XVIII. THE ESTIMATION OF TOTAL SULPHUR IN URINE.

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THE methods for the estimation of total sulphur in urine are less satisfactory than those available for determining many of the other urinary constituents. A high degree of accuracy is specially important when we are concerned with the amount of unoxidised or "neutral" sulphur in the urine. This can only be determined by subtracting the amount of sulphates from that of the total sulphur and any error in the latter value falls on the relatively small difference.

Benedict's [1909] method in which the oxidation is effected with a mixture of copper nitrate and potassium chlorate has been criticised on the ground that considerable losses frequently occur through spattering during the initial stages of the reaction. Denis [1910] has stated that out of forty analyses mechanical loss resulted in every case. She has proposed to substitute a solution of copper nitrate, ammonium nitrate and sodium chloride in place of Benedict's oxidising reagent, thereby reducing the vigour of the reaction.

In my hands Denis' modification has not proved so reliable as the original method. Decrepitation is certainly less troublesome but the results are frequently too high, the errors amounting at times to more than 10 mg. The following series taken from nearly a hundred analyses are fairly representative:

25 cc. urine (1) gave 0.0711, 0.0719, 0.0783, 0.0826 g. BaSO_4 ,
,, ,, (2) ,, 0.0864, 0.0901, 0.0865, 0.0899, 0.0877 g. BaSO_4 .

The residue, even after prolonged ignition always contained appreciable amounts of nitrate, this being probably due to sodium nitrate formed from the sodium chloride and copper nitrate since the latter is readily decomposed at temperatures lower than those used. Whether the presence of this nitrate would alone account for the high results I am not able to say. Considerable errors in this direction were observed by Kolthoff and Vogelensang [1919] using higher concentrations of both sulphate and nitrate.

Consistent results were usually obtained by Benedict's original method, using an electric hot plate as suggested by Givens [1917] although in spite of this precaution vigorous spattering sometimes occurred. Only slight traces of nitrate were found in the residues after ignition and it is probable that these arose from the small amounts of alkali salts in the reagent and in the urine itself. The necessity of igniting the residue at a red heat was however

a frequent cause of spoiled analyses through the cracking of the porcelain basins. Probably those used by Benedict were of better quality than are at present obtainable.

Errors due to the use of coal gas for igniting the residue.

Neither Benedict nor Denis appears to have recognised the possibility of errors from the presence of sulphur in the coal gas used for the ignition of the residue. Folin [1903] advised spirit burners for his original method and according to Liebesny [1920] errors of 8–10 % in the amount of neutral sulphur may arise from the use of gas. My own experience confirms this. In determining the blank of the oxidising solution referred to below, 2.5 cc. was found to yield 0.7 mg. BaSO_4 , using a spirit burner, and 2.4 mg. using an argand burner with coal gas. If this error were constant in amount it would be cancelled in subtracting the blank but such was not found to be the case. Probably the absorption of oxides of sulphur depends on the surface area of the residue as well as on the total quantity present in the gas.

The use of a spirit burner is therefore essential if accurate results are to be obtained.

Since copper nitrate, which forms the basis of Benedict's reagent, is decomposed at comparatively low temperatures it seemed possible that the necessity of igniting the residue at a red heat might be avoided if neither chlorate nor alkali salts were introduced. Other substances were therefore tried as a diluent for the copper nitrate and of these copper chloride was found to be the most satisfactory. In the presence of a suitable proportion of this salt spattering never occurs, while the nitrate can be completely decomposed by heating the residue over a small spirit stove of the simplest kind. The temperature of a good argand burner is sufficiently high but gas cannot be used.

The oxidising reagent finally adopted has the following composition:

Copper nitrate (cryst.)	40 g.
Copper chloride (cryst.)	15 g.
Water	to 100 cc.

2.5 cc. of this solution are added to 10 cc. of the urine in a 4-inch porcelain basin and evaporated to dryness on a water-bath or electric hot plate. The oxidation can be started on the hot plate, or over a very small spirit flame. It proceeds rapidly but smoothly, leaving a coherent residue which frequently swells up. The dish is then heated over a broad spirit flame for 20 minutes. A spirit stove of the common kind is suitable but a sound tin, half filled with methylated spirit answers very well. A better flame is obtained if a number of holes are punched about half-way up the tin. The residue is dissolved in 10 cc. of 2N HCl and diluted with 300 cc. distilled water. The sulphate is precipitated in the boiling solution with 10 cc. of a 5 % solution of barium chloride, dropped in very slowly by means of a dropping tube. The precipitate is allowed to stand overnight before being filtered.

In the following analyses the precipitates were weighed in platinum crucibles.

For 25 cc. urine, 6.25 cc. of the oxidising solution, 20 cc. 2N HCl, 400 cc. H₂O and 15 cc.-20 cc. of 5 % barium chloride solution were used. A blank determination must be carried out with the reagents.

Two series, each of eight estimations, of the total sulphur in a normal urine yielded the following results.

BaSO ₄ from 10 cc. urine		BaSO ₄ from 25 cc. urine	
1.	0.0451	1.	0.1140
2.	0.0454	2.	0.1143
3.	0.0460	3.	0.1135
4.	0.0454	4.	0.1141
5.	0.0456	5.	0.1138
6.	0.0457	6.	0.1133
7.	0.0461	7.	0.1141
8.	0.0454	8.	0.1148
Average 0.0456 g.		Average 0.1140 g. equivalent to 0.0456 g. for 10 cc.	

It is doubtful whether any gain in accuracy is achieved by the use of 25 cc. instead of 10 cc. of urine.

In all the above estimations the filtrates were examined for nitrates by the very sensitive diphenylamine test, slight traces being found in one or two cases only.

The accuracy of the method was checked by estimating the total sulphur in two solutions of cystine containing also 2 % of urea and a definite volume of N/10 H₂SO₄ neutralised by an equal amount of N/10 NaOH.

The sample of cystine, for which I am indebted to the kindness of Dr J. C. Drummond, was analysed by the Carius method:

0.1878 g. gave 0.3679 BaSO₄; calculated 0.3681 g. BaSO₄.

BaSO ₄ from 10 cc. solution A	BaSO ₄ from 10 cc. solution B
0.0671	0.0662
0.0670	0.0659
0.0675	0.0661
0.0675	0.0659
—	0.0659
—	0.0663
0.0673 g.	0.0661 g.
Calculated 0.0678 of which 0.0212 is from cystine.	Calculated 0.0668 of which 0.0202 is from cystine.

This method has been employed for some time by myself and others in connection with metabolism experiments in this laboratory and has proved satisfactory.

I wish to thank Miss M. Tazelaar and Miss M. H. Carr for much valuable help in carrying out the numerous analyses, only a very small proportion of which have been recorded above.

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