A COMPARISON OF THE METHODS FOR THE ESTIMATION OF TOTAL SULPHUR IN URINE

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It is well known that urine contains sulphur, not only in the form of sulphates, both inorganic and ethereal, but also in the form of less highly oxidised organic compounds, generally spoken of (following E. Salkowski's¹ suggestion) as 'neutral sulphur.' The organic compounds in question are very diverse and include thiocyanic acid and its salts,² cystine and closely related bodies,³ taurine and tauro-carbamic acid,⁴ methyl mercaptan,⁵ ethyl sulphide,⁶ thiosulphuric acid,⁷ sulphurous acid,⁸ urochrome,⁹ oxyproteic acid,¹⁰ uroproteic acid,¹¹ and uroferric acid.¹²

The quantitative determination of the total amount of sulphur present in the urine depends upon the fact that this neutral or unoxidised sulphur can be converted by the aid of suitable oxidising agents into sulphuric acid. The various methods in use differ mainly in the different oxidising agents chosen. They may be arranged in three groups:—

1. Virchow's *Archiv.*, Bd. LVIII, S. 472, 1873.
2. Leared, *Proc. Roy. Soc., Lond.*, Vol. XVI, p. 18, 1870; R. Gschleiden, *Tageblatt d. 47. Versammlung deut. Naturf. u. Aertzte in Breslau*, 1874 (quoted from E. Abderhalden's *Lehrbuch d. physiol. Chem.*, II Aufl., S. 343, 1909); I. Munk, *Virchow's Archiv.*, Bd. LXIX, S. 354.
3. Stadthagen, *Zeitsch. f. physiol. Chem.*, Bd. IX, S. 125, 1885; E. Goldmann u. E. Baumann, *ibid.*, Bd. XII, S. 254, 1888.
4. E. Salkowski, *loc. cit.*
5. M. Nencki, *Arch. f. exper. Path. u. Pharmac.*, Bd. XXVIII, S. 206, 1891.
6. J. J. Abel, *Zeitsch. f. physiol. Chem.*, Bd. XX, S. 253, 1894.
7. J. J. Abel, *loc. cit.*, for dog. Presch found in case of typhus that a compound which he isolated from the urine in small quantities gave sulphurous acid on distillation with acids. This is the only case I can find in which thiosulphuric acid has been noticed in urine of man. Quoted from A. Heffter, *Ergebnisse d. Physiol.* (Asher and Spiro), Jg. I, Abt. I, S. 458, 1902.
8. Strümpell, in case of fever, *Arch. d. Heilk.*, 1876. (Quoted from Dixon Mann, *Physiol. and Path. of Urine*, 2nd Edit., p. 18, 1908.)
9. St. Dombrowski, *Zeitsch. f. physiol. Chem.*, Bd. LIV, S. 204, 1907-8.
10. St. Bondzýnski u. R. Gottlieb, *Centralb. f. d. med. Wissensch.*, No. 33, S. 577, 1897; St. Bondzýnski u. K. Panek, *Ber. d. Deut. chem. Gesellsch.*, Jg. XXXV, S. 2959, 1902; St. Bondzýnski, St. Dombrowski u. K. Panek, *Zeitsch. f. physiol. Chem.*, Bd. XLV, S. 83, 1905; F. Pregl, *Pflüger's Archiv.*, Bd. LXXV, S. 87, 1899.
11. Max Cloetta, *Archiv. f. exper. Path. u. Pharmac.*, Bd. XL, S. 29, 1897.
12. O. Thiele, *Zeitsch. f. physiol. Chem.*, Bd. XXXVII, S. 251, 1903.

1. Methods in which the residue left upon evaporation of a certain definite volume of urine is effected by fusing with a mixture of sodium carbonate and saltpetre. These are merely applications to the urine of Liebig's original method of sulphur estimation. Full directions as to the procedure are given in Savelieff's¹ and Moreigne's² papers.

2. Methods employing concentrated fuming nitric acid as the oxidising agent. Schulz³ and Mohr⁴ have described methods utilising this reagent. Schulz originally carried out the process in a special closed apparatus, believing that volatile sulphur compounds might be formed, but in his last communication on the subject he states that this is not the case, and now carries out the oxidation in an open Kjeldahl flask. Konschegg,⁵ who also uses fuming nitric acid, adds a small quantity of potassium nitrate, probably in order to effect more complete oxidation.

3. Methods in which the oxidation is brought about by sodium peroxide. The introduction of this reagent, for the purpose of sulphur estimations, we owe to Hempel.⁶ It was subsequently used by Hoehnel,⁷ Glaser⁸ and by Asbóth,⁹ for the estimation of sulphur in organic materials. In a later paper Asbóth,¹⁰ who subsequently employed sodium peroxide for the estimation of sulphur in organic compounds, suggested that the method might also be useful for the determination of the total sulphur in urine.¹¹ Modrakowski¹² simplified the method by omitting the addition of sodium carbonate to the peroxide, which formed a part of the original process. More recently Folin¹³ has modified the Asbóth-

1. Savelieff, *Virchow's Archiv.*, Bd. CXXXVI, S. 197, 1894.

2. Moreigne, *Bull. de la Soc. Chim.* [3], XI, 975, 1894.

3. H. Schulz, *Pflüger's Archiv.*, Bd. LVII, S. 57, 1894; also *ibid.*, Bd. CXXI, S. 114, 1907-8.

4. P. Mohr, *Zeitsch. f. physiol. Chem.*, Bd. XX, S. 556, 1895.

5. A. Konschegg, *Pflüger's Archiv.*, Bd. CXXIII, S. 274, 1908.

6. W. Hempel, *Zeitsch. f. anorg. Chem.*, Bd. III, S. 193, 1893.

7. M. Hoehnel, *Archiv. d. Pharm.*, Bd. CCXXXII, S. 225, 1894.

8. C. Glaser, *Chemiker Zeitung*, Jg. XVIII, S. 1448, 1894.

9. A. von Asbóth, *Chemiker Zeitung*, Jg. XIX, S. 599, 1895.

10. A. von Asbóth, *ibid.*, Jg. XIX, S. 2040, 1895; A. Edinger recommended sodium peroxide for the estimation of organically combined sulphur before Asbóth's paper appeared. He used an aqueous solution of the peroxide. *Zeitsch. f. analyt. Chem.*, Bd. XXXIV, S. 366, 1895; *Ber. d. Deut. chem. Gesellsch.*, Jg. XXVIII, S. 427, 1895.

11. S. Lang used the Asbóth method to estimate total sulphur in urine, but gives no control analyses. *Zeitsch. f. physiol. Chem.*, Bd. XXIX, S. 305, 1900.

12. G. Modrakowski, *Zeitsch. f. physiol. Chem.*, Bd. XXXVIII, S. 561, 1903. F. Clark, *Journ. Chem. Soc.*, 1893, I, p. 1093, had previously suggested that sodium carbonate was unnecessary. Almost immediately after Modrakowski's paper, Neumann and Meinertz (*Zeitsch. f. physiol. Chem.*, Bd. XLIII, S. 38, 1904) advised the admixture of potassium carbonate.

13. O. Folin, *Journ. of Biolog. Chem.*, Vol. I, p. 155, 1906. See also T. B. Osborne, *Journ. of Am. Chem. Soc.*, Vol. XXIV, p. 142, 1902; J. A. Le Clerq and Dubois, *ibid.*, Vol. XXVI, p. 1108, 1904; W. L. Dubois, *ibid.*, Vol. XXVII, p. 729, 1905.

Modrakowski method in several particulars (especially with regard to the addition of a little water before the final fusion in order to obtain complete fusion with the aid of comparatively little heat, and to protect the crucible against corrosion). In all these methods the fusion is accomplished by heat applied to the exterior of the crucible, preferably by means of a methylated spirit burner.

Pringsheim, however, utilising an observation of Parr's,¹ showed that the oxidation may be most easily brought about by the introduction of a red-hot iron nail into the mixture. He first employed this method for the estimation of the halogens, phosphorus and arsenic,² and later, following Konek³ and other observers,⁴ to the estimation of sulphur⁵ in organic combination. Recently, Abderhalden and Funk⁶ have applied Pringsheim's method to the estimation of total sulphur in the urine.

It is obvious that in any trustworthy method, the results obtained with the same urine should agree *inter se*; and in the comparison of different methods, the method which gives the highest results will in the absence of other indications be the most correct. On both these points there are differences of opinion with regard to the different methods: thus Österberg and Wolf⁷ state that while the method of Asbóth-Modrakowski, carried out according to Folin's directions, invariably gives higher figures than the Schulz method, the results obtained by the latter method do not agree well *inter se*; Konschegg states that his modification of Schulz's procedure gives considerably higher figures than the latter; Abderhalden and Funk claim that the Pringsheim modification in their hands also gives higher figures than Schulz's method; and Gill and Grindley⁸ say that Konschegg's figures are invariably higher than those obtained by both Osborne's and Folin's modifications.

Taking all these statements into consideration, and bearing in mind that no corroborative observations have yet appeared on the Pringsheim method, it seemed advisable to investigate the degree of accuracy of the various methods in a series of urines.

1. S. W. Parr, *Journ. Am. Chem. Soc.*, Vol. XXII, p. 646, 1900.
2. H. H. Pringsheim, *Ber. d. Deut. chem. Gesellsch.*, Jg. XXXVI, S. 4244, 1903; *Amer. Chem. Journ.*, Vol. XXXI, p. 386, 1904; *Zeitsch. f. angewand. Chem.*, Bd. XVII, S. 1454, 1904.
3. F. von Konek, *Zeitsch. f. angewand. Chem.*, Bd. XVI, S. 516, 1903.
4. C. Sundstrom, *Journ. of Am. Chem. Soc.*, Vol. XXV, p. 184, 1903; J. D. Pennock and D. A. Morton, *ibid.*, Vol. XXV, p. 1265, 1903.
5. H. H. Pringsheim, *Ber. d. Deut. Chem. Gesellsch.*, Jg. XLI, S. 4267, 1908.
6. E. Abderhalden and C. Funk, *Zeitsch. f. physiol. Chem.*, Bd. LVIII, S. 331, 1908.
7. E. Österberg and C. G. L. Wolf, *Biochem. Zeitsch.*, Bd. IX, S. 307, 1908.
8. F. W. Gill and H. S. Grindley, *Proceed. of Soc. of Biolog. Chem.*, Vol. VI, p. 11, May, 1909.

In comparing the different methods, I have taken as my standard of agreement the one adopted by Abderhalden and Funk, namely, variations of 0.1 milligr. on either side of the mean value, when the sulphur is estimated in 10 c.c. of urine.

As there seemed to be no necessity of confirming the correctness of the old Liebig method,¹ I limited myself to the following, viz.:—those of Schulz, Konschegg, Asbóth-Modrakowski, and Pringsheim (Abderhalden-Funk modification).

Schulz's Method. The following are the analytical results I have obtained with four different urines:

URINE A

5 c.c. urine gave 0.0358 gr. BaSO_4 = 0.0098 gr. sulphur in 10 c.c. urine
 „ „ „ 0.0362 gr. „ = 0.0100 gr. „ „ „

URINE B

5 c.c. urine gave 0.0348 gr. BaSO_4 = 0.0096 gr. sulphur in 10 c.c. urine
 „ „ „ 0.0353 gr. „ = 0.0097 gr. „ „ „

URINE C

5 c.c. urine gave 0.0510 gr. BaSO_4 = 0.0140 gr. sulphur in 10 c.c. urine
 „ „ „ 0.0501 gr. „ = 0.0138 gr. „ „ „

URINE D

5 c.c. urine gave 0.0154 gr. BaSO_4 = 0.0042 gr. sulphur in 10 c.c. urine
 „ „ „ 0.0150 gr. „ = 0.0041 gr. „ „ „

It will be seen that (contrary to Osterberg and Wolf's statement) the figures obtained agree very well *inter se*, the maximum variation being only 0.2 milligr. Mohr, who used a method almost identical with that of Schulz, also obtained concordant results. My results by the Schulz method are, however, lower than those obtained by other methods, as will be immediately seen.²

Konschegg's Method. The following are my analytical figures:—

URINE A

5 c.c. urine gave 0.0378 gr. BaSO_4 = 0.0104 gr. sulphur in 10 c.c. urine
 „ „ „ 0.0375 gr. „ = 0.0103 gr. „ „ „

URINE B

5 c.c. urine gave 0.0369 gr. BaSO_4 = 0.0101 gr. sulphur in 10 c.c. urine
 „ „ „ 0.0384 gr. „ = 0.0106 gr. „ „ „

URINE C

5 c.c. urine gave 0.0528 gr. BaSO_4 = 0.0145 gr. sulphur in 10 c.c. urine
 „ „ „ 0.0524 gr. „ = 0.0144 gr. „ „ „

URINE D

5 c.c. urine gave 0.0165 gr. BaSO_4 = 0.0045 gr. sulphur in 10 c.c. urine
 „ „ „ 0.0163 gr. „ = 0.0045 gr. „ „ „

1. See however O. Folin, *Journ. of Biolog. Chem.*, Vol. I, p. 156, 1906.

2. This confirms the statement of Konschegg, Osterberg and Wolf, Abderhalden and Funk (*loc. cit.*) and of Sherman who used the nitric acid method for the estimation of sulphur in organic compounds. (*Journ. of the Amer. Chem. Soc.* Vol. XXIV, p. 1100, 1902).

Pringsheim's Method (Abderhalden and Funk's modification). The following are my analytical figures:—

URINE A

10 c.c. urine gave 0.0740 gr. BaSO_4 = 0.0102 gr. sulphur in 10 c.c. urine
 „ „ „ 0.0744 gr. „ = 0.0102 gr. „ „ „

URINE B

10 c.c. urine gave 0.0798 gr BaSO_4 = 0.0110 gr. sulphur in 10 c.c. urine
 „ „ „ 0.0800 gr. „ = 0.0110 gr. „ „ „

URINE C

10 c.c. urine gave 0.1032 gr. BaSO_4 = 0.0142 gr. sulphur in 10 c.c. urine
 „ „ „ 0.1043 gr. „ = 0.0143 gr. „ „ „

URINE D

10 c.c. urine gave 0.0322 gr. BaSO_4 = 0.0044 gr. sulphur in 10 c.c. urine
 „ „ „ 0.0318 gr. „ = 0.0044 gr. „ „ „

URINE E

10 c.c. urine gave 0.0756 gr. BaSO_4 = 0.0104 gr. sulphur in 10 c.c. urine
 5 c.c. „ „ 0.0383 gr. „ = 0.0106 gr. „ „ „

It will be seen, in confirmation of what Abderhalden and Funk state, that the figures are very concordant.

If the figures obtained by the Konschegg method are compared with the above obtained by the Pringsheim method, it will be observed that the Konschegg figures are a little higher (1 to 2.1 per cent.) than those obtained by this method, and 5.2 per cent.¹ on the average higher than those obtained by Schulz's method.

Asbóth-Modrakowski Method. Here different results were obtained, according to differences in the details of the *modus operandi*. In most of my estimations I carried out the process in the following manner:— I added the urine gradually to the sodium peroxide to avoid loss by spitting,² evaporated down on the water bath to complete dryness, and subjected the residue to complete fusion over a spirit burner, maintaining it in the fused condition for ten to fifteen minutes; cooled; added 1-2 c.c. of distilled water, and more peroxide and again subjected the mixture to complete fusion, prolonging the fusion for twenty to thirty minutes. I used between three and four grams of sodium peroxide for the preliminary fusion and eight grams for the final; the quantity of urine used was 25 c.c. Using this method I obtained, in all the estimations I performed, agreement between the individual determinations made on the same urine, and also higher results than by any of the other methods.³ On the average the figures were 8 per cent. higher than those

1. This difference is much less than that given by Konschegg himself.

2. Recommended by Modrakowski, *loc. cit.*

3. M. A. Desmoulière, *Journ. de Pharm. et de Chim.* [6th series], Tome XXIV, p. 294, 1906, states that Moreigne's and Modrakowski's methods give accurate results in estimation of total sulphur in urine; the single comparison of figures which he gives for the two methods shows a slight excess in favour of Modrakowski. This, taken in conjunction with Abderhalden and Funk's demonstration, that the Pringsheim method gives the same figures (really slightly lower) as the Na_2CO_3 — KNO_3 method, accords with my results.

given by the Konschegg method. I was unable to detect any evolution of sulphuretted hydrogen, either during the evaporation or the subsequent acidification of the fused mass, such as Gill and Grindley describe.

The following are my analytical figures:—

URINE B

25 c.c. urine gave 0.2036 gr. BaSO_4 = 0.0112 gr. sulphur in 10 c.c. urine
 „ „ „ 0.2053 gr. „ = 0.0113 gr. „ „ „

URINE C

25 c.c. urine gave 0.2724 gr. BaSO_4 = 0.0150 gr. sulphur in 10 c.c. urine
 „ „ „ 0.2798 gr. „ = 0.0154 gr. „ „ „

URINE D

25 c.c. urine gave 0.0966 gr. BaSO_4 = 0.0053 gr. sulphur in 10 c.c. urine
 „ „ „ 0.0970 gr. „ = 0.0053 gr. „ „ „

This method, as I have already described it, is in the main identical with that described by Folin, except that he does not mention the preliminary fusion, and maintains that ten minutes' final fusion is sufficient. The number of analyses I have carried out according to his directions is not great; they agree well *inter se*, but they are never higher than those obtained by Pringsheim's method, and in the one case where I can make the comparison they are lower than those obtained by Konschegg's method.¹ The following are my analytical figures:—

URINE A

25 c.c. urine gave 0.1860 gr. BaSO_4 = 0.0102 gr. sulphur in 10 c.c. urine
 „ „ „ 0.1858 gr. „ = 0.0102 gr. „ „ „

URINE E

25 c.c. urine gave 0.1858 gr. BaSO_4 = 0.0102 gr. sulphur in 10 c.c. urine
 „ „ „ 0.1880 gr. „ = 0.0103 gr. „ „ „

Putting all the results together, the following table shows the mean figures expressed in grams of sulphur per 10 c.c. urine.

Method	Urine A	Urine B	Urine C	Urine D	Urine E
Schulz	0.0099	0.0097	0.0139	0.0042	—
Konschegg	0.0104	0.0104	0.0145	0.0045	—
Pringsheim	0.0102	0.0110	0.0143	0.0044	0.0105
Asbóth-Modrakowski	—	0.0113	0.0152	0.0053	—
Asbóth-Modrakowski (Folin) ...	0.0102	—	—	—	0.0103

From the above table it will be seen that Schulz's method gives the lowest, whilst the Asbóth-Modrakowski method gives the highest figures; the Pringsheim, Konschegg and the Folin's modification of the Asbóth-Modrakowski method all give intermediate figures.

Conclusion. The sodium peroxide method carried out according to Asbóth-Modrakowski (as described above) gives the highest figures in the estimation of the total sulphur in the urine, and must therefore be considered to be the most trustworthy of the methods at present in use.

1. Gill and Grindley, *loc. cit.*, state that the difference is even greater than I have obtained.