the two compounds have been tested with the following results:—

	5 cc. urea (from above)	5 cc. allantoin	10 cc. allantoin
Kjeldahl	48.75 cc. 0.1 N acid	—	9.8 9.7 9.5
			9.7 cc. 0.1 N acid
Folin	—	4.5 4.5 4.6	9.7 9.8
		4.5 cc. 0.1 N acid	9.75 ,, ,,
Urease	48.5 cc. 0.1 N acid	0	0
	Hence 5 cc. allantoin = 4.87 cc. 0.1 N acid.		
	5 cc. urea + 5 cc. allantoin	5 cc. urea + 10 cc. allantoin	
Folin	53.4 53.6	57.5 57.3	57.4 cc. 0.1 N acid
	53.5 cc. 0.1 N acid		
Urease	48.5 48.5	48.5 48.5	48.5 ,, ,,
	48.5 cc. 0.1 N acid		
	∴ 5 cc. allantoin = 5.0 ,,	4.45 ,,	,,

Several analyses of dog's urine (volume for 24 hours made up to 1000 cc.) have also been made, the figures being in all cases cc. of 0.1 N acid:—

Total N (5 cc.)	Ammonia (25 cc.)	Urea + allantoin (5 cc.) less ammonia	Urea (5 cc.) less ammonia	Allantoin in 5 cc.
19.5	8.3	16.7 16.6	14.4 15.1	1.85
		16.65	14.8	
15.5	6.1	13.0 12.9	11.2 11.6	1.55
		12.95	11.4	
7.4	2.0	6.6 6.4	5.0 5.4	1.3
		6.5	5.2	
21.3	3.0	19.1 19.3	17.2 17.4	1.9
		19.2	17.3	

The difference between the figures in columns 3 and 4 gives the amount of allantoin.

The estimation of allantoin in urine by Wiechowski's method does not lend itself to routine work and our procedure is undoubtedly more rapid than that adopted by Miss Lindsay [1909].

SUMMARY.

The estimation of urea in urine is quickly and accurately made by decomposing it with urease (1 g. powdered soy-bean) at 35 to 40° for one hour. During this time the ammonia evolved is removed by an air current as in Folin's method for estimating ammonia. One gram of anhydrous sodium carbonate is then added and the air current is continued for another hour. Liquid paraffin B.P. is very convenient for lessening the frothing.

Since urease does not decompose allantoin and since both allantoin and urea are quantitatively decomposed by the magnesium chloride method of Folin, the amount of allantoin in those urines, which contain both compounds, is readily estimated by difference.

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