ON THE
ESTIMATION OF ALBUMEN IN URINE
BY A NEW METHOD.

ADAPTED FOR CLINICAL PURPOSES.

BY

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(Received January 6th—Read February 22nd, 1876.)

WHEN an albuminous urine is progressively diluted with water and tested from time to time with nitric acid the opacity induced by the acid becomes gradually fainter and fainter until at length it ceases to be visible. This point is reached when the diluted urine contains less than about 0·0014 per cent. of albumen. The more albumen the urine contains the more dilution, of course, it will require to reach the vanishing point of the reaction; and if we could fix this point with accuracy we should arrive at a simple method of estimating the quantity of albumen in urine. The urine could be diluted with water until it ceased to react with nitric acid, and the degree of dilution required to reach this point would be a measure of the proportion of albumen in the urine.

But it is not possible to fix the vanishing point of this reaction with accuracy. It fades away so gradually with increasing additions of water that it is quite impracticable to decide, within many degrees, the point at which it ceases to be appreciable;¹ and not only so, but the development of the reaction is more and more retarded as the dilution proceeds, until at length it only becomes visible after the lapse of several minutes after the acid is added.

The following experiment on a moderately albuminous urine illustrates the behaviour of albuminous solutions when tested with nitric acid under progressive dilutions with water.

<i>Degree of dilution with water.</i>	<i>Time after the addition of the acid when the reaction comes into sight.</i>
20 volumes, and all lesser dilutions.	Immediately.
30 volumes.	About 4 seconds.
40 "	" 8 "
60 "	" 10 "
90 "	" 20 "
110 "	" 30 "
120 "	" 38 "
140 "	" 50 "
160 "	" 1 minute.
180 "	" 1½ " faint.
200 "	" 1½ " very faint.
240 "	" 3 minutes, very faint.
280 "	" 6 " doubtful ; very faintly visible in 10 minutes.
300 "	No appreciable reaction.

Finding it thus impossible to make use of the normal zero, an endeavour was made to hit on an arbitrary point or line which would serve as a practicable zero to the

¹ The same appears to be the case with other precipitants of albumen—heat, alcohol, gallic acid, carbolic acid, and bichloride of mercury—all of which I have tried.

scale. After many trials it was found most advantageous to draw the line at a reaction coming into sight midway between half and three quarters of a minute after the addition of the acid: that is, to dilute the urine until it gave no reaction for thirty seconds after the contact of the acid, but showed a distinct opalescence at the forty-fifth second. The exact point aimed at was a reaction coming doubtfully into view at the thirty-fifth to the fortieth second, and appearing still dim, but unmistakable at the forty-fifth second. After a little practice it was found possible to strike this point with sufficient exactness to serve as a practicable zero to the scale.

Each dilution with a volume of water equivalent to the unit volume of urine employed was counted as one degree on the scale; and these degrees might be conveniently termed "degrees of albumen." Thus, a urine which required forty volumes of water to reach the zero reaction might be described as possessing 40 degrees of albumen, a urine requiring three hundred dilutions as possessing 300 degrees of albumen, and so forth.

The difficulty of the method is to hit correctly the zero reaction. When this point is approached, a little more or less dilution makes but a slight difference in the time when the reaction comes into view; and to obtain exact results—results which compare favorably with those obtained by the weighing process—the testing must be performed in a scrupulously uniform manner. The test-tube must be of a certain fixed diameter, the acid must be added in the right way and at the right moment. The operation should be performed by daylight, or if by gaslight an addition of about 5 per cent. must be made to the result. It is, therefore, necessary to describe the precise mode of operating by which the results which follow were obtained.

The test-tube employed had an interior diameter of five eighths of an inch (15 millimètres). The watch was set on the table before the operator, and beside it was placed the unstoppered bottle of nitric acid. The acid

was added by means of a pipette capable of holding, when immersed to the depth of about two inches, twelve to fifteen drops of acid. When these preliminaries were arranged 5 cc. of the urine to be tested were introduced into a measure graduated to 500 cc., and water was added to 200 or 300 cc.¹ The test-tube was then filled to the depth of about an inch with the diluted urine. The eye was now directed to the watch, and as the seconds hand approached one of the quarter minute strokes (*i. e.* 15th, 30th, 45th, or 60th) the pipette, previously immersed in the acid and covered with the forefinger, was lifted out and passed into the test-tube, which was held inclined at a wide angle, and on the stroke of the quarter minute its point was pressed against the lower side of the tube, half an inch above the level of the fluid, and the charge of acid delivered. If the manœuvre was properly performed the acid sank to the bottom of the tube and formed a distinct layer below the diluted urine. The test-tube was then held up between the eye and the light against some dark back-ground (such as a dark corner of the room, a book bound in black cloth, or a black sleeve), and as soon as the faintest opalescence appeared above the level of the acid the time of its appearance was noted. If this occurred within thirty seconds after the contact of the acid more water was added and the testing repeated as before. Thus, by repeated additions of water and repeated testing a close approximation to the zero reaction was obtained. A fresh dilution was then prepared, and, guided by the previous trials, two or three more testings with different dilutions were generally sufficient to indicate with exactness the degree of dilution required to produce an opalescence between thirty-five and forty-five seconds after the addition of the acid. If too much water was added in the first instance, and the reaction did not show itself until after the forty-fifth

¹ In ordinary clinical work the fluid drachm is the most convenient unit-volume, and the dilution can be carried out in the imperial pint measure graduated to ounces.

second, the operation was recommenced with less water, and then proceeded with as in the former case.

When the zero-reaction was determined, the degree of dilution required to produce it was noted and expressed in multiples of the unit volume of urine employed. Thus, if 5 c.c. of urine gave the zero reaction when diluted up to 400 c.c., *i.e.* at the eightieth dilution ($\frac{400}{5} = 80$) the urine was registered as possessing 80 degrees of albumen.

When the urine possessed (as often happened) more than 100 degrees of albumen it was subjected to a preliminary dilution with water in the proportion of 1 in 2 or 1 in 5, and the result afterwards multiplied by 2 or 5 as the case might be.

In operating on an untried urine it was first tested in the ordinary way with nitric acid in order to get a rough idea of the degree of dilution likely to be required to reach the zero reaction. The amount and density of the precipitate produced by the acid furnished a useful guide, and saved much time in the subsequent steps of the operation.

The next step in the inquiry was to determine the actual value in weight of albumen of each degree on the scale. This was accomplished by first ascertaining the degrees of albumen by the dilution method and then estimating the quantity of albumen by the weighing process. Selected urines were chosen suitable for the weighing process, and the analyses were performed with all the precautions recommended by Neubauer. These analyses indicated that each degree on the dilution scale corresponded to 0.0034 per cent. of albumen, *i.e.* to 0.0034 grammes of dry albumen in 100 c.c. of urine. The proportion of albumen in a urine was therefore obtained by multiplying the degrees of albumen by the coefficient 0.0034. For example, a urine which possessed 250 degrees of albumen contained 0.85 per cent. of albumen— $250 \times 0.0034 = 0.85$. From these data it was easy to calculate the daily loss of albumen by the urine. Suppose 1200 c.c. of urine to be voided in the twenty-

four hours, and that a sample of this urine showed 250 degrees of albumen, *i. e.* 0·85 per cent., then—

$$\frac{1200}{100} \times 0\cdot85 = 10\cdot2.$$

The daily loss of albumen was 10·2 grammes.

In our English measures the calculation is made as follows:—Suppose, as before, that the urine showed 250 degrees or 0·85 per cent. of albumen, and that the quantity voided in twenty-four hours was forty fluid ounces. Each fluid ounce of the British Pharmacopœia contains 437·5 grains, then—

$$\frac{437\cdot5}{100} \times 0\cdot85 \times 40 = 148\cdot75.$$

The daily loss of albumen was 148·75 grains.

The time required for the completion of an analysis by the dilution method varied from ten to twenty minutes.

The degree of accuracy possessed by this method may be judged of by the following table, in which the results obtained by it are compared with those obtained by weighing.

Degrees of albumen indicated by the dilution method.		Albumen per cent.	
		Calculated with the coefficient 0·0084.	Found by weighing.
Urine A— 30	...	0·102	0·106
B— 98	...	0·333	0·308
C—300	...	1·020	1·027
D—100	...	0·340	0·346
E— 43	...	0·146	0·135
F—150	...	0·510	0·503
G—116	...	0·394	0·381
H—270	...	0·918	0·924

The table shows that the dilution method compares favorably with the weighing process even in urines selected for their suitability to the latter process, but it excels it in the diminished time and trouble required for its performance and also in its more general applicability to all grades of albuminous urines. When the proportion of albumen is small the weighing process gives very untrustworthy results, but nearly all albuminous urines

met with clinically are susceptible of a few dilutions before the zero reaction is reached, and are therefore amenable to the method here described.¹

¹ In the course of the inquiry the question arose whether the relative proportion of the normal urinary ingredients to the quantity of albumen influenced the results. This point was tested by mixing albuminous urines with various known quantities of healthy urine, and then diluting the mixture with water until the zero reaction was reached. These experiments showed that if such mixtures required ten or more dilutions with water to reach the zero the proportion of albumen was exactly indicated, but below this point there was a slow progressive increase of deficiency, or apparent deficiency, in the quantity of albumen indicated. In other words, when the relative proportion of the normal urinary ingredients to the quantity of albumen became very great, the sensitiveness of the nitric-acid test was diminished, and the results came out somewhat below the true values. The error involved is, however, too small to need special correction.