sidered as sufficient proof of the general applicability and accuracy of the method.

The investigation was carried out under the guidance of the Director of these laboratories, Professor M. A. Rosanoff, whom the writer wishes to thank again for his friendly interest and assistance in the work. Acknowledgment is also due to Dr. W. L. Prager for his assistance in the early part of the work.

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[FROM THE LABORATORY OF PHYSIOLOGICAL CHEMISTRY, DEPARTMENT OF ANIMAL HUSBANDRY, UNIVERSITY OF ILLINOIS.]

THE DETERMINATION OF TOTAL SULPHUR IN URINE.

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In connection with the nutrition investigations of this laboratory, it became necessary to make a large number of total sulphur determinations in human urine. The sodium peroxide method as devised and described in detail by Folin[1] for the determination of total sulphur in urine was selected for this work. Some time after this method had been in use, it was noticed that more or less frequently the fusion resulting evolved hydrogen sulphide gas upon being acidified with the hydrochloric acid. The discovery of this fact was a great surprise and at the same time very discouraging since this method had already been used in determining the total sulphur in several hundred samples of urine.

This being the case much time and labor were devoted to studying the Folin method with the hope that by finding the proper method of procedure and observing the proper precautions the method could still be used in the investigation undertaken. The details of the method as described by Folin were closely and carefully followed in all respects, and repeated attempts were also made to modify the Folin method by increasing the time of fusion and by the addition of a greater quantity of sodium peroxide so as to insure the complete conversion of the sulphur of the urine into sulphuric acid. However as a result, the method still gave fusions which upon acidifying with the hydrochloric acid evolved hydrogen sulphide gas. It was found in this connection that the addition of two or three grams of sodium peroxide to the hot fused mass during the oxidation gave fusions which did not liberate hydrogen sulphide upon acidifying. It should be noted here that the Folin sodium peroxide method as applied to urine does not, like the Osborne[2] sodium peroxide method as used for the determination of sulphur in air-dried foods and other dry substances, specifically state to add sodium peroxide to the hot fusion

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[1] *J. Biol. Chem.*, 1, 157 (1906).

[2] This Journal, **24,** 142 (1902). *Ibid.*, **26,** 111 (1904).

to insure complete oxidation. This slight difference in the method of procedure, namely, the addition of small quantities of the sodium peroxide, yields a fusion which does not contain a part of the sulphur as sulphide.

In the meantime the paper of A. Konshegg[1] appeared in which he described the use of fuming nitric acid and potassium nitrate for the determination of sulphur in human urine. In view of the character of the results which we had obtained by the use of the sodium peroxide method, it seemed desirable to check the sodium peroxide methods by the method suggested by Konshegg.

The details of the nitric acid-potassium nitrate method as it has been used in this laboratory are as follows: Twenty-five cc. of the urine samples were measured from a burette into a 500 cc. Kjeldahl flask. Ten cc. of a twenty per cent. solution of potassium nitrate free from sulphur and twenty-five cc. of concentrated fuming nitric acid having a specific gravity of 1.53 were added to each urine sample. The Kjeldahl flasks with their contents were placed on an asbestos gauze over Barthel alcohol burners and the acid liquid evaporated. When near to dryness, in order to prevent bumping and splashing, the flasks were held over the naked alcohol flame with a suitable spring clamp and they were rapidly rotated while being evaporated to complete dryness. The flasks containing the evaporated residues were now further heated over the free flame until all brown fumes ceased to be liberated and the residues were liquid and of a white color. The flasks were removed from the flames and to prevent them from cracking they were rotated until the fall in the temperature of the hot liquid material caused it to solidify over the inner surfaces of the flasks. When the contents of the flasks had cooled the necks of the flasks were carefully and thoroughly washed with 25–50 cc. of hot distilled water. It is necessary to use hot water for this purpose instead of dilute hydrochloric acid because during the digestion a volatile material is condensed upon the neck or cooler parts of the flasks which is of a dark brownish color, and which will easily dissolve in hot water and not in dilute hydrochloric acid. If the dilute hydrochloric acid is added first, some of the condensation will merely be washed down into the flask in an insoluble form and any hot water added afterwards will not dissolve the condensed material. The dilute hydrochloric acid added after the hot water has dissolved the condensed product does not precipitate it.

After the necks of the flasks are washed with the hot water, 10 cc. of dilute hydrochloric acid, one to four, were added to dissolve the salts and the resulting solutions were transferred by means of hot water to a 250 cc. beaker. After thoroughly washing out the Kjeldahl flasks the final volumes of the liquids in the beakers were approximately 200 cc. If the entire process had been successfully conducted the resulting solu-

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[1] *Arch. ges. Physiol.*, **123**, 274–279 (1908).

tions were perfectly clear. To the clear solutions there were added 8 to 10 grams of chemically pure sodium chloride. The beakers were placed on the steam bath and heated ready for the precipitation. The addition of the sodium chloride was made to counteract the disturbing effect of the potassium salt on the barium sulphate precipitation and it was added in the ratio of four parts of sodium chloride to one part of the potassium salt. Folin[1] considered this proportion the best to use to prevent the interference of potassium salts. Tests were made in this laboratory which in general confirmed Folin's conclusion as to the counteraction which sodium chloride has upon the disturbing effect of the potassium salts. The results of these tests are given elsewhere in this paper.

To the hot acid liquids in the beakers 5 cc. of a 10 per cent. solution of barium chloride were added by means of a Folin dropper. The precipitated solutions were then allowed to stand undisturbed for 48 hours, after which they were filtered through S. and S. 9 cm. blue ribbon filters, the precipitates being thoroughly washed. The filters and precipitates were transferred to weighed porcelain crucibles. For the ignition the crucible lids were placed upon the crucibles so as to leave only very small openings and the crucibles and contents were placed on flat nickel crucible covers which were supported over Bunsen flames. In this manner, using low flames at first, the filter papers were dried and charred without danger of burning with a flame. After the paper had thoroughly charred, the crucibles and their contents were inclined on a pipe-stem triangle and carefully heated with a Bunsen flame until the barium sulphate was white.

Comparisons of the results obtained by the above-described acid nitrate method with those obtained by the Folin sodium peroxide method indicated clearly that the former method, without exception, gave more total sulphur in urines than did the latter method. The results of a considerable number of these determinations are given below in detail in Table I. It will be observed that the acid-nitrate method gives from 3.56 to 18.67 per cent. more sulphur in the urines than does the sodium peroxide method as usually applied to the determination of this element in urine. The average of the results here reported shows that the former method yields 10.31 per cent. more sulphur than does the latter method. Examination of the analytical data for the thirty-four individual determinations shows that the amount of sulphur obtained by the Folin method never even in a single case equals or exceeds the amount found by the acid-nitrate method.

The reagents used in the acid-nitrate method were repeatedly and thoroughly tested for sulphur by running parallel blank determinations which were carried through exactly as the determinations themselves with

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[1] *Journ. Biol. Chem.*, 1, 143 (1906).

the single exception that the urine was not taken in these cases. The reagents were free from sulphur compounds.

In consequence of the results thus obtained it was deemed desirable in this connection to test the influence of the presence of potassium nitrate, equivalent to that which was used in the acid-nitrate method, upon the precipitation of the sulphuric acid in the form of barium sulphate. In our experiments with potassium sulphate solutions, sodium sulphate solutions and a mixed sulphate solution of one part of potassium sulphate to two and one-half parts of sodium sulphate, the ratio in which these two metallic elements occur in normal urine, we have found the following facts in regard to the influence of the presence of potassium nitrate (equivalent to 2 grams), and sodium chloride upon the precipitation of the sulphuric acid in the form of barium sulphate. With sodium sulphate solutions the presence of the potassium nitrate considerably increased the weight of barium sulphate but the presence of the sodium chloride with the potassium nitrate in the ratio of four to one counteracted this influence of the potassium nitrate almost completely. With potassium sulphate solutions the presence of the potassium nitrate did not increase the barium sulphate weight to such an extent as it did with the sodium sulphate solutions, and the presence of the sodium chloride with the nitrate in the ratio of four to one did not materially counteract the potassium nitrate effect. With the solution of mixed sulphates as given above, the potassium nitrate had a decided effect on the weight of the barium sulphate precipitate, and the added sodium chloride with the potassium nitrate in the ratio of four to one almost completely counteracted the effect of the potassium nitrate. The data for this test were as follows: Weight of sulphate taken calculated as $BaSO_4$ = 196.0 mg. The weight of barium sulphate precipitated from a solution containing no added salts equaled 195.8 mg. The weight of barium sulphate precipitated from a solution containing two grams of potassium nitrate amounted to 206.7 mg. while the weight of barium sulphate precipitated from a solution containing two grams of potassium nitrate and nine grams of sodium chloride equaled 197.5 mg.

In this connection it should also be said that we chose to use the filter paper for filtration in preference to the Gooch filtration for the following reasons: First, after we had obtained sufficient data to satisfy ourselves that the filter paper method was practically as good as the Gooch method. Second, that with a large number of determinations to be made daily, a whole series of filtrations and washings could be made, simultaneously by one analyst with the filter paper method, which could not be done by using the Gooch crucibles, thus saving much time. However, where the saving of time is not an essential and necessary object, the use of the Gooch crucibles is probably preferable to the use of the filter papers, pro-

MILLIGRAMS OF BARIUM SULPHIDE. THEORETICAL 175.0 MG.

	Porcelain crucibles. One filter used.	Porcelain Gooch crucibles. First filtration.	Porcelain Gooch crucibles. Second filtration.
	174.9	175.5	175.5
	175.0	175.0	173.4
	174.6	175.0	174.0
	175.0	174.8	175.0
	175.2	175.0	174.8
	174.4	175.2	174.2
Average,	174.8	175.1	174.5

TABLE I.—RESULTS OF ACID-NITRATE AND FOLIN METHODS.

(Results expressed as grams of sulphur in urine per 24 hours.)

Laboratory No.	Acid-nitrate method. Gram.	Folin method. Gram.	Per cent. Folin method of acid-nitrate method. Per cent.	Laboratory No.	Acid-nitrate method. Gram.	Folin method. Gram.	Per cent. Folin method of acid-nitrate method. Per cent.
8072	0.696	0.659	...	8246	1.163	1.125	...
8072	0.725	0.660	...	8246	1.170	1.126	...
Average,	0.711	0.659	92.80	Average,	1.166	1.125	96.44
8171	0.726	0.592	...	8444	0.816	0.775	...
8171	0.752	0.638	...	8444	0.802	0.775	...
Average,	0.739	0.615	83.27	Average,	0.809	0.775	95.79
8469	0.822	0.737	...	8449	0.681	0.625	...
8469	0.814	...	...	8449	0.695	0.615	...
Average,	0.818	0.737	90.08	Average,	0.688	0.620	89.03
8472	0.890	0.746	...	8452	0.562	0.482	...
8472	0.913	0.788	...	8452	0.554	0.500	...
Average,	0.901	0.767	85.09	Average,	0.558	0.491	88.00
8478	0.754	0.700	...	8461	0.676	0.623	...
8478	0.733	0.721	...	8461	0.679	0.625	...
Average,	0.743	0.710	95.50	Average,	0.678	0.624	92.08
8480	0.752	0.619	...	8531	0.853	0.730	...
8480	...	0.604	...	8531	0.868	0.753	...
Average,	0.752	0.611	81.33	Average,	0.860	0.741	86.16
8484	0.851	0.753	...	8543	0.880	0.768	...
8484	0.845	0.775	...	8543	0.935	0.760	...
Average,	0.848	0.764	90.09	Average,	0.907	0.764	84.23
8485	0.851	0.728	...	8565	0.953	0.868	
8485	0.825	0.714	...	8565	0.931	0.904	...
Average,	0.838	0.721	86.02	Averoge,	0.942	0.886	94.06
8245	0.923	0.886	...				
8245	0.934	0.877	...				
Average,	0.928	0.881	94.85				

vided the proper asbestos pad is prepared and the detailed precautions as to Gooch filtration are carefully followed. The two methods of filtration gave the above result in tests made in this laboratory. The results in column three were obtained by filtering the barium sulphate directly upon the Gooch crucibles which still contained the barium sulphate from the first filtrations.

Since it was thus evident that the acid-nitrate method always gave higher results than the sodium peroxide method as it is usually applied to the determination of sulphur in urines, it became desirable to compare with the acid-nitrate method the modification of the Folin method mentioned above, namely, the addition of a few grams of the sodium peroxide to the fused mass, which it was found gave a fusion which did not yield hydrogen sulphide gas upon acidification. While this modification of the procedure proved that in no case was the sulphur left in the condition of a sulphide in the fused alkaline mass, it will be seen from the results given below in Table II that the acid-nitrate method gave more total sulphur in urines than did the modified Folin method. The acid-nitrate method gave from 0.41 per cent. to 15.30 per cent. more sulphur than did the

TABLE II.—RESULTS OF ACID-NITRATE AND MODIFIED FOLIN METHODS.
(Results expressed as grams of sulphur in urine per 24 hours.)

Laboratory No.	Acid-nitrate method. Gram.	Modified Folin method. Gram.	Per cent. modified Folin method of acid-nitrate method.	Laboratory No.	Acid-nitrate method. Gram.	Modified Folin method. Gram.	Per cent. modified Folin method of acid-nitrate method.
8327	0.902	0.850	...	8262	0.721	0.717	...
8327	0.885	0.855	...	8262	0.716	0.714	...
Average,	0.894	0.853	95.42	Average,	0.718	0.716	99.59
8332	0.575	0.525	...	8518	0.741	0.704	...
8332	0.588	0.531	...	8518	0.720	0.700	...
Average,	0.581	0.528	90.78	Average,	0.731	0.702	96.03
8558	0.858	0.810	...	8520	0.757	0.639	...
8558	0.882	0.818	...	8520	0.757	0.643	...
Average,	0.869	0.814	93.70	Average,	0.757	0.641	84.70
8359	0.597	0.583	...	8524	0.861	0.797	...
8359	0.597	0.587	...	8524	0.836	0.789	...
Average,	0.597	0.585	98.01	Average,	0.848	0.793	93.46
8364	0.769	0.749	...	8543	0.880	0.808	...
8364	0.769	0.756	...	8543	0.935	0.821	...
Average,	0.769	0.753	97.87	Average,	0.907	0.814	90.00
8233	0.813	0.766	...	8565	0.931	0.861	...
8233	0.813	0.773	...	8565	0.953	0.853	...
Average,	0.813	0.769	94.60	Average,	0.942	0.857	90.98

modified sodium peroxide method. The average of the results here given show that the former method yields 6.24 per cent. more sulphur than does the latter method. Examination of the analytical data for the twenty-four individual determinations here reported shows that the amount of sulphur obtained by the modified Folin method only in a single instance equaled the amount found by the acid-nitrate method. On the other hand, it will be observed that the modified method gives results which approach more nearly the results of the acid-nitrate method than does the original Folin method. According to the data presented in this paper the acid-nitrate method gives 10.31 per cent. more of the total sulphur present in urines than does the Folin method, while it yields only 6.24 per cent. more of the total sulphur than does the modified Folin method.

From the data thus obtained it seemed more than probable that during the evaporation of the alkaline solutions of the urines or during the heating of the solid residues resulting from the evaporation of the urines with sodium peroxide, some sulphur compounds are volatilized. That this supposition was true, is directly proven by the following experiment: Twenty-five cc. of a sample of urine which by previous analysis showed the acid-nitrate method to give more total sulphur than the Folin method were placed in a 500 cc. side-neck Jena distilling flask. The side-neck of the distilling flask was connected with a glass tube reaching nearly to the bottom of a 200 cc. Jena Erlenmeyer flask into which was placed about 75 cc. of concentrated fuming nitric acid. About 7–8 grams of sodium peroxide were introduced into the side-neck flask containing the urine and the neck of the flask was closed with a cork stopper. As soon as the most violent part of the reaction of the mixture had ceased a flame was applied to the distilling flask which rested on an asbestos wire gauze. The heating was carefully continued, not allowing the distillate to bubble too strongly through the nitric acid, until the material in the flask went to dryness. The wire gauze was then removed and the flask containing the alkaline mixture was heated with the direct flame until no more gas bubbled through the nitric acid. Ten cc. of a 20 per cent. solution of potassium nitrate was added to the nitric acid solution of the distillate and the resulting mixture evaporated to dryness and fused over an alcohol lamp as in the regular acid-nitrate oxidizing method. The residue was dissolved in about 200 cc. of the water and the solution acidified with hydrochloric acid and precipitated as usual with barium chloride. Precipitates of barium sulphate were thus obtained showing that during the evaporation and the heating of the evaporated residue of the urine with sodium hydroxide as required in the sodium peroxide method, volatile sulphur derivatives were evolved. As an example there is given herewith the complete data for one of these tests. Twenty-five cc. of human urine, laboratory No. 8452, gave by the acid oxidizing method 84.4 mg. of barium sulphate and the same quantity of this same urine gave by the Folin method 74.2 mg. of barium sulphate. The barium sulphate obtained by the distillation process as outlined above weighed 4 mg. This quantity of barium sulphate does not account for the entire difference between the weights of barium sulphate obtained immediately above. To account entirely for the difference by the sulphur recovered in the distillate would probably be impossible for the following reasons: First, the temperature attained by heating the alkaline mixture in the flask could not be made sufficient to entirely fuse the mass and therefore it is possible that not as much of sulphur was volatilized as in the Folin determination. Second, the residue remaining in the flask in the distillation process undoubtedly contained a portion of the sulphur in the form of a sulphide and it was proved by direct test that the fusion resulting from the Folin method evolved hydrogen sulphide gas.

This distillate experiment has been repeated several times with corresponding results. Further, blank determinations have been run upon this distillate method which gave no barium sulphate precipitate from the nitric acid distillate, demonstrating the fact that the sulphur obtained by this process did not come from the reagents employed.

This experiment proves conclusively that there is a loss of sulphur attending the determination of this element in urines where the sodium peroxide methods and probably in fact where any alkaline fusion methods are used for this purpose. This loss in many cases amounts to ten per cent. or more of the total sulphur existing in the urine. This fact therefore throws considerable doubt upon the true value of the data obtained in past experiments as to sulphur metabolism where the alkaline fusion methods have been used to determine the amount of sulphur in the urine. At the present time it is difficult to determine the exact nature of these errors since it is not known whether or not the losses of sulphur occurring in this determination in urines also occurs in the determination of this element in foods and feces. Experiments are now being made in this laboratory to determine the nature and extent of the losses, if any, occurring in the determination of sulphur in foods and feces. Results so far obtained show that the determination of the total sulphur in foods and feces by the method of procedure usually employed in the determination of total sulphur in urines gives fusions which more or less frequently evolve hydrogen sulphide gas upon being acidified.

We wish here to express our thanks to F. G. Allison for his valuable assistance in connection with the analytical work involved in this paper.

URBANA, ILL.