XLVII. THE VOLUMETRIC ESTIMATION OF TOTAL SULPHUR AND SULPHATES IN SMALL QUANTITIES OF URINE.

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Introductory.

A large number of publications have appeared from time to time upon the estimation of sulphates by the benzidine method of Raschig [1903]. The majority, if not all, of these communications have declared that, in general inorganic analysis, the method is one on which the utmost reliance is placed.

The method, which has as its basis the fact that benzidine sulphate is very insoluble in water, depends upon the precipitation of the sulphate present by means of a dilute solution of benzidine hydrochloride. The insoluble benzidine sulphate is then removed by filtration, and can be accurately titrated against standard alkali, as benzidine salts are readily dissociable. The application of the method to the analysis of biological material was not attempted until two papers appeared, almost simultaneously, by Rosenheim and Drummond [1914]¹, and Gauvin and Skarzynski [1913].

Both these papers dealt with the analysis of urine by this method. That of Rosenheim and Drummond contained a systematic investigation into the conditions necessary for accurate analyses to be made, and showed that the inorganic and ethereal sulphates in urine could be rapidly and accurately determined by the benzidine method. These authors also suggested that the method could be adapted for the analysis of very small quantities of urine and for the determination of total sulphur.

¹ Rosenheim and Drummond's results were communicated to the Biochemical Society at a meeting held on November 20th, 1913.

This was partially borne out by the independent findings of Gauvin and Skarzynski, who showed that, by applying the benzidine method to the solution of salts obtained after oxidising the sulphur in urine by various fusion methods, analyses of the total sulphur present might be made.

These two authors also confirmed the results of Rosenheim and Drummond for the analysis of the total and inorganic sulphates in urine, although they did not, apparently, investigate so closely the conditions necessary for accuracy.

Subsequently Raiziss and Dubin [1914] made use of the benzidine method in the determination of total sulphur in small quantities of urine, and in their communication made some criticisms of the titration of the benzidine sulphate with alkali.

These workers stated that the method was not trustworthy for the analysis of small quantities of urine, as they found that the quantity of sulphuric acid liberated from 10 cc. of urine was too small to be accurately titrated with 0.1 N alkali, and further, they were unable to obtain a sharp end-point to the titration, except after repeated additions of alkali and boilings. To surmount these apparent disadvantages, they devised an alternative method of titrating the benzidine sulphate, which was based on an observation that benzidine sulphate may be quantitatively oxidised by means of potassium permanganate.

Their paper only deals with the analysis of the total sulphur in urine by this method, applying the precipitation of the benzidine sulphate to the final solution of salts obtained after oxidising the urine by the method of Benedict [1909], as modified by Denis [1910]; and they claim considerable accuracy for the method even in the analysis of as small a volume as 2 cc. of urine. The present communication records the results of a series of experiments which were carried out to test whether the earlier method might not be used successfully for the micro-estimation of the sulphates in urine and the total sulphur in biological material in general.

Incidentally, the method devised by Raiziss and Dubin was investigated, mainly with the object of determining whether any great advantage lay in the titration of the benzidine sulphate with permanganate, which they advocate, instead of with alkali. Naturally, the much larger titration figure, which is obtained in the permanganate method, is a distinct advantage when small quantities of benzidine sulphate are being dealt with, but I have not been convinced that this is in itself sufficiently important to necessitate an alteration in the method.

The objections raised by the American workers to the titration of the benzidine sulphate with alkali appear to have little foundation, for, first, I have never yet, in the course of many hundred urinary analyses by this method, encountered an indefinite end-point to a titration; provided that during the filtration strict care is paid to certain details of technique. If the precipitated benzidine sulphate is allowed to suck dry upon the filter, it will form a glistening layer which will not disintegrate upon suspension in water, but will float about in the liquid in the form of small flakes, which are not readily attacked by the alkali during titration. Should the precipitate be once obtained in this form, an end-point by direct titration with alkali is only possible after "repeated boilings and additions of alkali." However, even in this case, a rapid and accurate titration may be made by dissolving the precipitate in an excess of standard alkali by warming, and then titrating that excess with standard acid.

The great importance of the details of technique, which must be observed during filtration, have been emphasised by almost all the investigators who have used the method; hence it is possible that Raiziss and Dubin failed to carry out the filtration in an entirely satisfactory manner.

Their other objection to the titration of the benzidine sulphate with alkali, namely, that 10 cc. of urine yields too small a quantity of sulphuric acid to titrate accurately with standard alkali, receives its answer in the results recorded in the present paper, which show that by the employment of a more dilute alkali, 0.02 N, very accurate analyses of much smaller volumes of urine than 10 cc. may be made.

The method employed by Raiziss and Dubin is as follows. The precipitated benzidine sulphate is filtered off upon an asbestos filter, and is then dissolved in a comparatively large volume of dilute sodium hydroxide, by warming. The solution thus obtained is cooled to room temperature, and, by the addition of a certain volume of concentrated sulphuric acid, is brought to an empirical temperature at which titrations are made with standard potassium permanganate.

In order to standardise the permanganate solution, and obtain its value in terms of sulphur, they carried out estimations upon a pure sulphuric acid solution, and they thus arrived at the factor 0.000099 to represent the value of 1 cc. 0.1 N potassium permanganate in grams of sulphur. Utilising this factor they calculate their results for the analysis of urine.

Upon trying the method, as described by them, I found that in the analysis of pure solutions of sulphates there is no difficulty in the technique. It was

my experience, however, that the end-point here really does leave much to be desired. This is particularly the case when one is titrating comparatively large quantities of benzidine sulphate.

The titration fluid passes through various shades of orange and yellow, until shortly before the end-point is reached one has an almost colourless solution, and I have often found it most difficult to determine precisely when I have obtained the permanent pink coloration, lasting for twenty seconds, which is advised by the authors.

The empirical temperature of the titration seems an unsatisfactory condition, and, in my experience, unreliable results may occur if one does not always titrate at the same temperature.

TABLE I.

Solution analysed	Cc. analysed	Weight of BaSO_4 in g.	Cc. 0.1N KHO	Cc. 0.1N KMnO_4	% S	Factor (g. of S equivalent to 1 cc. KMnO_4)
Solution 1	20	0.2402	—	—	—	—
0.1N H_2SO_4	„	0.2394	—	—	0.164	—
	10	—	9.99	—	—	—
	„	—	9.99	—	—	—
	„	—	9.99	—	0.1602	—
	„	—	10.00	—	—	—
	„	—	10.02	—	—	—
	0.5	—	—	8.44	—	—
	„	—	—	8.55	—	—
	„	—	—	8.49	—	0.0000944 g. S
	„	—	—	8.57	—	—
	„	—	—	8.52	—	—
Solution 2	50	0.0918	—	—	—	—
Sodium	„	0.0907	—	—	0.0249	—
sulphate	25	—	3.91	—	—	—
containing	„	—	3.92	—	—	—
0.0245 % S	„	—	3.91	—	0.0250	—
	„	—	3.93	—	—	—
	„	—	3.90	—	—	—
	2	—	—	5.39	—	—
	„	—	—	5.30	—	—
	„	—	—	5.30	—	0.0000939 g. S
	„	—	—	5.42	—	—
	„	—	—	5.30	—	—
Solution 3	20	0.0216	—	—	—	—
Sodium	„	0.0216	—	—	0.0148	—
sulphate	100	—	8.88	—	—	—
containing	„	—	8.91	—	—	—
0.0145 % S	„	—	8.92	—	0.0143	—
	„	—	8.90	—	—	—
	5	—	—	7.73	—	—
	„	—	—	7.70	—	—
	„	—	—	7.75	—	0.0000937 g. S.
	„	—	—	7.72	—	—

As a result of the analysis of many pure solutions of sulphates, I have not obtained a constant value to express the strength of the permanganate solution in terms of sulphur. The factor is an empirical one and considerably influenced by the experimental conditions. As a rule I have obtained somewhat lower figures than the 0.000099 given by Raiziss and Dubin. In the analysis of urine by this procedure, good results can be obtained if the permanganate is standardised against a pure sulphate solution under certain experimental conditions, and the urinary analyses are then conducted under identical conditions. The method, however, is in no way superior to the usual process, in which the benzidine sulphate is titrated directly in aqueous suspension with standard alkali.

Some of the analyses obtained upon pure sulphate solutions may be recorded here (Table I).

Having come to the conclusion that the permanganate titration suggested by Raiziss and Dubin possessed no advantages over the alkali titrations used by earlier authors, it was then necessary to determine first, whether the alkali titration process was adaptable to the estimation of small quantities of sulphates, and if so, to apply it to the estimation of the sulphates in small volumes of urine; and secondly, how best to utilise the process in the estimation of total sulphur.

Experimental.

1. THE ESTIMATION OF SMALL QUANTITIES OF SULPHATES IN PURE SOLUTION BY THE BENZIDINE METHOD.

In the first place, it was found that the precipitation of very small quantities of sulphates from pure solutions as benzidine sulphate was not quantitative unless the reaction of the fluid was carefully adjusted. Rosenheim and Drummond pointed out that an excess of hydrochloric acid prevented the quantitative precipitation of benzidine sulphate, and it was found that only a faint acid reaction must be present during precipitation. If this is so the benzidine sulphate separates quantitatively. The separation of the small precipitates thus obtained was attempted by several means, such as centrifugalisation, but it was finally found that the simple filtration method could not be improved upon. Naturally, the greatest care must be paid to the details of technique during filtration when very small precipitates are dealt with. The details are as follows:

A 5 cm. funnel is fitted into the neck of a 250 cc. filtering flask and carries

a small perforated porcelain filtering disc of 1 cm. diameter. A suspension of filter paper pulp is poured into the funnel and sucked dry upon the porcelain disc by means of a filter pump. The layer of paper should not be too thick; 1–2 mm. is sufficient to retain benzidine sulphate crystals. The benzidine sulphate precipitate can now be directly poured on to the filter, and filtered by the application of a partial vacuum. Care must be taken to prevent the precipitate at any time sucking dry upon the filter, otherwise, as previously stated, it will be obtained in a flaky condition and will not be attacked by the alkali during the titration [Raschig, 1903; Järvinen, 1913]. The pressure is therefore gently released at the moment before the precipitate sucks dry upon the filter. The vessel in which the precipitation has been carried out is then washed out into the funnel with two washings of about 2–3 cc. of a saturated solution of benzidine sulphate in distilled water, and the washings drawn through the filter by gentle suction, again taking care to prevent the precipitate being sucked dry. It is not advisable, especially when dealing with small precipitates, to wash the precipitate with distilled water, as do Raiziss and Dubin, because of the slight, but appreciable solubility of benzidine sulphate in water.

The precipitate, together with the filter paper and porcelain disc, are transferred by means of a clean glass rod into the vessel in which the precipitation was carried out, and the rod and funnel washed into it with a fine jet of distilled water.

The suspension of benzidine sulphate and filter paper, which, provided care has been taken during filtration, should show no flakes of benzidine sulphate, is then heated to boiling-point and titrated whilst hot with 0.02 N potassium hydroxide. As an indicator a dilute solution of phenolphthalein, or a drop or two of a saturated alcoholic solution of methyl red, can be used. Both indicators yield sharp end-points.

It is always necessary, when titrating benzidine sulphate precipitates with 0.02 N alkali, to carry out a blank estimation upon the reagents for each series of analyses.

Should the benzidine sulphate precipitate be obtained upon the filter in the form of flakes which cannot be titrated with alkali, all that is necessary to obtain an accurate result is to dissolve the precipitate in an excess of standard alkali by warming, and then to titrate the excess of alkali with standard acid, using either of the indicators mentioned above.

The following results, which have been obtained upon a pure solution of sodium sulphate, demonstrate the accuracy of the method.

TABLE II.

Analysis of a Solution of Sodium sulphate containing 0.0248 % S.

Cc. analysed	Weight of BaSO ₄ in g.	Cc. 0.1 N KHO	Cc. 0.02 N KHO	% S
50	0.0914	—	—	0.0250
"	0.0910	—	—	
25	—	3.91	—	0.0251
"	—	3.92	—	
"	—	3.91	—	
"	—	3.93	—	
10	—	—	8.04	0.0253
"	—	—	7.93	
"	—	—	8.00	
"	—	—	7.96	
"	—	—	7.96	
5	—	—	3.94	0.0249
"	—	—	3.88	
"	—	—	3.92	
"	—	—	3.94	
"	—	—	3.86	
2	—	—	1.56	0.0252
"	—	—	1.58	
"	—	—	1.59	
"	—	—	1.56	
"	—	—	1.60	

Having determined definitely that upon pure solutions the method yielded highly accurate results, the application was extended to the micro-analysis of the urinary sulphates.

2. ESTIMATION OF SMALL QUANTITIES OF URINARY SULPHATES.

2 a. Inorganic sulphates. The estimation of the inorganic sulphates in urine presented no difficulty whatever, it being quite sufficient to apply on a smaller scale the method as described by Rosenheim and Drummond for 20 cc. of urine. Accordingly the following technique was adopted and has yielded very accurate figures:

5 cc. of urine are measured into a 50 cc. beaker, acidified with 0.5 cc. of a 1 in 4 solution of hydrochloric acid (1 part conc. HCl to 3 of H₂O), and 20 cc. of the benzidine reagent are then added. After allowing the precipitate to settle for five minutes, it is filtered off upon a small filter-paper pulp filter in exactly the same way as has just been described for the estimation of pure sulphate solutions. The precipitate is transferred to the original beaker, and titrated at boiling-point with 0.02 N potassium hydroxide, using either phenolphthalein or methyl red as an indicator.

Quantities of urine as small as 2 cc. may be estimated by an exactly similar procedure, using proportional quantities of reagents. Analyses of the inorganic sulphates in two samples of urine are here shown. The control estimations were in all cases made by Folin's gravimetric method [1906].

TABLE III.

Sample	Cc. urine	Weight of BaSO_4 in g.	Cc. 0.1 N KHO	Cc. 0.02 N KHO	% SO_3
Urine 1	20	0.1069	—	—	0.183
	"	0.1067	—	—	
	20	—	9.10	—	0.182
	"	—	9.00	—	
	"	—	9.10	—	
	"	—	9.15	—	
	"	—	9.12	—	
	10	—	4.55	—	0.182
	"	—	4.55	—	
	"	—	4.55	—	
	"	—	4.55	—	
	5	—	—	11.55	0.183
	"	—	—	11.37	
	"	—	—	11.25	
	"	—	—	11.45	
	"	—	—	11.40	
	"	—	—	11.46	
	"	—	—	11.42	
Urine 2	20	0.0916	—	—	0.157
	20	—	7.70	—	0.153
	"	—	7.62	—	
	"	—	7.66	—	
	"	—	7.60	—	
	10	—	3.88	—	0.154
	"	—	3.88	—	
	"	—	3.80	—	
	"	—	3.82	—	
	5	—	—	9.76	0.154
	"	—	—	9.46	
	"	—	—	9.79	
	"	—	—	9.56	
	"	—	—	9.59	
	2	—	—	3.85	0.157
	"	—	—	3.87	
	"	—	—	3.96	
	"	—	—	4.00	
	"	—	—	4.00	

2 b. *The estimation of total sulphates.* This presented more difficulties than did the determination of the inorganic sulphates, mainly because the most careful regulation of the acidity after hydrolysis had to be made, in order that the precipitation of the benzidine sulphate should be complete.

In the earlier analyses, this was carried out as described by Gauvin and Skarzynski [1913], who, after hydrolysis of the ethereal sulphates in the usual manner, neutralised their solution with alkali and then reacidified it with the correct amount of hydrochloric acid. This procedure yielded fairly good results, but it was found, during some preliminary experiments, that there was no need to go to this trouble, as the ethereal sulphates in urine may be quantitatively hydrolysed by a far smaller proportion of hydrochloric acid than is usually employed.

Equal volumes of a sample of urine were hydrolysed by boiling with varying proportions of 1 in 4 hydrochloric acid, under identical conditions for 30 minutes. The analyses of total sulphates made on each sample, which are given below, demonstrate fully that the ethereal sulphates present in 20 cc. of urine are as completely hydrolysed by boiling for half an hour with 2 cc. of 1 in 4 hydrochloric acid as by boiling under identical experimental conditions with 15 cc. of the acid.

TABLE IV.

Urine hydrolysed for 30 minutes over constant source of heat.

Estimation	Cc. urine	Cc. 1 in 4 HCl	Weight of BaSO ₄ in g.	% SO ₃
Total	20	15	0.0824	
sulphates	20	15	0.0828	0.142
"	20	10	0.0827	
"	20	10	0.0829	0.142
"	20	7	0.0826	
"	20	7	0.0835	0.142
"	20	5	0.0830	
"	20	5	0.0826	0.142
"	20	3	0.0837	
"	20	3	0.0835	0.143
"	20	2	0.0834	
"	20	2	0.0836	0.143
Inorganic	20	—	0.0771	
sulphates	20	—	0.0778	0.133

This fact at once simplified the estimation of the total sulphates in urine on a small scale, and the method finally adopted as yielding the most reliable results is as follows.

5 cc. of urine are measured into a 25 cc. wide mouthed Erlenmeyer flask, and 0.5 cc. of a 1 in 4 solution of hydrochloric acid is added. A small glass

funnel inserted in the neck of the Erlenmeyer flask acts as a reflux trap to prevent spurting and undue concentration of the contents. Hydrolysis is then effected by boiling the liquid gently on a sand-bath for 30 to 40 minutes. The flask should be kept under observation during this period, and any appreciable diminution in the volume of the contents of the flask readjusted by the addition of a few drops of distilled water.

At the end of the period of time stated, the flask is removed from the sand-bath and allowed to cool. 20 cc. of the benzidine reagent are added, after the funnel and the neck of the hydrolysis flask have been washed down with a fine jet of distilled water. The precipitate is allowed to settle for five minutes, and is then filtered off and titrated in the manner already described.

The following urinary analysis will serve to show that the analysis of the total sulphates in as small a volume of urine as 2 cc. is rapidly and accurately made by this method.

TABLE V.

Cc. urine	Weight of BaSO ₄ in g.	Cc. 0.1 N KHO	Cc. 0.02 N KHO	% SO ₃
20	0.0824	—	—	0.142
"	0.0824	—	—	
10	—	3.56	—	
"	—	3.57	—	
"	—	3.54	—	0.142
"	—	3.58	—	
5	—	—	8.78	
"	—	—	8.80	
"	—	—	8.78	0.141
"	—	—	8.79	
2	—	—	3.50	
"	—	—	3.49	
"	—	—	3.46	0.140
"	—	—	3.47	

2 c. *Pathological urines.* Many pathological urines have been analysed by the method, and, in all but a few cases, have yielded highly accurate results. The presence of pigments in some pathological urines may render the hydrolysed urine dark in colour, and the precipitated benzidine sulphate will then often carry down much of the coloration with it. Usually the benzidine sulphate precipitate thus obtained is of a pale mauve colour, and this coloration is apt to interfere somewhat with the end-point of the titration. No means has yet been found of preventing this, but personally I have found little difficulty in deciding the end-point in such cases, if phenolphthalein is used as the indicator, and one has a little experience of the method.

The presence of albumin at first presented difficulties in the analysis of some urines, but I have found that if the method recently described by Tracey and Welker [1915] for the removal of albumin from urine is used, accurate results may be obtained in these cases.

To determine this, a sample of urine was taken and divided into two portions. To one was added about 0.7 % of albumin in the form of a serum albumin solution, whilst to the other was added an equal volume of distilled water. The urine containing the albumin was then mixed with an equal volume of aluminium cream suspension, prepared as described by Tracey and Welker, and filtered, whilst the other specimen was diluted with an equal volume of distilled water. Upon analysis the two specimens yielded the following figures, which clearly demonstrate that the removal of albumin from urine by means of its absorption with aluminium cream does not affect the sulphate content of the fluid.

TABLE VI.

Urine	Estimation	Cc. taken	Cc. 0.02 N KHO	% SO ₃
Treated	Inorganic sulphates	20	8.60	
		"	8.25	0.067
		"	8.34	
		10	4.17	
		"	4.21	0.067
		"	4.14	
Untreated	Inorganic sulphates	20	8.40	
		"	8.25	0.067
		"	8.20	
		10	4.20	
		"	4.10	0.066
		"	4.14	
Treated	Total sulphates	20	9.14	
		"	9.12	0.073
		"	9.10	
		10	4.55	
		"	4.51	0.072
		"	4.51	
Untreated	Total sulphates	20	9.22	
		"	9.20	0.073
		"	9.16	
		10	4.61	
		"	4.51	0.073
		"	4.50	

Some typical urinary analyses are here given, to show the high degree of accuracy attainable in analyses by this method.

TABLE VII.

Sample of urine	Estimation	Cc. urine	Weight of BaSO ₄ in g.	Cc. 0.1 N KHO	Cc. 0.02 N KHO	% SO ₃
Normal	Inorganic sulphates	20	0.0970	—	—	0.166
		10	—	4.16	—	
		"	—	4.16	—	
		"	—	4.20	—	0.167
		"	—	4.17	—	
		5	—	—	10.20	
		"	—	—	10.25	
		"	—	—	10.27	0.165
		"	—	—	10.27	
		2	—	—	4.18	
		"	—	—	4.17	
		"	—	—	4.20	0.167
		"	—	—	4.18	
	Total sulphates	20	0.1062	—	—	0.182
		10	—	4.56	—	
		"	—	4.56	—	
		"	—	4.55	—	0.182
		"	—	4.57	—	
		5	—	—	11.38	
		"	—	—	11.35	
		"	—	—	11.33	0.181
		"	—	—	11.38	
		2	—	—	4.64	
Normal	Inorganic sulphates	"	—	—	4.68	
		"	—	—	4.70	0.187
		"	—	—	4.71	
	Total sulphates	10	—	4.61	—	
		"	—	4.63	—	
		"	—	4.62	—	0.185
		"	—	4.66	—	
		5	—	—	11.78	
		"	—	—	11.58	
		"	—	—	11.60	0.187
		"	—	—	11.62	
		2	—	—	4.78	
		"	—	—	4.78	
	Total sulphates	"	—	—	4.68	0.188
		"	—	—	4.68	
		10	—	4.95	—	
		"	—	4.89	—	
		"	—	4.88	—	0.196
		"	—	4.84	—	
		5	—	—	12.46	
		"	—	—	12.38	
		"	—	—	12.50	0.198
		"	—	—	12.38	
	Total sulphates	2	—	—	5.11	
		"	—	—	5.08	
		"	—	—	5.12	0.204
		"	—	—	5.08	

TABLE VII (*continued*).

Sample of urine	Estimation	Cc. urine	Weight of BaSO_4 in g.	Cc. 0.1 N KHO	Cc. 0.02 N KHO	% SO_2
Tubercular	Inorganic sulphates	10	—	—	8.74	0.070
		"	—	—	8.72	
		"	—	—	8.70	
		5	—	—	4.36	0.069
		"	—	—	4.31	
		"	—	—	4.40	
		"	—	—	4.34	
	Total sulphates	10	—	—	9.95	0.080
		"	—	—	9.97	
		"	—	—	9.89	
		5	—	—	4.99	0.080
		"	—	—	4.96	
		"	—	—	4.95	
		"	—	—	5.00	
Nephritis; albumin removed by aluminium cream	Inorganic sulphates	10	—	4.62	—	0.195
		"	—	4.61	—	
		"	—	4.64	—	
		5	—	—	11.51	0.184
		"	—	—	11.48	
		"	—	—	11.56	
		"	—	—	11.49	
	Total sulphates	2	—	—	4.60	0.184
		"	—	—	4.61	
		"	—	—	4.56	
		10	—	5.25	—	0.208
		"	—	5.20	—	
		"	—	5.25	—	
		"	—	5.18	—	
		5	—	—	13.00	0.208
		"	—	—	13.07	
		"	—	—	13.07	
		"	—	—	13.01	
		2	—	—	5.20	0.208
		"	—	—	5.27	
		"	—	—	5.21	

3. THE DETERMINATION OF TOTAL SULPHUR.

Previous workers who have attempted to apply the benzidine method to the estimation of total sulphur have employed various oxidation methods; thus Gauvin and Skarzynski utilised various fusion methods, whereas Raiziss and Dubin employed the much more convenient process of Benedict, as modified by Denis [1910]. The American authors neutralised the final solution of salts obtained by this method, with sodium hydroxide, then

slightly re-acidified with hydrochloric acid and precipitated the sulphate present as benzidine sulphate in the usual manner.

The technique I have adopted for this determination resembles this method in its main points, and is as follows.

2 cc. of urine are measured into a 6 cm. porcelain evaporating basin, 0.5 cc. of Benedict's reagent is added, and the fluid evaporated gently to dryness upon the water-bath. The ignition of the residue is, however, carried out entirely by the use of a Barthel alcohol burner. This procedure was adopted as a result of some unpublished observations by Mrs M. C. Rosenheim.

TABLE VIII.

Cystine solution taken, cc.	Weight of BaSO ₄ in g.	Cc. 0.1 N KHO	Cc. 0.02 N KHO	% S
5	0.0380	—	—	0.055
"	0.0381	—	—	
10	—	3.25	—	0.052
"	—	3.25	—	
"	—	3.28	—	
"	—	3.20	—	
"	—	3.20	—	
"	—	3.23	—	
2	—	—	3.28	0.0518
"	—	—	3.26	
"	—	—	3.23	
"	—	—	3.20	
"	—	—	3.24	
"	—	—	3.23	

She informed me that the variable amount of sulphur found by her in the blank estimations which she made on the purest reagents obtainable were traceable to the sulphur-containing impurities present in London coal gas. I have been able to corroborate this, and have found that if one uses reliable reagents, and conducts the ignition solely with the use of a spirit burner, the blank estimations seldom, if ever, contain detectable quantities of sulphur.

When the residue has been completely ignited over the spirit burner, it is allowed to cool, and 1 cc. of 1 in 4 hydrochloric acid is added. The dish is covered with a clock-glass, and is warmed upon the water-bath until the residue is entirely in solution. Should evaporation to dryness occur before this is the case, another 1 cc. of acid should be carefully added and the warming continued. When the whole of the black residue is in solution, the dish is allowed to remain upon the water-bath, the cover is washed into the dish and the contents gently evaporated to dryness. 1 cc. of distilled water is now added and the contents quantitatively transferred

to a 25 cc. beaker. 10 cc. of the benzidine reagent are then added to precipitate the sulphate present. The subsequent filtration and titration of the precipitate are carried out in the manner already described.

Analyses carried out upon a solution of pure cystine, for which I am indebted to Dr O. Rosenheim, are given in Table VIII, and are sufficient to show that the method is reliable.

0.1960 g. cystine was dissolved in 5 cc. of hydrochloric acid and diluted to 100 cc. with distilled water; the solution contained 0.052 % sulphur.

The results of the application of the method to the analysis of total sulphur in urine are also given.

TABLE IX.

Sample	Cc. urine	Weight of BaSO ₄ in g.	Cc. 0.1 N KHO	Cc. 0.02 N KHO	% S
Urine 1	10	0.0481	—	—	0.066
	"	0.0486	—	—	
	10	—	4.15	—	0.066
	"	—	4.10	—	
	"	—	4.17	—	
	"	—	4.15	—	
	5	—	—	10.14	0.065
	"	—	—	10.16	
	"	—	—	10.10	
	"	—	—	10.18	
	2	—	—	4.10	0.065
	"	—	—	4.07	
	"	—	—	4.11	
	"	—	—	4.12	
Urine 2	10	0.0439	—	—	0.0604
	"	0.0442	—	—	
	10	—	3.80	—	0.061
	"	—	3.82	—	
	"	—	3.80	—	
	"	—	3.75	—	
	2	—	—	3.78	0.061
	"	—	—	3.80	
	"	—	—	3.83	
	"	—	—	3.78	

In conclusion I wish to tender my sincere thanks to Dr O. Rosenheim of King's College, for many helpful criticisms and suggestions during the course of this work.

SUMMARY.

1. The benzidine method of Rosenheim and Drummond for the estimation of urinary sulphates can be carried out upon as little as 2 cc. of urine, by titrating the precipitated benzidine sulphate with 0.02 N alkali.

2. The method is also applicable to the estimation of the total sulphur in small quantities of biological material, by applying the precipitation of the benzidine sulphate to the solution of salts obtained after oxidation of the material by Benedict's method.

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