grams per liter and the sulphate, calculated as ammonium sulphate, is usually below 4 grams per liter. The phosphoric acid of the urine as PO_4, is generally below 3 grams per liter, with the phosphate calculated as $HNa_2PO_4.12H_2O$, consequently below about 12 grams per liter. These amounts are therefore well within the weights of salts taken in the experiments. In addition the conductivity of the urine is rarely above 0.025 or 0.03 at most. From all this, taken in connection with the results of Table XI, it follows that with the salt of a urine known and the conductivity at 20° accurately determined, the conductivity of the non-chloride constituents may be found with a considerable degreee of certainty by diminishing by about 3 per cent. the chloride conductivity as calculated from tables for the given concentration, and subtracting this corrected salt conductivity from the observed urine conductivity. The remainder must give very nearly the true conductivity of the remaining substances in solution and must measure, in a manner, the amount of the electrolytes. The 3 per cent. correction for the chloride conductivity covers the average effect of the other salts present in normal urine. It is not to be supposed that the method would suffice to give a quantitative determination, but as an independent factor the non-chloride conductivity has a bearing and importance of its own, irrespective of the character of the salts which produce it.

NORTHWESTERN UNIVERSITY,
CHICAGO, November, 1903.

[FROM THE SHEFFIELD LABORATORY OF PHYSIOLOGICAL CHEMISTRY, YALE UNIVERSITY.]

THE DETERMINATION OF NITROGEN BY THE KJELDAHL METHOD.

BY ROBERT BANKS GIBSON.

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IN a recently published paper by Kutscher and Steudel,[1] the reliability of the Kjeldahl method for estimating nitrogen in various organic compounds of physiological-chemical importance has been called in question. These investigators have failed to obtain satisfactory results in the analysis of such substances as creatine, creatinine, uric acid, lysine and histidine by this widely

[1] Kutscher and Steudel: *Ztschr. physiol. Chem.*, **39**, 12 (1903).

used process; and they are thus naturally led to doubt the applicability of the Kjeldahl method to the determination of nitrogen in fluids and precipitates of animal (or vegetable) origin. The Kjeldahl method has become so useful in the prosecution of studies in metabolism, as well as in the direct analysis of a large variety of nitrogenous compounds that any criticism of its accuracy demands immediate consideration.

The Kjeldahl process is carried out differently in almost every laboratory. Kutscher and Steudel's method consisted in heating the substance (0.15-0.2 gram in the case of creatine) vigorously for about ten minutes with 10 cc. of concentrated sulphuric acid and a piece of copper sulphate; after cooling a little, potassium permanganate was added, the mixture again heated to boiling and then distilled in the customary manner. The results obtained in this way with creatine were extremely divergent and in nearly every case considerably below the theoretical values, the differences running as large as 7 per cent. There was, apparently, no fixed relationship between the discrepancies found and the duration of the boiling, or the quantities of copper sulphate and permanganate used. With creatinine from meat, the results were even more divergent. Although the data obtained from the analyses of uric acid show a closer correspondence with the theoretical requirements than in the preceding cases mentioned, yet the results varied from — 1.37 to + 0.67 per cent. Particularly noticeable is the failure to obtain duplicate analyses under identical conditions.

A critical examination of the protocols of Kutscher and Steudel shows that the period of heating employed by them—usually until the mixtures were clear—was considerably shorter than is customary with most analysts. In this country the use of permanganate has been abandoned almost entirely; in our own laboratory the Gunning modification[1] (with potassium sulphate) has been adopted for several years. The Gunning method was first officially studied in this country by the Association of Official Agricultural Chemists in 1891. It was adopted as an official alternative method in 1892 and since then has been used almost exclusively in the analysis of food products and fertilizers. It seeems to have been late in receiving recognition in physiological laboratories.

[1] Cf. Gunning: *Ztschr. anal. Chem.*, **28**, 188 (1889); Arnold and Wedemeyer: *Ibid.*, **31** 525 (1892); Winton: *Ibid.*, **32**, 478 (1893); *Chem. News*, **66**, 227.

The excellent results which we have obtained in the form of closely agreeing duplicate analyses in many hundreds of determinations on various materials (foods, urine, faeces, proteids, etc.) lead to the belief that the failure of Kutscher and Steudel is perhaps attributable to faulty technique rather than to an inadequacy of the Kjeldahl process in the cases studied. At Professor Mendel's suggestion I have, therefore, undertaken a considerable number of control analyses in which nitrogen was estimated in several typical physiological-chemical compounds, notably uric acid which is frequently determined by the Kjeldahl method and which Kutscher and Steudel failed to estimate with accuracy in this way. The inadequacy of the Kjeldahl-Gunning method in the case of a number of types of nitrogenous compounds is too well known to need consideration here. For creatine, creatinine and guanidine, the possibility of obtaining reliable results has lately been demonstrated anew by Beger, Fingerling and Morgen[1] and by Malfatti.[2] The latter in particular has called attention to the deficiencies and limitations in the use of permanganate to complete the oxidations. Extracts from our own protocols are given below. The decompositions were carried out with 20 cc. of sulphuric acid and 10 grams of potassium sulphate. The final titrations were made with N/5 or N/10 solutions. The length of time during which the flasks were heated (to boiling) varied considerably; the heating was, however, always continued for some time after the fluids first appeared colorless.

Uric Acid ($C_5H_4N_4O_3$).—The preparation (Kahlbaum's) used was recrystallized three times from concentrated sulphuric acid and dried at 105° C. The heated decomposing mixtures became colorless in from ten to thirty minutes. The heating was then continued over a hot flame for periods varying from forty-five minutes to two and one-half hours. For these titrations N/5 solutions were employed.

[1] Beger, Fingerling and Morgen: *Ztschr. physiol. Chem.*, **39**, 329 (1903).
[2] Malfatti: *Ibid.*, **39**, 467 (1903).

ANALYSES OF URIC ACID.

Quantity taken. Gram.	Nitrogen found. Gram.	Per cent.	Difference from theory. Per cent.	Duration of decomposition. Hours.
0.2140	0.07104	33.20	—0.18	1
0.1945	0.06482	33.33	—0.05	1
0.1945	0.06482	33.33	—0.05	1½
0.2081	0.06956	33.42	+0.04	1½
0.2061	0.06838	33.18	—0.20	2
0.2095	0.06985	33.34	—0.04	2
0.2464	0.08229	33.39	+0.01	3
0.6870	0.22940	33.39	+0.01	3
0.2807	0.09393	33.42	—0.04	2½
0.2992	0.09901	33.10	—0.28	2
0.2728	0.09072	33.26	—0.12	2½
0.2273	0.07637	33.58	+0.20	3
0.2739	0.09120	33.29	—0.09	3
	Average.....	33.33		
	Calculated	33.38		

Hippuric Acid ($C_9H_9NO_3$).—A well crystallized Kahlbaum preparation dried at 105° C. was used. The oxidizing mixtures became colorless in from one to one and one-half hours. The heating was continued for periods varying from one and one-half to three and one-half hours.

ANALYSES OF HIPPURIC ACID.

Quantity taken. Gram.	Nitrogen found. Gram.	Per cent.	Difference from theory. Per cent.	Duration of decomposition. Hours.
0.4794	0.03703	7.90	+0.06	3
0.3197	0.02488	7.78	—0.06	2½
0.9603	0.07518	7.82	—0.02	2½
0.7116	0.05535	7.78	—0.06	2½
0.6547	0.05076	7.76	—0.08	1½
0.5527	0.04387	7.93	+0.09	2
0.5293	0.04212	7.95	+0.11	3
0.6787	0.05298	7.80	—0.04	3½
	Average.....	7.84		
	Calculated	7.84		

Other Compounds.—A few analyses were made on preparations of leucine ($C_6H_{13}NO_2$), tyrosine ($C_9H_{11}NO_3$), urethane ($C_3H_7NO_2$), thiourea ($C_8H_{10}N_2S$), phenylmethyloxypyrimidine ($C_{11}H_{10}N_2O$) and *o*-aminobenzoic acid ($C_7H_7NO_2$), which have for the most part been recrystallized and dried at 105° C.

ANALYSES OF OTHER COMPOUNDS.

Substance.	Quantity taken. Gram.	Nitrogen found. Gram.	Nitrogen found. Per cent.	Nitrogen calculated. Per cent.	Difference. Per cent.
Tyrosine	0.3984	0.03137	7.87	7.76	+0.11
Tyrosine	0.3797	0.02990	7.87	....	+0.11
Leucine	0.3060	0.03256	10.53	10.70	—0.17
Urethane	0.5435	0.08614	15.84	15.76	+0.08
Urethane	0.3132	0.04973	15.87	....	+0.11
Thiourea[1]	0.4597	0.07813	16.68	16.86	—0.18
Phenylmethyloxypyrimidine	0.3814	0.05742	15.05	15.08	—0.03
Aminobenzoic acid	0.3145	0.03241	10.29	10.23	+0.06
Caseinogen	0.6362	0.09827	15.45	....	....
Caseinogen	0.4668	0.07223	15.49	....	....
Caseinogen	0.6109	0.09472	15.47	....	....
Caseinogen	0.4353	0.06736	15.47	...	....

The caseinogen used in the above analyses was a carefully purified preparation made according to Hammarsten's method. I. Munk[2] has already shown that the Kjeldahl method can be applied to give results comparable to those obtainable with the Dumas method with caseinogen, provided the heating of the decomposition mixtures is continued for a sufficiently long time. The figures here given indicate the possibility of obtaining comparable and reliable analyses even where the conditions are widely varied. In addition to these, reference may be made to a large number of determinations of the nitrogen content of various proteids carried out in our laboratory and also by Osborne[3] and his co-workers. In many cases these have been verified by the Dumas method.

The foregoing results afford no occasion to question the usefulness or accuracy of the Kjeldahl method as applied within a wide range of experimental conditions in the physiological-chemical laboratory. Indeed, it would be difficult to explain the numerous instances of duplicate analyses repeatedly made on the same substances, such as proteids, foods, etc., by different analysts under widely different conditions if the reactions involved were uncertain or variable in their character. When due care is exercised to procure a proper decomposition and oxidation of the

[1] When the decomposition mixtures obtained from thiourea were distilled while still straw-colored (and presumably incompletely oxidized), the results obtained were too low.

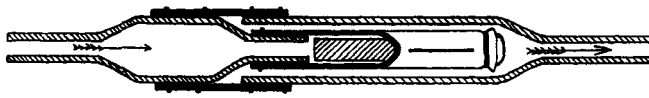
[2] I. Munk: *Archiv für Physiol.*, 1895. p. 551.

[3] Cf. various papers by Osborne in the Reports of the Connecticut Agricultural Experiment Station and This Journal, since 1892. Sadikoff has reported unfavorable results with some preparations of gelatins in *Ztschr. physiol. Chem.*, **39**, 411 (1903).

substances analyzed, uniformly satisfactory determinations can readily be obtained. For substances of unknown structure, however, the results furnished by the Kjeldahl process should not be accepted without verification by other methods.[1]

NOTES.

A Back Pressure Valve for Use with Filter Pumps.—The body of the valve is constructed of two pieces of glass tubing of fairly heavy gauge, drawn out as in sketch. The valve itself is an improvement on the old Bunsen valve, with a glass rod of slightly smaller diameter than the rubber tubing, wired on, to prevent collapse. Soft rubber tubing works best. The device has



given excellent service and can be made at little expense of time and material. It was originally devised for use with condensers in a laboratory where the water pressure sometimes gave out and the water ran back. It gives equally good service for both purposes. R. N. KOFOID.

The Conversion of Calcium Oxalate to the Sulphate.—The customary process of reducing a calcium oxalate precipitate to a sulphate by applying a flame directly to a platinum crucible, after saturating the precipitate with sulphuric acid, is a slow one at best, and there is great danger of losing a portion of the contents of the crucible on account of boiling over or spattering.

These difficulties may be avoided by the following method: The precipitate is placed into a platinum crucible and saturated with concentrated sulphuric acid in the usual manner. A porcelain crucible about one-half inch larger in diameter than the platinum crucible is then filled about half full with powdered asbestos, or calcium sulphate, and the platinum crucible is sunk into the asbestos until it clears the porcelain crucible by about one-fourth inch at the bottom. After covering the platinum crucible loosely,

[1] Since the above was written, an article by Sörensen and Pedersen (*Ztschr. physiol. Chem.*, **39**, 513 (1903)) has appeared with reference to Kutscher and Steudel's work. For a further criticism cf. also Schöndorff: *Arch. f. d. ges. Physiol.*, **98**, 130 (1903).